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Optimization of the quanti-qualitative production of basil destined for the preparation of "Genovese Pesto"

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List of Publications

1. **Ciriello, M.**, Formisano, L., Rouphael, Y., De Pascale, S., Kacira, M. Effect of daily light integral and photoperiod on yield, morpho-physiological characteristics and mineral composition of Genovese basil grown in vertical farming in two successive harvests. *Paper submitted to Scientia Horticulturae on 6th March* 2023.
2. **Ciriello, M.**, Kyriacou, M. C., De Pascale, S., and Rouphael, Y. An Appraisal of Critical Factors Configuring the Composition of Basil in Minerals, Bioactive Secondary Metabolites, Micronutrients and Volatile Aromatic Compounds. *Journal of Food Composition and Analysis*, **2022**, 104582.
3. **Ciriello, M.**, Formisano, L., El-Nakhel, C., De Pascale, S., and Rouphael, Y. Influence of Genetic Material, Successive Harvests and Hydroponic Cultivation on the Yield and Quality Characteristics of Genovese Basil: an Overview. *Paper submitted to Acta Horticulturae on 6th March 2022, accepted for publication on 26th November* **2022**.
4. **Ciriello, M.**, Formisano, L., Kyriacou, M. C., Colla, G., Graziani, G., Ritieni, A., De Pascale, S., and Rouphael, Y. Biostimulatory Action of Vegetal Protein Hydrolysate Compensates for Reduced Strength Nutrient Supply in a Floating Raft System by Enhancing Performance and Qualitative Features of" Genovese" Basil. *Frontiers in plant science*, *13*, **2022**, 906686.
5. **Ciriello, M.**, Formisano, L., El-Nakhel, C., Corrado, G., and Rouphael, Y. Biostimulatory Action of a Plant-Derived Protein Hydrolysate on Morphological Traits, Photosynthetic Parameters, and Mineral Composition of Two Basil Cultivars Grown Hydroponically under Variable Electrical Conductivity. *Horticulturae*, *8*(5), **2022**, 409.
6. **Ciriello, M.**, Formisano, L., Kyriacou, M., Soteriou, G. A., Graziani, G., De Pascale, S., and Rouphael, Y. Zinc biofortification of hydroponically grown basil: Stress physiological responses and impact on antioxidant secondary metabolites of genotypic variants. *Frontiers in Plant Science*, **2022,** *13*.
7. **Ciriello, M.**, Formisano, L., Zarrelli, A., Corrado, G., Kyriacou, M. C., De Pascale, S., and Rouphael, Y. Zinc biofortification of Genovese basil: influence on mineral profile and estimated daily intake in adults and children. *Food Research International*, **2022**, 112374.
8. **Ciriello, M.**, Formisano, L., El-Nakhel, C., Kyriacou, M. C., Soteriou, G. A., Pizzolongo, F., ... and Rouphael, Y. Genotype and successive harvests interaction affects phenolic acids and aroma profile of genovese basil for pesto sauce production. *Foods*, *10*(2), **2021**, 278.
9. Formisano, L., **Ciriello, M.**, El-Nakhel, C., Kyriacou, M. C., and Rouphael, Y. Successive Harvests Modulate the Productive and Physiological Behavior of Three Genovese Pesto Basil Cultivars. *Agronomy*, **2021**, *11*(3), 560.
10. **Ciriello, M.**, Formisano, L., El-Nakhel, C., Corrado, G., Pannico, A., De Pascale, S., and Rouphael, Y. Morpho-Physiological Responses and Secondary Metabolites Modulation by Preharvest Factors of Three Hydroponically Grown Genovese Basil Cultivars. *Frontiers in Plant*

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11. **Ciriello, M.**, Pannico, A., El-Nakhel, C., Formisano, L., Cristofano, F., Duri, L. G., ... and Rouphael, Y. Sweet Basil Functional Quality as Shaped by Genotype and Macronutrient Concentration Reciprocal Action. *Plants*, **2020**, *9*(12), 1786.

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Chapter 1
General Introduction

Italy is undoubtedly considered one of the leading countries in terms of high-quality food and wines, with a value in the agrifood sector of 575 billion euros in 2021[1]. In the national economic system, the agrifood production chain represents a strategic sector for territorial development and valorization of local specialties is considered a promising market strategy to overcome the challenges of globalization. The national Ministry of Agriculture lists more than 200 typical products (PDO, IGT, and TSG), including many specialty foods, usually related to the territories of production and cultivation or production characteristics, traditions, and biodiversity of the territories. The research presented in this Ph.D. thesis was carried out in collaboration with Barilla S.P.A. and aimed to fine-tune the production of basil for processing into "pesto Genovese" sauce by implementing sustainability principles and resource-efficient practices in the production process. The sustainability of a product is a value that consumers are increasingly asking for. To obtain pesto sauce, cultivation systems must have short crop cycles, ensure uniform growth, implement automation of some operations, guarantee hygiene, and foster the nutraceutical and functional quality of the product, which could add further value to the final product. The need to meet the urgent demands of the processing industry for a clean, tasty, and highly aromatic product is a challenge for basil growers, considering the large variability of aromatic plants in terms of flavor, especially in open field/soil-based agriculture. This challenge has led the scientific community and growers to focus on alternative intensive growing methods with controlled environmental and nutrient conditions, such as hydroponics[2,3]. The experimental work presented here is organized into 11 Chapters that cover the major specificities of basil growth, cultural variables that can affect the yield, aroma profile, organoleptic and processing characteristics, and possible strategies to enhance the nutritional value of pesto sauce (biofortification). Specifically, **Chapter 2** reviews the literature in which the genetic diversity of the *Ocimum* genus was critically analyzed from a phytochemical (mineral, vitamin, polyphenolic, and aromatic profile) perspective. This chapter also discusses how different pre-harvest factors interact with genotypes to better understand the potential of what is commonly called the "king of herbs." Despite the genetic variability of the *Ocimum* genus, which has led to the classification of well over 60 species[4] differing in growth aptitude, leaf morphology and pigmentation, and aromatic content[5], in western countries, the Genovese type is undoubtedly the best known and most appreciated. In Italian cuisine, the tender leaves of Genovese basil, in addition to their crucial role as a food garnish in iconic dishes of the culinary tradition of "Bel Paese" (e.g., the pizza Margherita and Caprese salad), are, due to a unique and pleasant aroma (characterized by the high content of linalool

eucalyptol and eugenol and the absence of estragole and menthol), the central ingredient of the famous green sauce known worldwide as "Pesto."[6,7] The distinctive quality features of Genovese type (aroma and phenolic profile) were critically analyzed and reported in a mini-review (**Chapter 3**) that focused on the primary pre-harvest practices typically performed for the industrial production of this aromatic plant. The growing demand for the agri-food industry, which is increasingly attracted to this sauce, has driven an increase of more than 60% in the cultivation of this leafy vegetable in Italy in recent decades[8]. Ordinarily, the cultivation of Genovese basil for "pesto" is carried out in open fields. Finally, according to the needs of the industrial processing chain, basil plants are harvested several times during the growing season (up to a maximum of three cuttings)[9-11]. In addition to ensuring early production, this agronomic practice reduces labor costs by avoiding multiple sowings during the growing season[9].

The cutting harvest system is another critical parameter that may affect the quality traits of Genovese basil. In **Chapters 4 and 5**, three basil cultivars were compared for open field industrial production of "Genovese pesto" in response to two successive harvests to evaluate the productive and qualitative aspects (characterization and modulation of the aromatic and phenolic profile). The results provided a valuable resource for growers and opened new research insights. Cropping systems, which are not dependent on changing seasonal conditions, can provide higher yields, improve nutraceutical and technological quality, reduce the incidence of pests and pathogens (and the use of pesticides), allow complete deseasonalization of production by shortening the crop cycle, and offer the possibility of growing in any environment where land use is impossible and the climate does not allow it[12,13]. Among the many hydroponic techniques, the floating raft system (FRS) is suitable for the large-scale cultivation of relatively small aromatic plants such as basil due to its ease of management, cost effectiveness, low input requirements, high resource use efficiency, and lower environmental impact[14,15]. Ordinary Genovese basil cultivars intended for industrial pesto production were selected for their ability to respond to traditional soil cultivation. Therefore, in **Chapter 6**, we evaluated the production and quality responses of the three cultivars (Aroma 2, Eleonora, and Italiano Classico) used in previous experiments (**Chapters 4 and 5**) and grown in FRS. In the same experiment, two different planting densities (159 and 317 plants m^{-2}) and the effects of successive harvests were evaluated (**Chapter 6**). In hydroponics, management of nutrient solutions is a critical preharvest factor that plays an imperative role in plant growth and development. The concentration and composition of the nutrient solution can modulate the production performance and plant nutritional and organoleptic features. Due to this, in **Chapter 7**, the impacts of three nutrient solutions with different macronutrient concentrations (1 dS m^{-1}, 2 dS m^{-1} and 3 dS m^{-1} at 25 °C) on the production, aroma and phenolic profiles of Genovese Aroma 2, Eleonora, and Italiano Classico basil cultivars grown in FRS were evaluated,

thus identifying the best combination of nutrient solution and Genovese basil cultivar to ensure a proper balance between production and quality while ensuring low environmental impact and paving the way for future work.

According to the guidelines of the European Commission, the need to reduce chemical-synthetic production inputs in the intensive agricultural sector while improving yields and quality has prompted the research community to become increasingly interested in biostimulants[16,17]. The combination hydroponic-biostimulant seems to be a winning strategy for producing vegetables with premium quality features, especially from a more ecologically sound perspective. As Colla et al.[18] reported, protein hydrolysates (PH) could be the key to addressing the urgent challenges of the agricultural sector. Considering the possibility in FRS of applying PH in contact with the roots (in NS) as a method to improve its efficacy, an experiment was conducted to evaluate the effects on the production performance and quality of two Genovese basil genotypes (Eleonora and Italiano Classico) grown in FRS with two concentrations of nutrients (1 and 2 dS m^{-1} at 25 °C) and the use of PH (Trainer®) directly in NS at two doses (**Chapters 8 and 9**). The ability to change the composition of the nutrient solution, enriching it with essential microelements for human health, better known as biofortification[9,19], makes hydroponics one of the most exciting and promising cropping systems. A successful hydroponic biofortification program could also be implemented in aromatic herbs to increase the concentration of desirable micronutrients (such as zinc and selenium) and secondary metabolites (such as phenolic acids and volatile aroma compounds) that are superior quality traits that consistently attract the interest of producers and consumers. Therefore, **Chapters 10 and 11** evaluated the impact of zinc biofortification on the bioaccumulation of this essential trace element, the yield, physiological responses, and the functional and nutraceutical quality of two Genovese basil cultivars (Aroma 2 and Eleonora) grown in an FRS system using biofortified nutrient solutions at different concentrations of zinc (12.5, 25.0, 37.5, and 50 μM). In **Chapter 12**, we evaluated the responsiveness of Genovese basil to vertical cultivation, focusing on the influence of light both in terms of photoperiod and quantity (DLI). The results highlight basil's good adaptability to more extensive cropping systems by satisfying industrial needs, paving the way for interesting scenarios from the perspective of economic, environmental and production sustainability.

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Chapter 2

An appraisal of critical factors configuring the composition of basil in minerals, bioactive secondary metabolites, micronutrients and volatile aromatic compounds

Abstract: Combining health-promoting nutrition with gastronomic novelty is a major trend currently driving the agri-food sector. Basil (*Ocimum basilicum* L.) is a genetically diverse aromatic vegetable crop that combines rich phytochemical composition and enticing sensory profile. The current review examines how genetic variation underlies the phytochemical composition, nutrient composition, and volatile aromatic compounds of basil. It further provides a critical assessment of preharvest factors that configure product quality, including nutrient modulation, controlled stress, biofortification, biostimulant and light management applications. Appropriate genotype selection may facilitate sustainable production of improved quality, whereas targeted preharvest applications combined with optimized light intensity and spectral quality may effectively increase the content of essential phytochemicals and micronutrients, while suppressing the accumulation of anti-nutritive agents. The application of biostimulants may further underpin the sustainability factor in basil production, especially under growth-limiting conditions. The current review constitutes a critical synopsis of all available scientific literature investigating key factors configuring the composition of basil in minerals, bioactive secondary metabolites, micronutrients and volatile aromatic compounds from 1996 to 2022. Topics warranting further research are highlighted, with emphasis placed in identifying optimal combinations within the genotype-environment-management interaction nexus that tap the physiological and molecular mechanisms responsible for improving plant performance and functional-sensory quality in basil.

Keywords: Biofortification; Biostimulants; Controlled stress; Light management; Nutrient management; Phytochemicals; *Ocimum basilicum* L.; Functional quality; Sensory quality.

1. Basil as an Upcoming Aromatic Vegetable in the Global Market

A Basil is an annual herbaceous plant of the *Lamiaceae* family native to India, currently cultivated and distributed worldwide mainly for its aromatic leaves. Its versatility as a culinary herb and leafy vegetable renders it a quintessential gastronomic component of soups and salads, a dried spice, and a key ingredient of tasty sauces. Perhaps the most acclaimed and internationally recognized of Italian sauces is "pesto", produced from sweet basil. The medicinal and nutritional properties of basil are associated with its rich content in biologically active compounds, including phenolic acids (rosmarinic, chicoric, caffeic, and *p*-coumaric), flavonoids (quercetin), anthocyanins, but also vitamins and minerals[1-4].

Recent research has highlighted the crucial role of phenolic compounds in preventing chronic and cardiovascular diseases due to its anti-inflammatory and anticancer properties. However, their structural complexity and polymerization affect their intestinal absorption, resulting in low bioavailability and uncertain therapeutic specificity. More than 90% of polyphenols are not absorbed in the small gut, accumulating in the large gut where they are metabolized by the gut microbiota into low molecular weight phenolic compounds that make them bioavailable[5]. In turn, bioavailable phenolic compounds can modulate the activity of the gut microbiota, performing a natural prebiotic function that promotes the growth and proliferation of beneficial bacteria[5,6]. However, the signature trait of basil is its aromatic profile, which draws its constitutive complexity and highly variable composition on broad genetic background[4]. Notwithstanding variation among the different chemotypes, the prevailing aromatic compounds comprise monoterpenes and phenylpropanoids[7]. The greatest challenge for modern agriculture is the need to produce food for the growing global population, projected to reach ten billion by 2050[8]. To meet this soaring demand, novel cultivation modules and techniques are required to enhance crop yield without compromising sustainability and quality. In recent decades moreover, the demand for horticultural products of nutraceutical value has also been rising, driven by the growing interest of modern society in health-promoting nutrition[8]. Many bioactive compounds (e.g., ascorbic acid, tocopherols, carotenoids, phenolic acids, flavonoids and, anthocyanins) account for significant antioxidant activity that mitigates the risk of chronic and cardiovascular diseases while stimulating cognitive health[9]. Basil is considered a potent functional food that has gained great economic importance thanks to its chemical composition and organoleptic properties. Considering that bioactive composition derives mainly from the genetic material used[10-12], the first part of this review illustrates the nutraceutical and functional properties of basil across species and cultivars. However, crop performance and phytochemical content beneficial to human health also depend on pre-harvest factors, such as the prevailing environmental conditions and crop management[8]. Plant nutrition influences the quality of basil[13,14].

Hydroponic systems allow greater control over the nutritional status of plants, thus effectively modulate their phytochemical profile[8,10], reduce the accumulation of antinutritive agents such as nitrates, and facilitate the application of positive stress to improve the quality of basil[11,15]. Moreover, soilless cultivation can effectively implement schemes for the biofortification of basil with micronutrients essential to human health[8]. Similarly, the management of light conditions significantly influences crop performance and the sensory and functional quality of basil[16]. Consequently, in the second part of this review we examine the scientific progress on crop management of basil to illustrate how environmental factors and cultivation practices impact product quality.

A literature review was conducted, integrating peer-reviewed papers, books, technical journals, and conference proceedings published from 1996 to 2022, including the phytochemical composition, functional and sensory quality of basil. In the Scopus electronic database, the following keywords were used (in the title/abstract): "Basil", "*Ocimum*", "genotype", "mineral profile", "nitrate", "ascorbic acid", "vitamin C", "vitamin E", "bioactive compounds", "carotenoids", "phenolic compounds", "phenolic profile", "flavonoids", "anthocyanins", "aromatic profile", "volatile essential oils", "nutrient management", "plant nutrition", "hydroponics", "soilless cultivation", "eustress", "biofortification", "biostimulant", "light quality", "light quantity", "light management".

2. Variation in the Composition and Functional Quality of Basil

2.1. Methods for Determining the Phytochemical, Vitamin, and Mineral Composition of Basil

The mineral, vitamin, and phytochemical composition of basil have been investigated in previous works with respect to genetic and agro-environmental factors. Different classes of potentially bioactive compounds have been profiled and quantified by different analytical methods. The methods for determining mineral and vitamin (ascorbic acid, vitamin E and carotenoids) concentrations, and the aroma profile have been summarized and reported in **Table 1**, whereas the methods used for phenolic compounds are presented in **Table 2**.

Table 1. List of analytical methods used for the determination of mineral profile, Ascorbic Acid (AA), tocopherols (Vit. E), carotenoids, and volatile organic compounds (VOCs) in basil. Abbreviations: Atomic absorption spectrometry (AAS); Ion chromatography (IC); Iodometric method (IDM); High-performance liquid chromatography (HPLC); Spectrophotometric (SPM); Reverse-phase High-performance liquid chromatography (RP-HPLC); Solid-phase microextraction (SPME); Gas Chromatography/mass spectrometry (GC/MS); Gas chromatography–electron impact mass spectrometry (GC–EIMS); gas chromatography/flame ionization detection (GC/FID).

Compound	Determination method	Reference
Mineral Profile	AAS	17-19
	IC	10,20
Ascorbic Acid	IDM	21
	HPLC	22
	SPM	23
Tocopherols (Vitamin E)	RP-HPLC	23
	HPLC	24
Carotenoids	HPLC	25,26
	SPM	27-31
Volatile organic compounds	SPME and GC/MS	10,20,32,33
	GC–EIMS	34
	GC/MS and GC/FID	35-38
	GC/MS	39-44

Table 2. The content of phenolic acids, anthocyanins and total phenolics in popular basil species and cultivars. Abbreviations: High-performance liquid chromatography (HPLC); High-performance liquid chromatography tandem mass spectrometer combined with a quadrupole time-of-flight mass spectrometer (LC-MS Q-TOF); Liquid Chromatography with tandem mass spectrometry (LC-MS/MS); Spectrophotometric (SPM); High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD); Liquid Chromatography coupled to Diode-Array Detection with tandem mass spectrometry (LC-DAD-MS/MS); Reversed-fase High-performance liquid chromatography (RP-HPLC); High-performance liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS)

Basil species and cultivar	Phenolic Acids													Anthocyanins	Determination method	Reference
	Rosmarinic acid	Chicoric acid	Caftaric acid	Caffeic acid	Ferulic acid	*P*-Coumaric acid	Caffeoyltartaric acid	Quercetin rutinoside acid	Gentisic acid	Salvianic acid A	Salvianolic acid K	Salvianolic acid L	Fertaric acid			
	mg g^{-1} dry weight (mg g^{-1} fresh weight) [μmol g^{-1} fresh weight]													mg g^{-1} dry weight		
O. basilicum L. 'Genovese'	0.04	0.05		0.04	0.005											
	0.11	0.06		0.05	0.005										HPLC	20
	0.14	0.7		0.05	0.003											
	0.42	0.23		0.09	0.017	0.007										
	0.37	0.3		0.11	0.017	0.007									HPLC	10
	0.64	0.33		0.09	0.016	0.006										
	0.56			0.33		3.71									LC-MS Q-TOF	1
	0.80	0.02	0.02	0.08		0.026	0.024	0.6							LC-MS/MS	45
														0.08	SPM	46
	1.50	3.69		0.55											HPLC-DAD	2
	25.5														HPLC	47
	8.81			0.215											LC-DAD-MS/MS	48
	0.12	0.05	0.12	0.16	0.33	0.07	0.12	0.59							LC-MS/MS	49
	16.73	9.76	4.97	0.124											HPLC	30
	1.00	0.261											0.04		HPLC	50
	1.71	2.15	0.21	0.77										0.52	HPLC	51
	7.93	1.09												0.33	HPLC	52
	0.048			0.15	0.06										RP-HPLC	23
	(1.17)	(0.248)	(0.033)												HPLC-DAD-ESI-MS/MS	53
	[14.85]	[1.19]	[0.35]								[0.23]	[1.46]	[0.12]		HPLC-DAD-ESI-MS/MS	54
	0.0047	0.0040	0.038	0.028	0.0020											
	0.0054	0.0048	0.030	0.028	0.0040										HPLC	55
	0.0045	0.0033	0.021	0.028	0.0016											
	0.0070	0.0027	0.028	0.034	0.0026											
	0.015	0.033		0.016	0.003											
	0.152	0.050		0.023	0.012										HPLC	32
	0.326	0.205		0.021	0.006											

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Cont. Table 2

Basil species and cultivar	Phenolic Acids													Anthocyanins	Determination method	Reference
	Rosmarinic acid	Chicoric acid	Caftaric acid	Caffeic acid	Ferulic acid	*p*-Coumaric acid	Caffeoyltartaric acid	Quercetin rutinoside acid	Gentisic acid	Salvianic acid A	Salvianolic acid K	Salvianolic acid L	Fertaric acid			
	mg g^{-1} dry weight (mg g^{-1} fresh weight) [μmol g^{-1} fresh weight]													mg g^{-1} dry weight		
O. basilicum L. var. purpurascens 'Dark Opal'	44.3			0.212											LC-DAD-MS/MS	48
	6.69	2.01												0.60	HPLC	52
	48														HPLC	56
	[12.45]	[0.93]	[0.18]							[0.28]	[0.18]				HPLC-DAD-ESI-MS/MS	54
	0.0175	0.0037	0.0241												HPLC	55
O. basilicum L. 'Red Rubin'	33														HPLC	56
	(0.84)	(0.05)	(0.062)	(0.046)											HPLC-DAD-ESI-MS/MS	57
	1.392	0.107	0.048	0.090					0.407							3
	[15.00]	[1.09]	[0.29]	0.0276	0.0054						[0.21]	[0.53]		0.72		58
																54
O. basilicum L. 'Cinnamon'	7														HPLC	56
	4.79	1.70	0.26	0.16										0.48		51
	(1.38)	(0.746)	(0.039)	(0.062)											HPLC-DAD-ESI-MS/MS	57
O. basilicum L. var. thyrsiflorum 'Siam Queen'	0.26	2.78	0.49	0.32										0.50	HPLC	51
	(2.41)			(0.0022)				(0.012)							HPLC-DAD-ESI-MS/MS	59
O. basilicum L. 'Lettuce leaf'	29.0														HPLC	56
	0.0041	0.0020	0.0223	0.0223	0.0007											
	0.0029	0.0026	0.0238	0.0334	0.0017										HPLC	55
	(1.58)			(0.0033)				(0.0066)							HPLC-DAD-ESI-MS/MS	59
O. basilicum L. var. minimum 'Small leaved'	21.0			0.464											LC-DAD-MS/MS	48
	0.0029		0.0178	0.0362	0.0022											
	0.0056	0.0026	0.0286	00427	0.0023										HPLC	55
	0.0022	0.0020	0.0219	0.0254	0.0014											
O. basilicum L. 'Purple ruffles'	23.0														HPLC	56
	1.087	0.058	0.071	0.058					0.354						HPLC	3
O. basilicum L. 'Osmin purple'	29.0														HPLC	56
	0.10	0.46	0.10	0.16										0.98	HPLC	51
	1.471	0.343	0.035	0.121											HPLC	51
O. basilicum X *O. americanum* 'Sweet Dani'	0.06	0.07	0.11	0.14										0.58	HPLC	51

2.2. The Mineral Composition of Basil

Vegetables in general, and basil in particular, can contribute balanced amounts of minerals to the daily diet[60]. However, the genetic diversity of the genus *Ocimum* and prevailing agroenvironmental may also affect basil's mineral content significantly. The study by Ličina et al.[60] shows that calcium is one of the most abundant minerals in basil, yet strongly influenced by genotype, as it ranged from 3.65 g kg^{-1} dry weight (dw) in 'Finoverde' to 1.92 g kg^{-1} dw in 'Holy red' and 'Blue spice', with the renowned 'Genovese' registering 3.40 mg g^{-1} dw. With reference to the United States Department of Agriculture[61] database for basil, the average calcium concentration is 22.4 mg g^{-1} of dry product.

Genotype also strongly influences the concentration of phosphorus and magnesium in basil[17]. Phosphorus and magnesium concentration in cultivars Genovese, Lettuce, Purple Opal, and Compact were 0.99-0.80 mg g^{-1} and 0.84-0.73 mg g^{-1} dw, respectively[60], whereas the work by Nurzynska-Wierdak et al.[19] reports significantly lower phosphorus and magnesium concentration of 0.3 and 0.15 mg g^{-1} dw, respectively. In the USDA database, values for phosphorus concentration in basil are near 560 mg g^{-1} and for magnesium 470-820 mg g^{-1} fresh weight (fw). Nutritional interest in potassium stems from its ability for lowering blood pressure. The Genovese and Lettuce cultivars, favoured in Italy for pesto production and fresh consumption respectively, were reported to have potassium concentration of 1.13 and 1.76 mg g^{-1} dw[60]. Majkowska-Gadomska et al.[17] recorded much higher values in green varieties with an average of 2.18%. The USDA data register the range of variability as 1.28 to 5.65 mg g^{-1} fw of potassium. Contrary to potassium, the high intake of sodium contributes to hypertension, hence reducing sodium intake is widely recommended. In sweet and purple basil, the sodium concentration varied between 0.05 and 0.01 mg g^{-1} dw[17]. The USDA reports that the sodium concentration in basil is generally 0.03-0.04 mg g^{-1} fw. Considering that the recommended daily sodium intake is 1.2-1.5 g for adults[9], the sodium contribution from basil is very low.

The iron content of basil is genetically configured[60], but it is also strongly influenced by the planting time of the seedlings[17]. The work of Majkowska-Gadomska et al.[17], shows that spring transplanting almost doubled the iron concentration in sweet basil (0.69 mg g^{-1} dw) compared to summer transplanting (0.34 mg g^{-1} dw). The most iron-rich basil cultivars were Compact and Lettuce, with values of 3.58 mg g^{-1} dw and 1.585 mg g^{-1} dw, respectively, as opposed to 1.01 mg g^{-1} dw for Genovese[60]. According to USDA data, 100 g of fresh basil contains 3.17 mg of iron, the intake of which can provide about 20% of the recommended iron daily requirement (8-18 mg day^{-1} per adult). In the Genovese, Lettuce, Compact, and Purple Opal cultivars, zinc concentration ranged 0.12-0.57 mg g^{-1} dw[60]. Golcz & Seidler-Łożykowska[62] reported similar values for sweet basil of about 0.69 mg g^{-1} dw. Organic fertilization with 40% vermicompost increased zinc

concentration from 0.08 to 0.1 mg g^{-1} compared to the untreated control[18]. However, basil cannot be considered a good source of zinc based on USDA data, as 100 g of fresh basil provides only 9% of the recommended daily requirement (8-11 mg day^{-1} per adult)[9].

The mineral profile of basil contains compounds that promote health but also antinutritive agents that can be toxic in excessive quantities[22]. Nitrate is one of these agents, with leafy vegetables overall constituting the group of foods that contributes most to dietary nitrate intake[10]. However, the European Commission has not set a threshold limit for nitrates in basil, although it accumulates nitrates in its tissues. Aside from genotypic sources of variation, nitrate concentration also varies among different plant parts[10,20] and is strongly influenced by environmental, nutritional, and physiological factors[22]. Nitrate varied from 0.24 to 4.28 mg g^{-1} fw in different basil cultivars grown in protected environment, with the purple-pigmented ones Purple Opal and Red Rubin showing the highest concentration of 3.88 and 4.28 mg g^{-1} fw, respectively[22]. Recent studies on Genovese basil cultivars Italiano Classico, Eleonora, and Aroma 2 showed that conventional cultivation in the open field yielded a mean nitrate concentration of 0.92 mg g^{-1} fw [10] compared to 3.11 mg g^{-1} fw obtained in a floating system[20]. Nonetheless, the possibility of managing accurately the concentration and composition of the nutrient solution (NS) in soilless culture renders it a valuable tool for reducing nitrate accumulation in plant tissues[8,15].

2.3. Vitamins and Bioactive Secondary Metabolites

2.3.1. Ascorbic Acid

Unable to synthesize vitamins, humans rely on dietary intake to prevent severe metabolic disorders. The ones contained in basil are vitamins C, E, and the precursors of vitamin A (carotenoids). Ascorbic acid is crucial for human health as it is implicated in immune and antioxidant activity[63]. Due to its high solubility in water, it is easily discarded by the body, so it is necessary to consume this vitamin regularly through the daily diet[63]. Dumbrava et al.[21] observed that basil leaves are richer in ascorbic acid (0.271 mg g^{-1}) than rosemary leaves (0.185 mg g^{-1}). In a study conducted on seven basil cultivars, including two of red pigmentation, the ascorbic acid ranged from 0.105-0.222 mg g^{-1} fw [22]. The green cultivar Ohře produced by Seva Moravia had the highest ascorbic acid (0.220 mg g^{-1} fw), while the Mánes compact cultivar with dark green leaves had the lowest (0.105 mg g^{-1} fw). The same study demonstrated the effect of growing season on ascorbic acid content. Using the Genovese reference cultivar Mammolo, Murátiková & Neugebauerová[22] showed that the summer level (0.072 mg g^{-1} fw) was half of that obtained in spring (0.140 mg g^{-1} fw). Both cultivation technique and time of harvesting have considerable influence on the ascorbic acid concentration of basil leaves. Sgherri et al.[23] showed that the leaf concentration of reduced ascorbate (bioactive form of ascorbic acid) in hydroponically grown basil (about 20 μmol g^{-1} dw) was near twice that of basil

grown on soil (about 10 μmol g^{-1} dw). They also observed that performing earlier harvest by 15-days in the floating system to obtain softer plant tissues resulted in further increase of the reduced ascorbate form to about 40 μmol g^{-1} dw.

2.3.2. Vitamin E

As the main fat-soluble antioxidant obtained exclusively from dietary sources, vitamin E gathers increasing interest from the food, pharmaceutical, and cosmetic industries. It is used to improve the shelf-life and stability of foods, while it also demonstrates photoprotective, antihypertensive, neuroprotective, anti-inflammatory, and antioxidant activities[24], moreover it curbs the risk of coronary heart disease and degenerative diseases[9]. Vitamin E is composed of 4 tocotrienols and 4 tocopherols, all of vegetable origin, of which α-tocopherol is the most abundant and biologically active[9]. The primary forms of vitamin E found in Genovese basil are α- and γ-tocopherol[23] as also evidenced by Fernandes et al.[24] on 'Red Rubin'. According to the USDA, the α-tocopherol concentration per 100 g of fresh basil was 0.8 mg, while that of γ-tocopherol was only 0.16 mg. Cultivation technique and especially the time of harvest strongly influence the α- and γ-tocopherol concentration in basil[23]. Harvested 20-days after sowing in a floating system, basil leaves recorded the highest α- and γ-tocopherol concentrations (0.015 and 0.020 mg g^{-1} dw, respectively) compared to the open field harvest 35 days after sowing.

2.3.3. Carotenoids

Vitamin A refers to both retinoids and carotenoids, and it is essential to the human body as it influences the visual, immune, and reproductive functions. Retinol comes mainly from animal sources, whereas carotenoids come from plant foods[9]. Carotenoids are accessory photosynthetic pigments present in considerable concentrations in yellow-orange plants and dark leafy greens, and they also have a complex biochemical role in the human body[25]. It is well-known that physiological, biochemical, and genetic traits of plants such as geographic origin, species, mode and time of harvest, part of the plant analyzed, and post-harvest management can influence the accumulation and bioaccessibility of these valuable bioactive compounds[26]. β-carotene has the highest pro-vitaminic activity among the carotenoids that can be metabolized into vitamin A. Basil can accumulate high levels of carotenoids with concentrations mainly influenced by genetic and environmental factors[25]. In fact, in agreement with the previous statement, Batra et al.[27] showed the strong impact of genotype on carotenoid biosynthesis in two morphotypes of *Ocimum sanctum* with the highest values recorded for 'Sri Tulsi' (4.01 mg g^{-1} fw). Similarly, Saran et al.[64] observed in 11 accessions of holy basil for industrial application a wide variability on carotenoid biosynthesis, with values ranging from 5.4 to 8.5 mg g^{-1} fw. According to the USDA nutrient database, carotenoid concentrations in sweet basil are 0.05 mg g^{-1} fw for lutein+zeaxanthin, and 0.03 mg g^{-1} fw for β-carotene.

Kopsell et al.[25] have extensively analysed the concentration of β-carotene and lutein in 8 cultivars of sweet basil both in the open field and protected environments. In the open field, the ranges of β-carotene and lutein content were 0.05-0.06 and 0.07-0.08 mg g^{-1} fw, respectively, while in the protected environment the corresponding ranges were 0.03-0.04 and 0.06-0.08 mg g^{-1} fw. In contrast, Daly et al.[26] observed on an Israeli basil ecotype a higher β-carotene content (0.18 mg g^{-1} dw) than lutein+zeaxanthin (0.07 mg g^{-1} dw), demonstrating the influence of genotype on the bioaccumulation of these compounds again. According to Kopsell et al.[25] red-pigmented cultivars accumulated more carotenoids in the protected environment, while green-pigmented cultivars showed the highest carotenoid concentration when grown in full sun. On the contrary, Daly et al.[26] did not find significant differences in β-carotene and lutein concentration for the Greek cultivar as a function of the cultivation system (greenhouse vs open field) while for 'Red Rubin' open field cultivation increased lutein (+31.30%) and decreased β-carotene (–19.10%).

In addition, light interception as influenced by leaf position on the plant can affect the quality and quantity of carotenoids in sweet basil. Wongsen et al.[31] showed that the β-carotene content was higher in the younger top leaves that were less shaded than the middle and bottom leaves. Despite the above, Stagnari et al.[30] did not observe significant differences in carotenoid concentration (on average, 1.24 mg g^{-1} fw) for both upper and lower leaves of Genovese basil (‘Emily’) under different shading with colored plastic films (yellow, green and blue). Not least, the data reported by Proshkin et al.[28] emphasize that for the cultivar Red Rubin, carotenoid concentration increased linearly as the crop cycle progressed. However, this response was not observed for the cultivars Gastronome and Markus, emphasizing the influence of genetic material on carotenoid biosynthesis in relation to the growth cycle. Similarly, Sakalauskaitė et al.[29] observed on basil 'Cinnamon' that carotenoid content (on average, 0.34 mg g^{-1} fw) was not significantly affected by phenological stage (3-4 leaf pair stage vs flowering stage).

2.3.4. Phenolic Composition

2.3.4.1. Phenolic Compounds

Phenolic compounds are an important group of secondary metabolites responsible for the plant defense and stress response systems[1,7]. Phenols are also known for their positive impact on human health, but also influencing the sensory and nutritional properties of food[65]. In addition to imparting greater antioxidant activity than ascorbic acid and vitamin E, phenolic compounds protect cells during the early stages of cancer development[65,66]. Phenolic compounds are divided into different subgroups[65] according to their chemical structures. The phenolic subgroups, characteristic of basil, are phenolic acids and flavonoids.

Basil contains high levels of phenolic acids, with rosmarinic acid being the most abundant[51,53,67]. Rosmarinic acid is a caffeic acid ester with several critical biological properties, such as antioxidant, anti-inflammatory, and immunostimulant activity[68]. According to the study by Nguyen et al.[52], the average concentration of rosmarinic acid found in the cultivars Sweet Thai, Dark Opal, and Genovese were 5.06, 7.45, and 5.72 mg g^{-1} dw, respectively. Regardless of the cultivar ('Italiano Classico', 'Red Rubin' and 'Dark Opal'), Prinsi et al.[54] showed that rosmarinic acid was the most accumulated phenolic compound, accounting for more than 80% of the sum of the individual phenolic acids. Similar results were reported by Majdi et al.[40] on cinnamon (*O. basilicum* cv. Cinnamon) and lemon basil (*Ocimum* × *citriodorum*) and by Zeljković et al.[44] on 12 basil genotypes with rosmarinic acid values that ranged from at 2653.24 μmol g^{-1} to 3686.49 μmol g^{-1} in the Ohre and Chládek cultivars, respectively. The influence of genotype was also confirmed by Kiferle et al.[68] who observed that rosmarinic acid concentration varied approximately from 4 to 63 mg g^{-1} dw among 'Superbo', 'Genovese' and 'Dark Opal'. Despite lower values compared to the above author, Bajomo et al.[55] in a recent study of 27 basil cultivars confirmed the strong genetic variability on rosmarinic acid bioaccumulation, observing a variation from 0.022 mg g^{-1} dw for Spicy Globe to 0.175 mg g^{-1} dw for Dark Opal. Although the findings of Scagel et al.[57] confirm that rosmarinic acid is predominant, regardless of genetic material, significant differences were found between Sweet Broadleaf and Siam Queen cultivars. Although Kiferle et al.[68] state that hydroponic cultivation offers the possibility to significantly increase the biosynthesis of this secondary metabolite recent studies by Ciriello et al.[10,20] revealed higher rosmarinic acid production in basil plants grown in open field compared to those grown in hydroponics. The higher rosmarinic acid in open field plants could be attributed to the less favorable soil, climatic, and nutritional conditions prevailing compared to hydroponics, which induced oxidative stress that fostered the accumulation of secondary metabolites[23,67], reported higher values in the phenolic profile of 23 Iranian basil cultivars, with the highest concentration of rosmarinic acid reaching near 100 mg g^{-1} dw. The authors hypothesized that these outlying values could be the response to the intense dry heat that characterizes the geographical area of cultivation. In addition to environmental conditions, the biosynthesis of bioactive compounds in basil leaves can be affected by cultural practices such as successive cuts. Ciriello et al.[10] demonstrated that successive harvests increased the rosmarinic acid concentration of Genovese basil eight-fold. Juliani et al.[56] evaluated the accumulation of rosmarinic acid at different phenological stages of basil crop and found that 'Dark opal' exhibited the highest concentration at the full bloom stage. By contrast, the highest accumulation of rosmarinic acid in the 'Italian Large Leaf' was in the post-flowering stage. Several studies report chicoric acid as the second most abundant phenolic acid in basil leaves[51-53]. The average concentration of chicoric acid in 'Sweet Thai', 'Dark Opal', and

'Genovese' was 1.24, 1.74, and 2.50 mg g^{-1} dw, respectively[52]. Kwee & Niemeyer[51] observed greater variability in the concentration of chicoric than rosmarinic acid, with the values found in 15 cultivars examined ranging from 0.03 to 2.78 mg g^{-1} dw, obtained respectively from the 'Spice' and 'Siam Queen'. Similarly, Bajomo et al.[55] observed on 27 basil cultivars that the genetic effect strongly influenced the accumulation of chicoric acid, with the highest values recorded by the cultivar Rosie. In contrast, it was impossible for the cultivar Globette to quantify the content of this crucial phenolic compound. Although rosmarinic acid is consistently referred to as the predominant phenolic acid in basil leaves, the cultivars Nufar F1 and Siam Queen had a 5-10 times higher concentration of chicoric acid than rosmarinic acid. Similarly, the results obtained from the varietal comparison of Genovese basil grown in a floating system highlight the influence of genotype on the production of phenolic acids. While the phenolic profile of cultivars Aroma 2 and Italiano Classico corroborated the existing literature on the predominance of rosmarinic acid, cultivar Eleonora presented a higher content of chicoric acid[10], as reported by Ciriello et al.[32] for the same cultivar in the autumn-winter crop cycle, confirming the key role of genotype on the quality and quantity of phenolic profile. Regarding caffeic acid, considerable variability was recorded in its concentration, ranging from 0.004 to over 0.7 mg g^{-1} dw [67]. In support of the above, a varietal comparison of different types of basil conducted by Bajomo et al.[55] showed that caffeic acid ranged from 0.47 to 0.16 mg g^{-1} dw in the Jolina and Persian cultivars, respectively. The same authors pointed out that the genetic component also affected caftaric acid accumulation, with values ranging from 0.16 to 0.41 mg g^{-1} dw, Persian and Cardinal cultivars, respectively. Additionally, caftaric acid has recently been identified at concentrations of 0.02 and 0.03 mg g^{-1} dw in Thai basil and Genovese basil cultivars, respectively[53]; whereas higher values ranging from 0.09 mg g^{-1} dw for 'Spice' and 'Sweet Salad' to 0.49 mg g^{-1} dw for 'Siam Queen' were also reported[51]. The content of *p*-coumaric and ferulic acids in most of the cultivars examined by Javanmardi et al.[67] was in the range of 0.01-0.25 mg g^{-1} dw. Significantly lower values of ferulic acid were recorded on hydroponic basil by Ciriello et al.[32] in fall-winter (0.07 mg g^{-1} dw) and by Ciriello et al.[20] in spring-summer (0.42 mg g^{-1} dw). Apart from emphasizing the crucial role of genotype, this discrepancy in results highlights the previous statements for rosmarinic acid. To support this hypothesis, further work by Ciriello et al.[10] shows that the same cultivars grown in the open field had on average a higher concentration of ferulic acid (0.017 mg g^{-1} dw) than those obtained in hydroponics. The same authors reported significant differences for p-coumaric concentration among the Genovese cultivars tested, with the lowest (0.006 mg g^{-1} dw) and highest (0.007 mg g^{-1} dw) values recorded in 'Italiano Classico' and 'Eleonora', respectively. Recent work on basil 'Italiano Classico', 'Red Rubin' and 'Dark Opal' by Prinsi et al.[54] showed the evidence of other phenolic acids. Specifically, the three cultivars tested did not differ significantly for the content of

salvolinic acid K; on the contrary, 'Italiano Classico' was characterized by the highest concentration of salvianolic acid L, while Dark Opal was characterized by a peculiar accumulation of salvianic acid A. Majdi et al.[40] identified on cinnamon (*O. basilicum* cv. Cinnamon) and lemon basil (*Ocimum* × *citriodorum*) the salvianolic acid B isomers.

2.3.4.2. Flavonoids

Nine basil cultivars examined by Grayer et al.[69] contained as main flavonoids a mixture of quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside. In addition, luteolin 5-*O*-glucoside, luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, and kaempferol 3-*O*-malonylglucoside were also identified, but not quantified. Genotype was the main source of flavonoid variation, as the mean total flavonoid content of 'Lettuce Leaf' (0.40 mg g^{-1} dw) was significantly lower than that of 'Sweet Dani' (1.86 mg g^{-1} dw). Flavonoids, particularly flavonoid glycosides, are known as chemotaxonomic markers for plants belonging to the family *Lamiaceae*, particularly the genus *Ocimum*[69]. These accumulate at the beginning of the vegetative phase and increase during the flowering phase to reach maximum concentration at late flowering[70]. Prinsi et al.[54], by LC-ESI-MS/MS analysis, indicated that the main flavonoids of basil 'Italiano Classico', 'Red Rubin', and 'Dark Opal' are cyanidin derivatives. However, the most significant differences were attributable to the higher concentration of dihydroquercetin-3-*O*-glucoside and naringenin-7-*O*-glucoside in Dark Opal and cyanidin-3-rutinoside and apigenin 7-galacturonide in Red Rubin. The impact of genotype on the biosynthesis of these compounds is confirmed in the study of Zeljković et al.[44], where the cultivars Bush, Napolitano leaf lettuce, Litra, and Mammouth were characterized by higher concentrations of apigenin, naringenin, rutin, and quercetin, respectively. Anthocyanins, a subclass of flavonoids responsible for the red pigmentation of basil, perform various functions within the plant, ranging from defensive and protective roles to stimulation of growth, development, and reproduction[3]. In addition to their benefits for plants, anthocyanins possess important antioxidant properties directly related to preventing human diseases. Their consumption is associated with neuroprotective, antioxidant, and anti-inflammatory effects on human health, moreover they can be used as coloring agents by the food industry[24]. The mean total concentration of extractable anthocyanins for broadleaf basil cultivars Purple Ruffles, Rubin, and Dark Opal ranged from 0.17 to 0.19 mg g^{-1} fw, as opposed to merely 0.06 mg g^{-1} fw for the small leaf cultivar Purple Bush[71]. The 14 anthocyanin pigments identified in these cultivars, comprised 11 cyanidins and 3 peonidins. The highest concentration of total anthocyanins was observed shortly before flowering, although the seedlings had composed their full anthocyanin profile from the eighth day after sowing. Lee & Scagel[53] reported a total anthocyanin concentration of only 0.65 mg g^{-1} fw for the Purple Petra cultivar, whereas Flanigan & Niemeyer[3] reported much higher values in eight purple basil cultivars assessed that ranged from 7.55 mg g^{-1} dw for Red Rubin to 16.6 mg g^{-1} dw for Purple

Ruffles. Scagel et al.[59] instead detected an anthocyanin concentration of (0.0099 mg g^{-1} fw) in the Siam Queen cultivar in contrast to 'Sweet Broadleaf' in which the anthocyanin concentration was below the detection limit. As suggest by Flanigan & Niemeyer[3] variation in the levels of anthocyanins reported may not be exclusively attributable to genetic differences but may include other contributing factors such as the different instrumentation and protocols employed, the different harvest times and fundamental differences in growing conditions.

2.3.5. Aroma Profile

Leaf The quality of basil is determined by its physicochemical, sensory, and bioactive traits, as well as its shelf-life. In addition to the phenolic class of secondary bioactive metabolites, basil is rich in volatile essential oils and other aromatic compounds that define the characteristic aroma and flavour of the plant[10,34]. The aromatic composition is highly dependent on genetic, developmental, and environmental factors[43,72]. Besides being a crucial sensory component, the essential oil of basil is also known for its insecticidal, antimicrobial, and antioxidant properties[4,10,43], for which it finds important applications of considerable economic value in the food, pharmaceutical, and cosmetic sectors. The volatile aromatic compounds isolated and identified in basil, belong mainly to phenylpropanoids produced from shikimic acid and terpenes produced from mevalonic acid[7]. The most diffuse and cultivated chemotype in the Mediterranean region is the sweet basil, generally characterized by aromatic compounds such as linalool (21-70% of the oil), estragole (1-49% of the oil), eugenol (1-25% of the oil), eucalyptol (2-18% of the oil), and methyl-eugenol (0.1-3% of the oil)[35,36], which give this leafy vegetable its unmistakable flavor. Elementi et al.[35] reported the aromatic profile of two types of sweet basil traditionally used in Italy are the Genovese and Lettuce leaf, both having linalool as their main aromatic component, with a higher average content in Genovese (54.3%) than Lettuce leaf (35.9%). The key discriminant between the two cultivars is estragole, which accounts for about 30% of aromatic content in Lettuce leaf while for Genovese it is just over 1%. Other key aromatic components of sweet basil, such as eugenol (0.3-25%) and eucalyptol (5-21%), are also higher in Genovese. The study by Miele et al.[72] conducted on one of the most widely used cultivars of basil to produce Genovese pesto, 'Il Gigante Genovese', shows that the aromatic profile of this cultivar is strongly influenced by the phenological stage and plant height at the time of harvest. Methyl-eugenol was the main aromatic constituent in the early stages of the cultivation cycle (plant height about 10 cm), reaching 59% of the essential oil composition. As methyl-eugenol is suspected to have carcinogenic activity, the authors suggest using for pesto production plant material that exceeds 16 cm in height, therefore significantly reduced methyl-eugenol content.

Singh & Chaudhuri[73] reported a high percentage of methyl eugenol in Indian basil essential oil, although as supported by Patel et al.[38] the main compound in South Indian

basil oil was estragole, with content ranging from 38.20 to 82.23%, depending on cultivar and growth conditions.

Recent studies by Ciriello et al.[10,20], found that the principal aromatic constituent of three Genovese basil cultivars collected during the phenological stage of flower induction in Mediterranean environment (Italy) was linalool, which comprised about 40% of the essential oil. Similarly, Milenković et al.[37] observed among 57 compounds analyzed by GC-MS that the aromatic composition of basil Genovese cultivars grown in southern Serbia was characterized by a predominant content of linalool (50.3%), followed by eugenol (15.3%) and eucalyptol (12%). Surprisingly, this last compound, which has pronounced biological activity, was predominant in two cultivars of Genovese basil in recent studies by Ciriello et al.[32,33]. The same authors highlighted how the specific enzymes implicated in the biosynthesis of eucalyptol and linalool, which share the same precursor (geranyl pyrophosphate [GPP]), could be influenced by genotype × environment interactions.

Although the 12 genotypes analyzed by Zeljković et al.[44] belonged to the linalool chemotype with values ranging from 29.8% (Mánes) to 65.4% (Litra), the aromatic composition was strongly influenced by genotype. Among all, the cultivar Mánes had the highest content of eugenol, an essential compound with antioxidant activity, while the cultivar Napolitano a Foglia di Lattuga had the highest content of estragole (18.5%). The influence of genotype was even more highlighted in the content of the oxygenated monoterpene geraniol, which in the Bejo and Bush cultivars was the fourth most abundant compound while it was absent in the large sweet cultivar.

Although Tsasi et al.[74] reported that the flavor profile of the Cinnamon cultivar grown on the island of Kefalonia (Greece) was characterized by a dominant estragole content (up to 75%), Majdi et al.[40] showed a high linalool content for the same basil cultivar grown in Portugal, confirming the influence of climatic conditions on the biosynthesis of these aromatic compounds. The same oxygenated monoterpene was also the predominant compound in the aroma profile of lemon basil. In contrast, citral, which imparts a characteristic lemon aroma, was only the fifth compound in relative abundance (about 5%)[40]. In contrast, Tangpao et al.[42] observed a different aroma profile for the lemon cultivar, characterized by higher estragole, citral, and neral values. This discordance is mainly attributable to the fact that the quality and quantity of essential oils strongly depend in addition to genotype on growth conditions, different agronomic techniques, number of harvests and harvest time[37].

Similarly, for holy basil (*Ocimum tenuiflorum* L.) a variable aroma profile was recorded, characterized by the predominance of b-caryophyllene and methyl eugenol[39,75]. The latter compound was also principal in white holy basil ('Rama'), followed by α-cubebene and α-copaene[42]. The near total absence of linalool and the high

presence of estragole in Thai basil cultivars (*O. basilicum* var. thyrsiflorum) is responsible for an unmistakable aniseed aroma[41,42].

Finally, Wogiatzi et al.[43] highlighted the varietal variation in aromatic composition between two cultivars of narrow-leaved basil, *O. basilicum* var. minimum (Finissimo Verde a Palla, and Larosa Emanuele-Sementi) and two cultivars of broad-leaved basil, *O. basilicum* var. latifolia (Genovese and Large Leaf Basil). In general, broad-leaved cultivars showed higher linalool concentration (3.8 mg g^{-1} dw) than narrow-leaved cultivars (2.8 mg g^{-1} dw), while the latter had a higher eugenol concentration.

3. Preharvest Management Practices Impacting Performance and Product Quality

3.1. Nutrient Management

3.1.1. Mineral Nutrition

Plant nutrition has important effects on crop performance, and quality, modulated by mineral element, chemical form, genetic material, environmental conditions, agricultural practices, and application methods (**Table 3**). Nitrogen is crucial for plant growth as it is fundamentally involved in various metabolic processes, thus its deficiency impairs plant development[76,77]. In a recent study, Acharya et al.[76] reported that the fresh marketable yield of Genovese basil 'Eleonora' increased quadratically with nitrogen rate, attaining maximum yields at 161 and 141 kg ha^{-1} nitrogen in the low tunnel and open field, respectively. Sifola & Barbieri[14] subjected three Italian cultivars (Mostruoso mammouth, Genovese profumatissimi and Napoletano a foglia di lattuga) of *Ocimum basilicum* (L.) to the dose of 300 kg nitrogen ha^{-1}, obtaining a significant increase (about 85%) in the essential oil content of the leaves. This finding suggests that the increase in essential oil yield obtained through nitrogen fertilization derives not only from increased foliar biomass but also from increased leaf oil content. This is attributable to improved oil biosynthesis and the cardinal role of nitrogen in the development and division of cells containing essential oil: glandular trichomes and secretory ducts[78].

Table 3. Implications of nutrient management (mineral nutrition, nutrient deprivation and controlled stress techniques) for crop performance and functional-sensory quality of basil.

Basil Cultivars	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Gecom'	Greenhouse	Three nutrient solutions differing in the NaCl concentration (either 0, 20, or 40 mM) on morphological growth and quality parameters.	NaCl impacted plant height and leaf colorimetric parameters only at the highest saline concentration (40 mM). Quercetin rutinoside acid and chicoric acid reached a 2× and 10× higher concentration at 40 mM NaCl than control. In comparison, at 20 mM NaCl, the highest content of ferulic acid was recorded (5× higher than in control).	45
'Local cultivar'	Open Field	Three phosphorus supply (0.1, 0.2, and 0.3 g $CaHPO_4$/kg soil) on production and quality performance under drought-stressed (60% field capacity) and well-irrigated (90% field capacity) conditions.	Increased phosphorus availability resulted in a linear increase in dry weight, rosmarinic, and caffeic acid content under both water stress and well-irrigated conditions.	79
'Genovese'	Growth chamber	Three different levels of mineral nutrition (high, medium, and low) in the nutrient solution.	The nutrient-limiting condition resulted in a 294% increase in total antioxidant activity and a significant increase in total flavonoids content and rosmarinic and caffeic acids concentration.	48
'Sweet lemon' *'Cinnamon'* *'Red Rubin'*	Greenhouse	Comparison of Steiner nutrient solutions with different potassium levels (7, 9, 11, and 13 mmol/L)	Increased concentration of potassium in nutrient solution has resulted in a significant increment in the total content of phenols, flavonoids, anthocyanins, and vitamin C for the three basil cultivars.	58
'Gecom'	Greenhouse	Sub-irrigation application of 4 nutrient solutions with different electrical conductivities (2.2, 2.5, 2.8, and 3.1 mS cm^{-1})	The use of a more concentrated nutrient solution (3.1 mS cm^{-1}) compared to the control (2.2 mS cm^{-1}), improved the concentration of soluble solids (°Brix) and increased the content of ascorbic acid (vitamin C) by 46%.	13

Cont. **Table 3**

Basil Cultivars	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Mammolo'	Greenhouse	Two salinity levels in nutrient solution (1 and 40 mM NaCl) during two growing seasons (summer and autumn).	The induced salt stress increased the phenol content by 11% and 26% in the summer and autumn seasons, respectively, while it reduced the nitrate content of the leaves by 16% and 22% in the summer and autumn seasons, respectively.	12
'Dark Opal' *'Sweet Thai'* *'Genovese'*	Greenhouse	Five Hoagland nutrient solutions with different doses of potassium (1.0, 2.0, 4.0, and 5.0 mM)	Linear increase in potassium content in nutrient solution enhanced the antioxidant activity by an average of 70%. Similarly, chicoric and rosmarinic acids concentration increased by 115 and 48%, respectively.	52
'Genovese'	Greenhouse	Comparison of Hoagland nutrient solutions with different nitrogen levels (0.1, 0.5, and 1.0 mM)	Reduction in the level of nitrogen in the nutrient solution has resulted in an increase in the total phenol content and a significant increase in the concentration of rosmarinic acid.	80
'Mostruoso mammouth' *'Genovese profumatissimo'* *'Napoletano a foglia di lattuga'*	Open Field	Comparison of three different doses of nitrogen (0, 100, and 300 kg nitrogen ha^{-1}), administered before the transplantation phase as ammoniacal nitrogen (26%)	The dose of 300 kg nitrogen ha^{-1}, resulted in a significant increase (about 85%) in the essential oil content of the leaves.	14

For Genovese basil, the intensity of green colour is a quality trait preferred by consumers and the processing industry, which seems directly related to nitrogen availability as nitrogen stimulates the synthesis of proteins and chlorophylls. The response to nitrogen fertilization, however, seems intricately linked to genetic material, as demonstrated for example in the lower nitrogen use efficiency of the 'Genovese' cultivar compared to other cultivars tested by Sifola & Barbieri[14]. Excess nitrogen, just like deficiency, is deleterious and promotes the spindling of young plants, increases susceptibility to phytophages and diseases, reduces the absorption of phosphorus, calcium, and magnesium and leads to exceeding foliar accumulation of nitrates[81]. Considering that the rates of nitrogen fertilizer applied on vegetables is generally higher than the actual needs of the crop, the application of slow-release fertilizers would allow plants to take up the necessary nutrients without significant and environmentally deleterious losses. Souri et al.[77] observed that the use of slow-release urea pellets improved basil average yield compared to the use of conventional urea.

Besides nitrogen, potassium is also among the most abundant mineral elements in plants with important effects on production and quality. Nguyen et al.[52] noted that increasing potassium concentration in the nutrient solution had a significant impact on the accumulation of phenolic compounds in the leaves of basil cultivars Dark Opal, Sweet Thai, and Genovese. The highest concentration of rosmarinic and chicoric acid (9.32±3.95 mg g^{-1} dw and 2.41±0.70 mg g^{-1} dw) was obtained at the maximum concentration of 5 mM potassium in the NS, while at 1 mM potassium the lowest concentration of 6.28±4.10 mg g^{-1} dw and 1.12±0.45 mg g^{-1} dw, respectively, was obtained. The key role of potassium in promoting the biosynthesis of secondary plant metabolism products has also been demonstrated by Salas-Pérez et al.[58], who observed a linear increase of several bioactive compounds as the concentration of potassium in the NS increased. The flavonoids, and ascorbic acid concentration were 5.38 mg of quercetin equivalents (QE) g^{-1} extract and 0.17 mg ascorbic acid (AA) g^{-1} dw at potassium concentration of 7 mM; whereas corresponding levels at 13 mM potassium concentration were 6.64 mg QE g^{-1} and 0.18 mg AA g^{-1} dw. As with nitrogen, excess potassium should be limited, as it may cause a reduction in calcium ion translocation resulting in physiological decompensation. Another essential element in the biosynthesis of secondary plant metabolites is phosphorus. Zare et al.[79] observed that increasing phosphorus levels in soil (0.1, 0.2, and 0.3 g $CaHPO_4$ kg^{-1} soil) led to increased PAL activity in sweet basil leaves resulting in increased rosmarinic and caffeic acids.

3.1.2. Nutrient Deprivation and Controlled Stress Techniques for Enhancing Quality

Compared to soil cultivation, hydroponics offers the possibility to accurately modulate the biosynthesis and production of bioactive compounds through appropriate management of nutrient solution composition, chemical forms of the elements as well

as salinity and electrical conductivity[20] (**Table 3**). In particular, the cultivar Napoletano cultivated with double nutrient availability showed an improvement in colorimetric values (L*–a*–b*) and an increase in leaf antioxidant activity[82]. However, it must be considered that an excessive increase in the strength of the NS has negative effects; as pointed out by Maggio et al.[82] and Morano et al.[13], doubling the nutrient availability also increased foliar nitrate concentration. To address this problem, several strategies have been proposed and are successfully implemented in hydroponics: nitrate deprivation a few days (2-15 depending on species) before harvest, partial substitution of nitrate by ammonium nitrogen or calcium nitrate substitution by calcium chloride[11,15]. The latter technique was used in a recent study by Corrado et al.[15] in which the use of nutrient solutions with a 40:60 nitrate:chloride ratio (80:20) led to an important reduction in nitrate (–50.35%) while the yield was reduced by 15.1%, compared to the control. Another NS management technique capable of increasing the production of secondary metabolites in basil is nutrient limitation[80]. In this context, Jakovljević et al.[48] noted that under nutrient limiting conditions (3.79 mM nitrate) and in the absence of ammonium, the Genovese cultivar developed a higher content of flavonoids, rosmarinic, and caffeic acid, as well as higher total antioxidant activity, compared to treatments with a high nutritional intake (15.14 mM nitrate). In addition to modulating mineral concentrations in the nutrient solution, moderate stress application may be an alternative technique to improve the nutritional value of basil[11,45] (**Table 3**). The work carried out by Rouphael et al.[12] shows that increasing the salinity of the nutrient solution from 1 to 40 mM sodium chloride increased the total phenolic and hydrophilic antioxidant activity of 'Mammolo' by 17 and 87%, respectively, and drastically reduced (–84%) the foliar nitrate content. Despite the improvement of functional quality, salinity reduced crop productivity, which was more pronounced in the summer (–30%) than in autumn (–20%). This difference can be attributed to higher temperature and solar radiation that raise the level of evapotranspiration. Similarly, in a recent study on 'Gecom', application of moderate salt stress (20 mM sodium chloride) did not alter leaf visual quality, reduced nitrate content (–23%), and increased hydrophilic antioxidant activity, total phenol content, and ferulic acid content, while only marginally reducing yield[45]. It can be inferred that the potential for improving basil quality through controlled stress, is influenced by the severity of the stress, the genotype, and environmental conditions, as well as the interaction of these factors. The balance between these factors must be optimal for the application of the abovementioned management techniques to be considered sustainable tools for improving product quality while minimizing the loss of marketable production.

3.2. Biofortification Applications

Nearly one-third of the world's population, located mostly in developing countries, faces important micronutrient deficiencies known as 'latent hunger'[8]. The biofortification of horticultural crops with micronutrients present a promising approach for solving this problem. Generally, the enrichment of leafy vegetables with essential and beneficial micronutrients is implemented by foliar sprays, however in the case of soilless culture applying these micronutrients directly in the nutrient solution presents increased effectiveness and practicality[83]. A daily intake of 55 μg selenium can have important long-term benefits on human health, as this micronutrient is implicated in immune system function and influences positively thyroid hormone metabolism[4]. However, high doses of selenium can have toxic effects both on humans and plants. In this respect, basil is a species that cannot accumulate selenium excessively as very high doses of selenium salts have a deleterious effect on yield; hence it can be considered a relatively safe species for applying selenium biofortification. The absorption and bioaccumulation of selenium in plants are not only dependent on the genetic material but are also strongly influenced by the chemical form and concentration of selenium used in biofortification programs (**Table 4**)[83].

Table 4. Implications of biofortification applications for crop performance and functional-sensory quality of basil.

Basil Cultivar	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Tigullio'	Greenhouse	Addition of iodine (10 μM KI) in nutrient solution on the growth and accumulation of foliar I in two hydroponic techniques (floating system and aeroponics).	Biofortification with iodine did not change fresh leaf production and nitrate content. At the second harvest, plants grown in the aeroponic system showed a higher accumulation of total phenols (6603 mg GAE kg^{-1} fw) because of biofortification with 10 μM KI. Biofortification with iodine increased foliar iodine content independently of the growing system, with the highest values obtained at the second harvest.	84
'Tigullio'	Greenhouse	Selenium addition in nutrient solution (0, 4, 8, and 12 mg selenium L^{-1}) on the yield and quality of sweet basil leaves at two successive harvestings.	Regardless of the selenium dose used in the nutrient solution, no significant reduction in yield was observed. In contrast, the maximum dose (12 mg L^{-1} selenium) increased the total phenols and rosmarinic acid content by 66.66 and 130.64%, respectively. At the first harvest, the selenium content in the leaves increased as a function of the dose used. In contrast, at the second harvest, in addition to being lower than at the first cut, it did not differ between biofortified treatments.	47
'Superbo'	Open field	Daily application by fertigation of nutrient solutions with KI or KIO_3 at different concentrations (0, 0.1, 1.0, and 10 mM).	Increasing concentrations of both iodine salts has gradually enhanced the accumulation of iodine in the leaves, resulting in increased antioxidant power, total phenols, and production of rosmarinic and cinnamic acid.	34

Cont. **Table 4**

Basil Cultivar	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Tigullio' 'Red Rubin'	Greenhouse	Biofortification in a floating system with different concentrations of iodine in nutrient solution (0.1, 10, 50,100, and 200 μM) supplied as potassium iodide (KI) and potassium iodate (KIO_3).	The foliar iodine content is increased with the iodine concentration in the nutrient solution. Both treatments (KI and KIO_3) of 100 and 200 μM resulted in an increase in the total phenol content and antioxidant capacity in the ripe leaves of both cultivars.	85
'Tonus'	Greenhouse	Biofortification with sodium selenate applied in nutrient solution or as foliar spray at three different concentration levels (2.0, 5.0, and 10 μM).	Compared to foliar treatment, the application in nutrient solution has increased the selenium content in the leaves, essential oil, and total phenols content.	4
'Tigullio'	Greenhouse	Floating system application of nutrient solutions containing different levels of sodium selenate (4, 8, and 12 mg L^{-1}).	The use of biofortified nutrient solutions has induced a dose-dependent increase in selenium absorption rate. The selenium concentration in leaves increased during the vegetative phase to decrease before and after flowering.	83
'Red Opal'	Open Field	Foliar treatment with two concentrations of sodium selenate (25 and 50 mg m^2) in function of cutting effect.	Selenium biofortification generally resulted in a dose-dependent increase in the total phenol content, while antioxidant activity was only positively influenced after the first cut.	86

It has been shown that applying selenium in the form of sodium selenate increases the concentration of this micronutrient in shoots more than when applied as selenite, which concentrates mainly in the roots[87]. Being very similar in chemical terms to sulphate, selenate is likely absorbed actively by various sulphate transporters, while selenite uptake is passive[87]. The addition of sodium selenate to the nutrient solution at three concentrations (4, 8, and 12 mg selenium L^{-1}) led to a dose-dependent increase in selenium concentration (2.8, 7.9, and 16.9 μg g^{-1} fw) in basil without causing any harmful effect on biomass production[83]. Similarly, the foliar application of selenium at 25 mg m^{-2} or 50 mg L^{-1} did not affect adversely the production parameters of sweet basil crop. Skrypnik et al.[4] compared the effect on basil of two types of selenium application (foliar spraying versus NS application) with three levels of selenium concentration (2.0, 5.0, and 10.0 μM). The untreated basil plants were characterised by a low selenium concentration (0.014 μg g^{-1} fw). By adding 'only' 2 μM of selenium in the NS, the authors observed that the selenium content of the leaves increased 75 times, while foliar application at the same dose increased the content of this micronutrient 39 times compared to the control[4]. Edelstein et al.[88] suggested that selenium concentrations in sweet basil nutrient solution should not exceed 0.25 mg L^{-1} (i.e., 3.2 μM) as it decreases the plant yield. However, Skrypnik et al.[4] observed that even with selenium doses as high as 5 and 10 μM, in both foliar and nutrient solution applications, the plant yield was not significantly different from the control. Considering that Skrypnik et al.[4] recorded a selenium concentration in basil of 1.61 μg g^{-1} fw with the addition of 10 μM selenium in the NS, the optimal recommended daily intake of 55 μg could be provided by about 35 g of basil biofortified with 10 μM selenium.

Even though selenium does not belong to the class of essential trace elements for plants, scientific evidence shows that its application to plants has a positive influence on certain physiological processes, which effectively enhance the quality of plant material obtained[4,47]. Barátová et al.[86] observed that the total concentration of polyphenols in 'Red opal' increased with the foliar application of 50 mg selenium L^{-1} at the rate of 25 mg m^{-2}. Similarly, Puccinelli et al.[47] observed that the maximum dose of sodium selenate in the nutrient solution (12 mg L^{-1}) increased the concentration of total phenols and rosmarinic acid by 66.7% and 130.6%, respectively. The same authors observed that increasing doses of sodium selenate in the nutrient solution (0, 4, 8, and 12 mg Se L^{-1}) linearly increased the selenium concentration in 'Tigullio' basil leaves (up to 123.23 mg selenium kg^{-1}) at the first harvest, however no differences were detected between biofortification treatments at the second harvest. Furthermore, basil leaves treated with 4 and 8 mg selenium L^{-1} had lower ethylene production rates, thus extended shelf-life. This could be attributable to the selenium's ability to replace sulfur, leading to the production of selenium-adenosylmethionine and justifying the lower production of this hormone. Finally, Skrypnik et al.[4] observed that the biofortification treatment of basil with

selenium increased the essential oils content, independently of the method of microelements administration (i.e., foliar application or directly in the NS; **Table 4**).

Iodine belongs to the group of essential and beneficial micronutrients in human nutrition as it plays a crucial role in thyroid function[84]. Therefore, the deficiency of this micronutrient constitutes a serious global health issue responsible for several diseases, including hypothyroidism. The recommended dietary intake of iodine is 150 μg day^{-1} for adult men, 250 μg day^{-1} for pregnant women, and 90-120 μg day^{-1} for children[34]. Most foods have a low iodine content, between 30 and 100 μg iodine kg^{-1} fw, so using iodised salt has always been the main strategy adopted to supplement low iodine diets[34]. However, considering that a large part of the population in most industrialized countries is at high risk of cardiovascular disease, the excessive use of iodized table salt as a source of iodine could exacerbate their health condition. As plants represent the first link in the human food chain, the possibility of directly enriching plants with microelements through 'biofortification' has come under investigation in recent years[34]. Based on current knowledge, iodine can be absorbed by roots through ion channels and chlorine transporters fed by proton pumps and can enter directly into foliar cells through stomata openings and cuticular wax. However, it should be considered that due to its phytotoxicity, the use of high concentrations of iodine might cause significant reduction in the biomass produced[34]. Scientific evidence suggests that plant response to iodine varies depending on the chemical form of the salt used (iodoacetate - ICH_2COO^- > iodide - I^- > iodate - IO_3^-, in order of phytotoxicity), the concentration applied, the growth system and the plant material[85]. The responses of two basil cultivars with different pigmentation (Tigulio and Red Rubin) to hydroponic conditions of increasing iodine concentrations in the nutrient solution (0.1, 10, 50, 100, and 200 μM) were compared by Incrocci et al.[85]. The 100 μM potassium iodide treatment caused significant reduction in plant height and foliar area by 64 and 68% respectively for 'Tigulio', and by 33 and 13% for 'Red Rubin', which indicates the increased tolerance of 'Red Rubin' to iodine toxicity, most likely due to its higher phenolic concentration. Nutrient solutions containing 10 μM potassium iodide did not influence plant growth but increased the foliar iodine concentration significantly compared to the untreated control which averaged 24 mg g^{-1} dw; in particular, the content of this microelement increased to concentrations of 295 and 420 mg g^{-1} dw for 'Tigullio' and 'Red Rubin', respectively. Puccinelli et al.[84] further compared the effects of biofortification with iodine (10 μM potassium iodide) using two different growing systems: aeroponic and floating. Adding potassium iodide to the nutrient solution increased the phenolic concentration of 'Tigullio' basil leaves on average by 11%. Iodine concentration in the leaves of control plants was below the detection limit, whereas it ranged from 9.76 to 23.58 mg kg^{-1} fw in potassium iodide-treated plants. The aeroponic system increased the foliar iodine concentration on average by 17.0% due to the more efficient supply of nutrients to the

roots. Regardless of the growing system, iodine concentration was higher (+89.7%) at the second harvest due to a longer period of iodine supplementation and larger leaf area, meaning higher transpiration. Kiferle et al.[34] observed dry weight reductions of about 20% and 30% when 'Superbo' plants were treated with iodate and iodine, respectively, applied at the maximum dose of 10 mM. On the other hand, the higher concentration of potassium iodide/potassium iodate (10 mM) improved the phenolic concentration and almost doubled the antioxidant power of the leaves. There were no reductions in the growth rates of plants treated with 0.1 and 1 mM potassium iodate/potassium iodide concentrations, while the foliar accumulation of this micronutrient increased for both salts in a dose-dependent manner. Based on the results obtained, Kiferle et al.[34] reported that the consumption of about 1 g of bio-fortified basil (with 1 mM of potassium iodide) corresponds to 67 μg of iodine, which would account for about 44% of the recommended daily intake per adult (150 μg day^{-1} for adult men).

3.3. Biostimulant Applications

The main challenge for modern agriculture is to reduce inputs drastically and move towards environmentally and economically sustainable agroenvironmental systems. The identification of organic molecules and microorganisms capable of modulating the primary and secondary metabolism of plants and improving crop performance, especially in sub-optimal environments, provides in recent years a new impetus for achieving this goal[89]. Many of these substances and microorganisms, generally termed as biostimulants, can activate physiological and molecular mechanisms, improve the efficiency of nutrient and water use, and stimulate the production of nutraceutical compounds as they also positively interfere with secondary metabolism[8] (**Table 5**).

Table 5. Implications of microbial and non-microbial biostimulant applications for crop performance and functional-sensory quality of basil.

Basil cultivar	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Gecom'	Greenhouse	Effect of three nitrogen equivalent rates (0.05, 0.15, and 0.25 g N kg^{-1}) of a vegetal-derived protein hydrolysate (V-PH) an animal-derived protein hydrolysate (A-PH) on the morphological, physiological, and biochemical responses	V-PH increased photosynthesis and color status, ion content, yield, and quality, especially when supplied at a rate ranging from 0.1 to 0.15 N. While the changes in the physiological development of basil plants induced by A-PH were less effective than those exerted by V-PH, and even negative when 0.25 N A-PH was used. In fact, the latter induced an overall reduction in plant growth and yield	[89]
'Gecom'	Greenhouse	Effect of arbuscular mycorrhiza fungi- and microbial-based biostimulant (*Rhizoglomus irregulare* BEG72 + *Funneliformis mosseae* BEG234 + *Trichoderma koningii* TK7 + *Bacillus megaterium* MHBM77 + *Bacillus megaterium* MHBM06) on yield of basil subjected to two mild salinity stresses (25 mM and 50 mM) and non-stressed control.	At both salinity levels (25 and 50 mM), the multispecies biostimulant inoculate increased, on average, leaf area (+22.65%), potassium content (+28.75%), and concentration of chicoric acid (+14.2%) and quercetin rutinoside (+69.76%), in contrast to chlorine, which decreased (26.59%), compared to non-inoculated saline conditions.	[49]
'Genovese Gigante'	Greenhouse	Inoculation of *Glomus intraradices* (arbuscular mycorrhizal fungi) on the morpho-quality characteristics of basil under different irrigation levels (100% of field capacity and mild water stress: 60% of field capacity).	At the second harvest and under water stress conditions, *Glomus intraradices* inoculation increased fresh yield (42.49%) and resulted in the highest percentage of linalool (55%), compared to non-inoculated plants.	[90]

Cont. **Table 5**

Basil cultivar	Growing conditions	Treatment factors	Modulation of crop performance and functional-sensory quality	Reference
'Crispum'	Greenhouse	Protected crop applications at different concentrations (2.5, 5, 10, and 20%) of protein hydrolysates obtained from two Moringa cultivars (*Moringa oleifera* and *M. peregrina*).	The use of *M.O* and *M.P* extracts at 10% increased anthocyanin content by 30% and 13.5%, respectively. In the same way, total carbohydrates increased by 80% and 14%.	91
'Purple Ruffle'	Greenhouse	Application of brown seaweed extracts (*Ascophyllum nodosum*) at different doses (5 and 7 mg L^{-1}) following two different application methods (drench and foliar)	The use of seaweed extracts has increased the percentage of essential oils, although applications in drenched soil at maximum dosage have defined a significant increase in linalool and estragole content.	92
'Nano Compatt' 'Red Bordaux'	Greenhouse	Inoculation with *Bacillus subtilis* and/or *Glomus irradicans* with different salt stress levels (0, 1,000, 2,000, and 4,000 ppm of NaCl).	Double inoculation with *Bacillus subtilis* and *Glomus irradicans and* providing both cultivars with greater salinity tolerance have increased the percentage and yield of essential oil.	93
Local cultivar	Greenhouse	Effect of single or combined application of different PGPRs (*Pseudomonas putida*, *Azotobacter chroococcum*, and *Azospirillum lipoferum*) on *Ocimum basilicum*.	Due to a synergistic action, the treatment with all three bacterial species, as well as providing both cultivars with greater salinity tolerance, has increased the percentage and yield in essential oil.	94
'Genovese'	Growth chamber	Comparative analysis of the effects induced by three AM fungi: *Glomus mosseae*, *Gigaspora margarita*, *Gigaspora rosea*.	The basil plants colonized by *G. rosea* had the highest α-terpineol content and the highest amount of essential oil. Mycorrhization with *G. margarita* and *G. rosea*, on the other hand, increased the percentage content of eugenol.	95

The most widely used plant biostimulants are seaweed extracts, obtained mainly from brown algae[92]. In a recent study, Elansary et al.[92] observed that the use of *Ascophyllum nodosum* (L.) seaweed extracts significantly improved the production and quality of basil cultivar Purple Ruffle. The authors observed that the application in wet soil of this biostimulant at the concentration of 7 ml L^{-1} increased the content of essential oil. The main differences concerned linalool and estragole, two of the most important and refined aromatic components of sweet basil. Another important group of biostimulants are protein hydrolysates (PHs), which in recent years have received increasing attention for their positive effects on the performance of many vegetables and for the economic and environmental sustainability of their production process[89]. Hassanein et al.[91] applied on sweet basil protected crops different concentrations of PHs (20%, 10%, 5%, and 2.5%) that were obtained from two species of Moringa, *M. oleifera* (M.O) and *M. peregrina* (M.P). Applying 10% of M.O. and M.P. PHs through irrigation under saline stress conditions increased the weight of fresh buds by 50% and 109% and the anthocyanin concentration by 30% and 13.5% compared to the untreated control. Nutritional improvement by microbial biostimulants, particularly mycorrhizal symbionts and plant growth-promoting bacteria (PGPR), has been reported several times on several vegetables[8]. Battini et al.[96] observed how PGPRs modulate the concentration of nutraceutical compounds in basil that are significant for the human diet. Plants inoculated with two PGPRs (*Sinorhizobium melilot* and *Streptomyce ssp*.) showed higher concentrations of rosmarinic acid as these PGPRs were able to trigger overexpression of genes (TAT, HPPR, and CS3'isol) that upgraded the biosynthesis of this phenolic acid characteristic of sweet basil. The improvement in plant growth due to PGPR, on the other hand, is closely linked to increased atmospheric nitrogen fixation, production of auxiliary hormones, and selective control of pathogens[1]. Ordookhani et al.[94] have observed that 'a microbial consortium' formed by *Pseudomonas+Nitrotobacter+Azosprillum* improved the percentage of volatile oils in *Ocimum basilicum*. In particular, the relative percentage content of essential oils in basil treated with the three PGPRs (0.82%) was about double that found in the control (0.40%). Using a microbial consortium consisting of *Trichoderma afroharzianum* T22+*Azotobacter chroococcum*_76A resulted in a rosmarinic acid concentration twice that of the control, without a negative impact on the fresh yield of Genovese basil[1]. The authors observed that adding a biopolymer (carboxymethyl cellulose) to the microbial consortium had a positive effect on production as it may have served as a valuable source of nutrients for the microbial consortium and for improved plant fitness. Biostimulants of microbial nature also comprise arbuscular mycorrhizal fungi (AMF). Scientific evidence has shown that root symbiosis with aAMF can modify the number of glandular trichomes in the leaves of aromatic plants such as basil by increasing the concentration of certain essential oils[95] (**Table 5**). These changes in the aromatic profile have been confirmed by

both electronic nose analysis and near infrared spectroscopy (NIR)[95]. On this aspect, Copetta et al.[95] observed that the inoculation of cultivar Genovese with *Gigaspora rosea* resulted in a higher concentration of eugenol (348.628 μg g^{-1}) compared to non-inoculated plants (296.529 μg g^{-1}). The inoculation with AMF (*G. intraradices*) fully preserved the aromatic quality of 'Genovese Gigante' under water-stress conditions and mitigated depressive effects on production, thus confirming the crucial role of mycorrhizal symbiosis in the defense of plants against abiotic stresses[90]. On the other hand, under controlled water stress conditions (60% of field capacity), Zare et al.[79] observed a significant increase in total phenols and rosmarinic and caffeic acids in sweet basil plants inoculated with *Glomus hoi*. As proposed by the same authors, the shifting of secondary metabolism induced by AMF could relate to cytological alterations that would promote the biosynthesis of phenolic acid precursors. In the case of salinity stress, several studies have shown that inoculation with AMF can improve the physiological performance of stressed plants and enhance yield and quality[97]. Indeed, Zuccarini & Okurowska[97] have observed that under saline stress conditions, basil plants inoculated with *G. intraradices* showed higher growth rates than controls. In general, mycorrhizal colonization led to the reduction by 27.2 and 45.3% of sodium in the leaves and roots. The allocation of sodium in the aerial parts leads to toxic effects that alter the functionality of cell membranes and impair crop performance, hence the reduction of sodium through symbiosis with *G. intraradices* imparted basil greater resistance throughout the production cycle[97]. In general, basil plants inoculated with *B. subtilis*+AMF showed higher values of essential oils and minerals such as nitrogen, phosphorus, and potassium. At the same time, the percentage content of sodium was lower compared to other treatments. As suggested by Abdel-Rahman et al.[93], these results were due not only to the mycorrhizal symbiosis, which regulates the uptake of sodium from the soil and its compartmental accumulation in the roots, but also to the presence of bacterial exopolysaccharides (EPS), produced by PGPR strains such as *B. subtilis*, which bind the sodium, thus making it less available for root uptake. Saia et al.[49] (**Table 5**) evaluated the crop performance and quality of 'Gecom' grown at two salinity levels (low: 25 mM NaCl; high: 50 mM NaCl) combined with inoculation using a multispecies microbial biostimulant (*Rhizoglomus irregulare* BEG72+*Funneliformis mosseae* BEG234+*Trichoderma koningii* TK7+*Bacillus megaterium* MHBM77+*Bacillus megaterium* MHBM06). At both salinity levels, the multispecies biostimulant inoculum increased potassium content, and the concentration of chicoric acid and quercitin rutinoside, compared with non-inoculated control.

3.4. Light Management

Light quality (spectrum), quantity, and photoperiod, strongly influence photosynthetic activity[98], and plant productivity[99]. Plants respond to different light conditions with physiological and biochemical adaptations, which in turn cause changes

in morphological-anatomical traits[99]. Dou et al.[100] noted that the phenolic content of basil plants was positively correlated to the linear increase in daily light integrals (DLI); hence, the antioxidant capacity of basil leaves at a DLI of 17.8 mol m^{-2} day^{-1} was about 75% higher than the DLI of 9.3 mol m^{-2} day^{-1}. However, excessive amounts of light energy can cause damage to Photosystem II [101]. Roofing materials significantly influence light intensity in the greenhouse, moreover photoselective films can change the quality of intercepted light with important implications on the yield and quality of production[8]. Regarding this aspect, Chang et al.[99] observed that in a protected environment, excessive shading (75%) reduced the photosynthesis rate and oil content and modified the oil composition of basil leaves. Increased irradiance on control treatments receiving partial shading (25%) increased the relative content of linalool and eugenol, whereas excessive shading increased methyl eugenol, which given its suspected carcinogenic action, is certainly not positive from a qualitative point of view. However, Ilić et al.[36] observed in a recent study that basil grown in shade (40% shade index) had higher eugenol content while plants directly exposed to solar radiation had the highest linalool content. On the other hand, substantial reduction in light intensity could cause a health-threatening peak in the nitrate content of leafy vegetables, as nitrate reductase activity is activated by high light intensities[8]. Maggio et al.[82] observed that a 50% reduction in greenhouse irradiation significantly increased the nitrate concentration of basil cultivars Napoletano and Genovese, while the nitrate concentration of plants grown without shading remained safe for human consumption.

In protected environments, the application of photo-selective plastic films influenced basil growth and morphology as an adaptive response to altered solar radiation[30], probably modulated by shifting in the red-far red ratio (R:FR). Although Stagnari et al.[30] observed that the height and fresh biomass of basil improved with photo-selective nets, the application of coloured films always reduced the concentration of rosmarinic and caftaric acids. In particular, the use of green plastic films drastically reduced the rosmarinic acid concentration but was partly compensated by significant increase in caffeic acid. The need to cultivate where natural light is insufficient (e.g., northern latitudes and indoor cultivation) and the quest for techniques that enhance vegetable quality has led to a surge in interest for the use of artificial lighting. The introduction of light-emitting diodes (LEDs) has in this respect facilitated considerable progress[102]. Considering that plants can respond to changes in light quality through different types of photoreceptors, the use of LED technologies with highly defined and modifiable spectral properties (250 - 1000 nm) renders this application even more interesting[98]. The red and blue bands of the light spectrum are the main energy sources that drive photosynthesis[103]. The close relationship between light spectral quality and plant primary and secondary metabolism has encouraged studies on the manipulation of light in controlled environments for modulating phytochemical composition[8]. Increase was

reported in fresh marketable yield of 'Dark Opal' and 'Caesar' basil under a 4:1:1 red:blue:green compared to 1:1:1 and 2:1:1 light treatments that downgraded the crop's commercial value[98]. Pennisi et al.[103] showed that basil fresh biomass and leaf chlorophyll concentration improved by increasing the red:blue ratio (R:B) up to 3, as red light has a greater influence on biomass production through the phytochrome photoreceptor. In the same study, basil plants cultivated with R:B≥2 showed 25% improvement of WUE compared to R:B=0.5. This can be attributed to the better yields obtained with higher R:B ratio and the reduction of stomatal conductance with R:B≥2, given that red light is less efficient than blue in promoting stomatal opening.

It is further known that the quality of light influences the synthesis of secondary metabolites in aromatic herbs but to date, the scientific literature offers scarce references as to the 'optimal' spectral composition. Nevertheless, Verma et al.[104] and Kyriacou et al.[105] have shown that blue light, alone or better in combination with other spectral bands, increases the production of beneficial compounds, such as phenolics, since its high energy content stimulates the activity of PAL. Taulavuori et al.[106] observed a four-fold increase in the concentration of rosmarinic acid in 'Genovese Gigante' with additional blue light compared to plants grown under HPS lamps. Similarly, continuous treatment with blue light compared to control (HPS) significantly increased the cinnamic acid concentration[106]. Hosseini et al.[16] demonstrated how different LED light spectra (white light and 70:30% R:B) influence the antioxidant components of sweet green basil. Similarly, the light spectral quality is a determining factor in the synthesis of essential oils, however these effects are not completely clear as pertinent literature remains conflicting. For example, Ivanitskikh & Tarakanov[107] reported an increase in the essential oil content of basil plants grown under an LED spectrum of R:B=2, while Hosseini et al.[16] reported maximum essential oil content in basil grown under red light or an R:B ratio 70:30. In particular, red light significantly increased the content of limonene, α-pinene, and β-myrcene. In line with the above, Pennisi et al.[103] observed stunted growth and a relative reduction of linalool in basil grown with R:B=0.5, compared to all treatments where R was dominant. Another quality aspect strongly influenced by the R:B spectral ratios is nitrate content. Piovene et al.[102] observed that the nitrate content in sweet basil cultivated under R:B=0.7 was significantly lower (635 mg kg^{-1} fw) than that obtained with R:B=5.5 (1,015 mg kg^{-1} fw). Although the range of visible radiation (400-700 nm) is commonly considered the most important for photosynthetic activity, photoreceptors in plants can detect and respond to shorter and longer wavelengths outside the photosynthetically active radiation range[108]. In direct sunlight, UV wavelengths represent high-energy radiation conventionally considered a plant stress factor. However, it has been shown recently that additional UV-B radiation acts as eustress elicitor inducing several positive responses in plants, including the synthesis of UV-absorbing compounds, such as anthocyanins and phenolic acids[2,108]. It is important to

note that such responses to UV radiation, in addition to being dose-dependent, vary widely between species and cultivars. As confirmed by Dou et al.[109] in a recent study on 'Red Rubin' and 'Improved Genovese Compact', the application of UV-B radiation for 1 h day^{-1} for 2-days under a PPFD of 224 μmol m^{-2} s^{-1} ensured an excellent compromise between yield and quality for 'Improved Genovese Compact', in contrast with what was observed for 'Red Rubin' in which additional doses of UV-B decreased the total phenolic concentration and reduced yield. In addition, regardless of genotype, the same authors observed that the increase in phenolic concentration under UV-B radiation was greater in plants grown under low PPFD (160 μmol m^{-2} s^{-1}) than those grown under high PPFD (224 μmol m^{-2} s^{-1}). In addition, Mosadegh et al.[110] showed that phenolic concentration in 'Genovese' grown under low PPFD but with additional UV-B radiation was significantly higher than in plants grown with high PPFD but without UV-B radiation. Therefore, additional UV-B radiation could represent a simple, fast, and cost-effective alternative to improve the nutraceutical composition of basil, especially in controlled environments where such radiation is completely absent[110]. However, considering that the continuous use of enhanced UV-B light throughout the entire cultivation cycle leads to a strong reduction in the biomass produced, a two-step elicitation strategy with UV-B is proposed for cultivation protocols. Plants should be grown firstly under optimal light conditions to achieve a satisfactory production level; secondly, additional UV-B light should be introduced to increase the production of desired phytochemicals. Finally, a recent study showed that the use of UV-B could improve the post-harvest nutritional quality of basil, as significant increase in all polyphenols was recorded 7 days post-harvest[2].

4. Conclusions

The upcoming trend for health-promoting nutrition alongside gastronomic novelty drives the growing demand for high-quality products in the entire agri-food sector. In this context, consumers rediscover and re-evaluate minor species such as basil that promote human health and well-being owing to their rich phytochemical composition and enticing sensory profile. Appropriate genotype selection through the wide genetic diversity inherent in basil may facilitate effective and sustainable production of improved quality without compromising yield. As reported in this review, the nutritional quality and crop performance of basil is profoundly influenced by environmental factors, particularly light which can be effectively optimized in protected environments. Similarly, plant nutrition management and practices such as biofortification constitute strategic tools for improving basil production, for increasing the content of essential phytochemicals and micronutrients, and for controlling the accumulation of anti-nutritive agents. Furthermore, the application of biostimulants could underpin sustainability in production, especially under growth-limiting conditions such as high soil salinity. In this respect, the scientific community should

focus on the interaction nexus of genotype-environment-management to identify the optimal combinations that tap the physiological and molecular mechanisms responsible for improving plant performance and functional-sensory quality in basil.

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Chapter 3
Influence of Genetic Material, Successive Harvests and Hydroponic Cultivation on the Yield and Quality Characteristics of Genovese Basil: an Overview

Abstract: Basil (*Ocimum basilicum* L.) is an aromatic plant recognized for its wide genetic diversity that combines a rich phytochemical composition and a tantalizingly distinctive aroma. Despite its exotic origin, is cultivated worldwide for its sought-after leaves. Its biochemical variability has endowed this aromatic herb with many uses, including pharmaceutical, cosmetic and culinary. In Italy, "Genovese" basil leaves are used to make pesto, a condiment that has attracted the interest of consumers and producers worldwide. The current review explores Genovese basil's technological, biochemical and sensory properties for agro-industrial pesto production, highlighting how genetic material may play a key role. It provides a critical evaluation of the effect of successive harvests on crucial parameters emphasizing the impact of this agronomic practice according to the different genetic materials on the yield components. Finally, alternative growing systems such as soilless cultivation on Genovese basil's productive and qualitative traits were described. These cultivation systems could represent the solution to the growing needs of the agro-industrial sector, which requires a higher quality product with standardized technological characteristics all year round.

Keywords: Pesto; Genotype; Soilless cultivation; Aromatic profile; Phenolic acids; Cut effect

1. Introduction

During history, basil has reached a prestigious rank among the aromatic plants belonging to Lamiaceae, universally recognized as the "king of herbs."[1] The popularity and relevance of the *Ocimum* genus are mainly ascribed to its wide genetic variety. The diverse species of *Ocimum* spp. (more than 60), which differ in growth habitus and color, possess considerable bioactive compounds used in pharmacology, cosmetics, and gastronomy[2]. Basil has a high concentration of phenolic compounds that provide broad-spectrum protection against chronic diseases and recognized antimicrobial and antifungal activity[3]. Like many other leafy vegetables, basil cannot be considered a primary source of protein and minerals due to low daily intake[4]. However, the attraction in foods rich in healthy bioactive compounds has increased consumers' demand for this leafy vegetable[4,5]. For example, Italy has increased the area cultivated with basil by almost 90% in the last decades[6]. Among the basil varieties, the "Genovese" has gained a prestigious status quo in Italy, and considered a European Union (EU) Protected Designation of Origin label (Reg. 611/2010)[7]. The present review aimed to investigate and assess the crucial role of cultivar selection in the production of "Genovese" basil for food processing and the agroindustry, taking into account the influence of the mechanical stress of the cut. Given the increasing interest and professional use of hydroponics for intensive leafy vegetable cultivation, the productive and qualitative results of alternative to soil growing systems were analyzed in detail.

2. Quality Attributes of Genovese Basil

The "Genovese" basil leaves are essential ingredients for delicious traditional Italian dishes such as pizza Margherita, Caprese salad, and pesto sauce. In "Genovese" basil for pesto production, the aroma is undoubtedly the crucial trait for the distinctive determination of taste[8]. Without hints of lemon and mint, the recognizable taste is attributed to characteristic essential oils, especially terpenes and phenylpropanoids, stockpiled in glandular trichomes[6]. Terpenes are biosynthesized by condensation of isopentenyl diphosphate (IPP) and dimethyl diphosphate (DMAPP), while phenylpropanoids are biosynthesized by the shikimate pathway[3]. As corroborated by several studies[6,7,9,10], the aroma profile of "Genovese" basils was characterized by the predominant concentration of monoterpene linalool. However, Ciriello, et al.[11] observed that a dominant presence of eucalyptol characterized 'Italiano Classico'. This finding highlights how genetic variability, even among cultivars belonging to the "Genovese" type, can significantly affect the aroma profile. As suggested by the same authors, this finding is the result of the specific genotype × environment interaction, as both eucalyptol and linalool share the same precursor. In addition to its unique sensory qualities, Genovese basil has always been attributed to its nutraceutical and medicinal properties due to its richness in biologically active compounds, mainly polyphenols.

Polyphenols are recognized for their antioxidant function that can reduce the incidence of chronic and vascular diseases while stimulating cognitive well-being[12]. Several studies focused on phenolic concentration have further confirmed the effects of genetic variability on the secondary metabolism of "Genovese" basil. Hydroponic studies[7,11] revealed that "Genovese" basil cultivars could differ in total phenolic acid concentration (with values ranging from 66.5 to 587.5 μg g^{-1} dw) but also in quality. Although rosmarinic acid is recognized as the dominant phenolic compound in "Genovese" basil[13,14] Ciriello et al.[7,11] observed a higher concentration of chicoric acid in 'Eleonora'. Biochemically, rosmarinic acid is a phenylpropanoid (caffeic acid ester), biosynthesized through the L-phenylalanine and L-tyrosine amino acid pathway. It has strong antioxidant activity that can exert a beneficial effect on human health, preventing diseases such as diabetes and melanoma of the skin as well as regulating melanin production and tyrosinase activity. However, basil cultivars also differ in the concentration of less representative phenolic compounds. Regardless of growing conditions, comparing three Genovese basil cultivars for pesto production[6,15] showed significant differences in ferulic and caffeic acid concentrations. The highest values were obtained from cultivars grown in open fields. As argued by the authors, open-field conditions would boost the production of these phenolic acids. Nicoletto et al.[16] pointed out that Genovese basil cultivars are also characterized by high p-Coumaric acid content. On average, the three basil cultivars grown in open field tested by Nicoletto et al.[16] had p-Coumaric concentrations of 92.56 mg kg of fresh weight. Like the other phenolic compounds, p-Coumaric is also an important antioxidant linked to the cinnamic acid biosynthetic pathway[17].

3. Response to Successive Harvests

Regardless of the growing system, usually, "Genovese" basil for pesto is harvested up to three times per crop cycle, in the pre-flowering phase, depending on latitude. This technique ensures the early production of tender leaves rich in glandular trichomes[18] and reduces labor costs because more harvests are obtained with one sowing (or transplanting). However, the mechanical stress of the cut triggers physiological responses that are not yet fully understood, that are influenced by different growth conditions and genotypes[4]. However, the mechanical stress of the cut triggers physiological responses influenced by the growing conditions and genotypes used4. Although Corrado et al.[4] observed a significant decrease in yield in the second cut, many studies conducted in hydroponics and the open field showed an opposite response, that is, an increase in yield regardless of genotype[6,15,16,19,20]. Specifically, studies conducted by Formisano et al.[19] in the open field and by Ciriello et al.[15] in hydroponics reported an increase in yield of 148.43 and 37.53%, respectively, attributable to an increase in the number of leaves per plant. The same trend was observed for dry biomass. This finding could be due to an efficient and well-established root system that would promote a faster

regrowth of the roots after the first harvest[21]. Furthermore, as Skalák et al.[22] suggested, the cut could have increased cytokinin concentration, improving leaf primordia emission. The increase in the number of leaves per plant and the emission of lateral buds inevitably lead to a significant leaf/stem ratio change[16]. This last parameter is crucial for the industrial production of pesto since excessive fibrousness, due to a higher dry matter and stem content, extends the processing time and leads to blackening of the pesto[2]. However, as Nicoletto et al.[16] pointed out, extending cultivation beyond the second cut has adverse effects on yield. Specifically, the authors reported an 40.5% (on average) reduction in fresh yield at the third cut compared to the second cut.

Like other abiotic and biotic stresses, the cut plays a critical role in the sensory and nutritional characteristics of the "Genovese" basil[23,24]. As observed by Nicoletto et al.[16] and Ciriello et al.[7], successive harvests increased the concentration of total phenolic compounds, regardless of the "Genovese" cultivars and the growth conditions. The same authors suggested that the mechanical stress of the cut may have promoted phenylalanine ammonia lyase (PAL) activity, stimulating a greater assignment of photosynthates to the shikimic acid pathway, as indicated by the higher yield registered at the second harvest in both experiments[25]. These results are also confirmed by the recent work of Ciriello et al.[6] on three "Genovese" basils grown in the open field. The authors observed an increase in the cichoric acid concentration of 517% and even 1128% for rosmarinic acid after the cut in 'Italiano Classico'. The reported results confirm that the cut is a practical tool for improving basil antioxidant activity, an aspect that is more than crucial given that a higher concentration of phenolic acids in pesto could reduce oxidative processes and extend shelf life. As observed for phenolic compounds, Milenković et al.[9] found that the cut, regardless of the shading conditions, increased the content of aromatic compounds. Regarding the aromatic profile, several authors[21,26] have observed an increase in the linalool content in response to cut, even if, as noted by Ciriello et al.[6], the other oxygenated monoterpenes typical of the "Genovese" basil (eucalyptol and β-cis-ocimene) showed the opposite trend. Concerning the concentration of nitrates, the reviewed literature showed that the accumulation of this antinutrient as a cut function is mainly related to genotype[4,6,7,16]. Ciriello et al.[15] observed a significant reduction of these minerals after cut with respect to tissue accumulation of P, K, Ca, and Mg. As argued by the authors, this reduction could be attributable to the increase (about double) in dry matter, explaining the decrease in macronutrient concentration. This is partially confirmed by data reported by Formisano et al.[19] and Nicoletto et al.[16] on cultivars of Genovese basil grown in open fields. The latter reported an increase in P after the cut. The differences observed by Ciriello et al.[15] could be attributed to the different growth systems (hydroponic).

4. Discussion Evaluation of the Productive and Qualitative Performance of Hydroponically Grown Genovese Basil

The high annual variability and the pressing need of the processing industry for safe, uniform and aromatic "Genovese" basil have compelled producers to use innovative growing systems. Soilless cultivation systems, in addition to ensuring well-replicated and deseasonalized production, would allow the requalification of uncultivable and degraded soils and urban areas[11,27]. Furthermore, total freedom from agricultural soil and complete control of growth conditions would reduce expensive and unsustainable soil disinfection practices, restricting the use of agrochemicals[28]. Today, more than half of the production of "Genovese" basil production in Italy is founded on soilless growing techniques, mainly hydroponic[13]. The most common hydroponic systems used to produce leafy vegetables are the nutrient film technique (NFT) and the floating raft system (FRS). A comparative investigation between NFT and FRS of Walters and Currey[29] showed no production differences on 35 basil cultivars, highlighting that the choice between the two systems is based solely on application, management, and economic aspects. Specifically, while the NFT system uses smaller volumes of nutrient solution per growing cycle, the FRS system does not require the constant use of pumps to deliver the nutrient solution. The same authors underlined how the absence of solid support for plants had forced producers of "Genovese" basil to reevaluate selection criteria, focusing on yield and quality but also on growth habitus and morphology (short internodes, high leaf-stem ratio and good branching)[29]. The productive benefits of growing in hydroponics compared to standard cultivation on agricultural soil were highlighted by Žlabur, et al.[27] on three different basil genotypes (Genovese, Dark Opal and Minimum) and by Ciriello, et al.[11] on three cultivars of "Genovese" basil (Aroma 2, Eleonora, and Italiano Classico). Ciriello et al.[11] showed that FRS produced an average yield almost seven times higher than the one recorded by Saha et al.[30] on 38 basil cultivars grown on agricultural soil. As also supported by Maboko and Du Plooy[31], this result could be explained by the high density of basil cultivation in FRS (317 m^{-2})[11]. Regarding the cultivar "Genovese", Žlabur et al.[27] observed that FRS cultivation resulted in yields almost 35% higher than cultivation obtained from the same cultivar grown in the open field. This result was partially attributable to the most significant leaf area and height recorded by FRS-cultivated plants, suggesting that this growing system may have guaranteed the achievement of the genetic potential of this cultivar. Not least, hydroponic cultivation, in addition to shortening the cultivation cycle by almost half compared to conventional cultivation, guaranteed significant reductions in dry matter, a crucial technological parameter for basil "Genovese" for the agroindustry[27]. The positive influence of hydroponics on the dry matter of the "Genovese" basil was recently confirmed by Ciriello et al.[7]. Regardless of the cultivar, Ciriello et al.[7] showed, on average, significantly lower values than those obtained by Nicoletto et al.[16] on three

genotypes of "Genovese" basil grown on agricultural soil. Although optimizing growth conditions guarantees the highest production, drastic reduction in exposure to abiotic and biotic stressors does not stimulate adequate defensive responses, which are crucial for the accumulation of secondary metabolites Sgherri et al.[32].

However, managing and modulating the different preharvest factors would also enhance the functional quality of hydroponic horticultural products. Regarding this aspect, Ciriello et al.[11] reported on three cultivars of "Genovese" basil that nutrient solutions with different macronutrient concentrations (1, 2, and 3 dS m^{-1}) significantly affected the α-bergamotene, trans-2-hexenal, and eugenol[33]. Furthermore, the use of the most diluted nutrient solution (1 dS m^{-1}) resulted, regardless of the cultivar, in higher production of caffeic acid and cichoric acid 11 because, as suggested by Kiferle et al.[34], deprivation of nutrients can elicit specific increases in phenolic compounds by up-regulation of PAL activity. Jakovljević et al.[35] showed that the limitation of nutrients significantly increased the antioxidant activity in "Genovese" basil.

However, the different concentrations of macro and micronutrients in the nutrient solution did not result in significant differences in the main yield and biometric parameters of Genovese basil either in a fall or summer cycle[36,37]. This highlights that basil is a moderately tolerant species to salinity, with an optimal range ranging from 1 to 5 dS m^{-1} [29].

One of stressor that is useful in hydroponics to improve the quality performance of basil is saline stress. Corrado et al.[13] and Ciriello et al.[38] observed a significant reduction in nitrates and a significant increase in phenolic compounds (with a minimal reduction in yield) in "Genovese" basil grown with low concentrations of NaCl. Similarly, Rouphael et al.[39] observed a reduction in leaf area (44%) and fresh basil yield (30%) under salt stress (40 mM NaCl), with more negative effects in summer than in fall (–16 and –20% for leaf area and yield, respectively). In addition, 40 mM NaCl increased total phenolics (17%) and antioxidant activity (87%) but reduced nitrate concentration by 84%, compared with the non-saline control. In addition, a reduction in total phenols was observed but an increase in nitrates in the summer crop compared to the fall. Archangi et al.[40] observed an increase in essential oil content as salt stress increases. However, the same authors pointed out that this increase in essential oils is sustainable only for moderate levels of NaCl (maximum 40 mM) as at higher levels (120 mM) there is an excessive and deleterious reduction in fresh biomass.

The possibility of modulating nutrient composition has prompted the research community to become increasingly interested in biofortification practices with nonessential but beneficial minerals for human health[41-43]. The application of selenium in basil, in addition to significantly increasing the concentration of selenium in plant tissues without a reduction in yield, increased the content of essential oils[43], while

Puccinelli, et al.[44] observed an increase in rosmarinic acid of up to 130% after the application of 12 mg L^{-1} sodium selenate. Similarly, Incrocci et al.[45] observed an increase in iodine concentration in basil leaves after applying 10 µM potassium iodine without finding a reduction in yield. The same dose resulted in a 50% increase in phenols in the "Genovese" basil[46]. Not least, the use of biostimulants in hydroponic represents a promising and successful strategy for the production of leafy vegetables with premium quality attributes[47]. Specifically, Ciriello et al.[37] observed a linear increase in leaf number, fresh and dry yield of two Genovese basil cultivars as a function of biostimulant dose. In the same work, the use of a protein hydrolysate in nutrient solution also increased the total polyphenol concentration. This result, as claimed by the authors, could be attributable to specific organic molecules that, in addition to being precursors of specialized metabolites, act as activators of key enzymes in the biosynthesis of phenolic compounds[48,49].

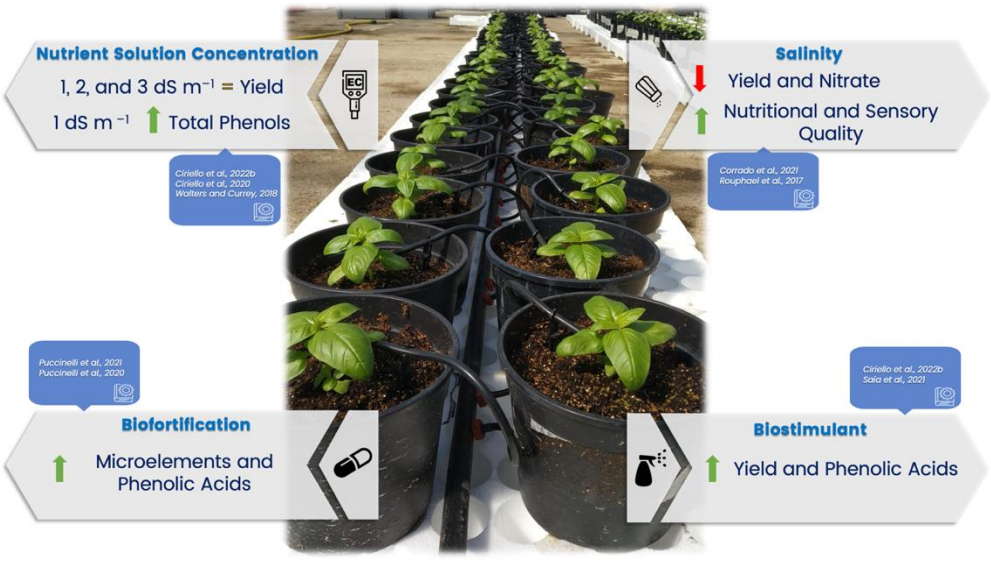

Figure 1. Effects of management techniques on production and quality of Genovese basil

5. Conclusions

The upcoming trend for a healthy diet and gastronomic novelty drive the growing demand for high-quality products across the food industry. In this context, consumers and the agrifood industry are reevaluating and rediscovering minor species such as basil, whose rich phytochemical composition promotes human health and well-being. As reported in this review, the nutritional quality and performance of basil crops are deeply influenced by the genetic aspect and typical agronomic practices such as

successive harvesting. Despite the wide genetic variability of basil, in this review, we have focused more on the Genovese type, the flagship of Italian gastronomy.

Similarly, the possibility to grow this leafy vegetable in alternative systems is a great advantage for the scientific community, producers, and consumers. Management of plant nutrition and practices such as biofortification are strategic tools to improve basil production, increase the content of essential phytochemicals and micronutrients, and control the accumulation of antinutritive agents.

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Chapter 4

Genotype and Successive Harvests Interaction Affects Phenolic Acids and Aroma Profile of Genovese Basil for Pesto Sauce Production

Abstract: Basil (*Ocimum basilicum* L.) is an essential ingredient of the Mediterranean cuisine due to its distinctive aroma. Genovese basil leaves are used to prepare "pesto", a condiment that has always caught the interest of consumers and producers. Usually, basil for industrial processing is harvested more than once to extract a higher yield. However, successive cuts can affect quality traits that play a crucial role in defining the product's final sensory profile. This research was aimed to evaluate the impact of cut on the quantitative and qualitative properties of three Genovese basil cultivars (Aroma 2, Eleonora and Italiano Classico) grown in an open field. Nitrate content, phenolic acids and aromatic profile were determined by ion chromatography (IC), high-performance liquid chromatography (HPLC), and gas chromatography coupled to a mass spectrometer (GC/MS) analysis, respectively. The second harvest increased fresh biomass and total phenolic acids content by 172% and 413%, respectively, with Italiano Classico recording the highest values. The combination of second-cut Aroma 2 yielded the lowest nitrate (473.8 mg kg−1 of fresh weight) and Eugenol (2.4%) levels. In the second harvest, Eleonora showed an increase in eugenol and trans-α-bergamotene of 75.3% and 48.2%, respectively; whereas, eucalyptol and β-cis-ocimene decreased by 34.4% and 51.6%, respectively. Although successive harvests may increase basil yield and quality overall, the cultivar-dependent response to successive cuts needs to be accounted for in order to accomplish standardization of industrial "pesto" sauce.

Keywords: Sweet basil; Mediterranean diet; Nitrate content; Linalool; Rosmarinic acid; Chromatographic analysis; Volatile compounds

1. Introduction

Over the last decades, the growing interest in a healthy lifestyle has motivated consumers towards wholesome eating choices, where natural products with high nutritional value and high quality are an integral part of the daily diet[1]. There is no doubt that a balanced regime is the key to psychophysical well-being because a diet based on unhealthy nutritional models represents a high-risk factor for the onset of obesity and related diseases[2]. Moreover, recent investigations have highlighted the impact of nutrition in altering the gut microbiome, which is critical in modulating and transforming nutrient intake, with direct consequences for people's health[3]. Since the 1950s, many studies on eating habits carried out in Mediterranean regions provided insight on the benefits of healthy and well-balanced nutrition, based on the consumption of fruit, vegetables, legumes, and cereals[4]. This led to the development of a novel nourishment model (i.e., the Mediterranean diet) declared as part of the Intangible Cultural Heritage of Humanity by UNESCO. In the Mediterranean diet, which finds its cradle in Italy, vegetables and leafy herbs are the protagonists of every meal, providing fiber, minerals, vitamins, and antioxidants, making it popular and appreciated worldwide[4]. In traditional Mediterranean cuisine, aromatic herbs are a must-have ingredient, exalting the dishes' organoleptic features, increasing their palatability, and providing antioxidants that assist in the reduction of chronic and cardiovascular diseases[5].

On top of that, aromatic herbs belonging to Lamiaceae are recognized as an affluent reserve of specialized metabolites that attract the interest of academic researchers[6]. Among the aromatic species, basil (*Ocimum basilicum* L.) stands out for its unique and pleasant aroma, as well as high mineral and vitamin content and low protein and lipid content[7]. Historically employed in folk medicine[8], basil is grown and marketed on a global scale[6] for its low caloric and high bioactive compounds content[9]. Its use as a fresh or dried herb made this polyhedral leafy herb ideal for seasoning soups, salads, meat, fish, and traditional Italian recipes[10]. Basil is indeed the main ingredient of the famous green sauce typical of the Riviera Ligure (Italy), known as "pesto". Its preparation requires tender leaves of the basil cultivar Genovese D.O.P. (Protected Designation of Origin), distinguished by a high content of linalool and eugenol and the absence of estragole, which confers the unmistakable and well-appreciated flavor[11]. Basil is a functional food that draws the consumers' and the pharmaceutical industries' attention due to its remarkable organoleptic properties. In the last decade, the high demand for fresh basil leaves from industry and the fresh market has pushed Italian farmers to increase the cultivated area by almost 90%, with a total production of 7800 tons (ISTAT)[12].

The richness in essential oils, which confers its distinct aroma, makes basil appreciated in gastronomy and in the pharmaceutical and cosmetic fields[13,14]. Essential oils, which are biosynthesized by specialized leaf epidermal outgrowths (i.e., glandular

trichomes), belong to different classes of compounds with the most significant fraction represented by terpenes (e.g., oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes) and phenylpropanoids, which have a proven antioxidant property[15]. In addition, basil is also characterized by a high phenolic content[16], providing broad-spectrum protection against chronic diseases[17]. Moreover, phenolic compounds have antifungal and antimicrobial activity[18,19] so much as to be considered multitargeting drugs[20]. High nutraceutical value is attributable to phenolics like rosmarinic, caffeic, and chicoric acids[21,22], among which the former is the most abundant in basil and has higher antioxidant activity, thus acting as a radical scavenger[23].

Considering that vegetables constitute the primary source of nitrate exposure in the human diet[24], its high content represents a critical anti-nutritional factor. Basil, like other leafy vegetables, accumulates nitrate in its tissues[25]. Although the regulation n° 1258/2011 of the European Commission has not set a threshold value for basil, its leaves can accumulate nitrate values higher than 5000 mg kg^{-1} of fresh weight[26]. Pharmacologically, nitrate has very low toxicity, but if ingested, it is reduced by saliva and digestive system into nitrite and N-nitrose compounds (e.g., nitrosamines), which oxidize hemoglobin into methemoglobin, interfering with oxygen transport and causing in children a pathology known as methemoglobinemia[27,28].

However, the high genetic variability of the genus Ocimum and intensive plant breeding undertaken over the years make farmers uncertain about the best suitable basil cultivar for their needs. In conventional cultivation, Genovese basil plants for "pesto" are harvested more than once during the growing season (up to three times)[10,29,30]. Taking into account that the biosynthesis of desired specialized metabolites can be stimulated by biological, physical, and chemical agents, the mechanical stress induced by successive cuts may have a considerable impact on the secondary metabolism of different genotypes[31]. Nevertheless, at present, there are no reports in the literature that unveil the cut effect on the quality traits of Genovese basil.

In this regard, our research was aimed to characterize three basil cultivars for the industrial production of "pesto Genovese" in response to two successive harvests. Production, nitrate accumulation, aromatic and phenolic profiles were evaluated. To the authors' knowledge, this is the first research investigating these quality aspects, profiting the food industry, and paving the way for future studies.

2. Materials and Methods

2.1. Plant Material and Experimental Design

This The field experiment was carried out in 2019 during the spring-summer season at the experimental site of the Department of Agriculture of the University of Naples "Federico II" in Bellizzi (Salerno, Italy). Three basil cultivars, Aroma 2 (Fenix), Eleonora

(Enza Zaden), and Italiano Classico (La Semiorto) (**Figure 1**), were transplanted on 6 June, 2019 with a density of 250 plants m^{-2}. The experiment was designed as a factorial combination of three cultivars and two harvests, where the cultivars were arranged in a randomized block design, with three repetitions. Each experimental plot covered an area of 2 m^2 and was set 0.5 m apart from the other plots. A drip irrigation system facilitated fertigation management. The experimental trial lasted 55 days, during which the two harvests (CT1 and CT2) were carried out at 34 and 55 days after transplanting (DAT), respectively. Fifty plants per plot were harvested in the pre-flowering stage, leaving two internodes to ensure an adequate vegetative regrowth. For the determination of dry weight (dw), the fresh weight of leaves and stems were recorded and then were dried to constant weight in a forced-air oven at 70 °C for about three days. Part of the fresh plant samples was im-mediately frozen in liquid N and then stored at −80 °C for volatiles and phenolics de-termination.

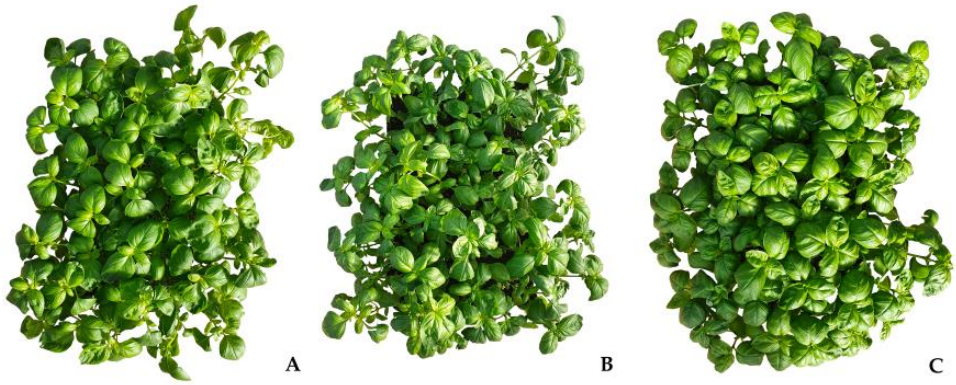


Figure 1. Genovese basil genotype used in the experiment. Aroma 2 (A), Eleonora (B), and Italiano Classico (C).

2.2. CIELAB Color Leaf Measurement

Basil Color coordinates were recorded using a Minolta Chroma Meter CR-300 (Minolta Camera Co. Ltd., Tokyo, Japan). At each harvest date, ten measurements were taken on the upper surface of young expanded leaves of ten plants per experimental unit. As described by the International Commission of Illumination (CIE), the color was expressed with degree of lightness (L*), greenness (a*), and yellowness (b*) values, chroma, and hue angle. Chroma is the color saturation quantitative attribute representing the degree of visual difference from neutral grey of the same lightness. Higher chroma values indicate a higher color intensity perceived by humans. The hue angle describes the qualitative color attribute with respect to the red/green (+a*/−a*) and yellow/blue (+b*/−b*) axes.

2.3. Nitrate Content Determination

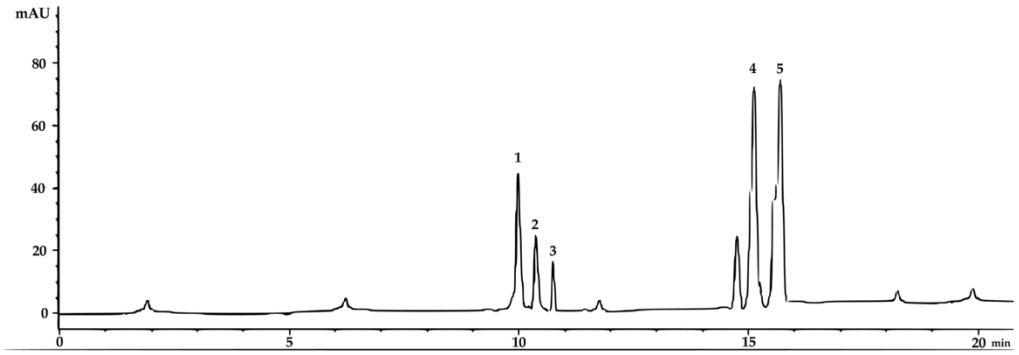
According to the method of Rouphael et al.[32], to determine the nitrate content, the dried samples were milled with a MF10.1 cutting-grinding head mill (IKA®, Staufen im Breisgau, Baden-Württemberg, Germany) and sieved with MF0.5 sieve (0.5 mm hole size; IKA®, Staufen im Breisgau, Baden-Württemberg, Germany). Fifty milliliters of purified water with Arium® Advance EDI pure water system (Sartorius, Goettingen, Lower Saxony, Germany) was added to 250 mg of dry sample and then placed in a SW22 shaking water bath (Julabo, Seelbach, Baden-Württemberg, Germany) at 80 °C for 10 min and centrifuged at 6000 rpm for 10 minutes with a R-10M centrifuge (Remi Elektrotechnik Ltd., Mumbai, Maharashtra, India). The supernatant was taken, filtered using a syringe filter with a 0.45 µm pore size (Whatman International Ltd., Maidstone, Kent, UK), and analyzed by Ion Chromatography (IC). Nitrate determination was performed with an IONPAC® ATC-HC 9 × 75 mm anion trap (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), an IONPAC® AG11-HC 4 × 50 mm guard column (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), and an IONPAC® AG11-HC 4 × 50 mm IC column (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), using a 1 mM–50 mM hydroxide gradient with a flow of 1.5 mL min^{-1}. Auto Suppression Recycle Mode with a temperature of 30 °C were used. The results were obtained as g kg^{-1} dw, then based on each sample dry matter, were converted to mg kg^{-1} of fresh weight (fw).

2.4. Profile Phenolic Acids: Extraction, Determination, and Quantification

The free phenolic acids extraction and quantification (i.e., caffeic, rosmarinic, chicoric, p-Coumaric, and ferulic acids) were performed by High-Pressure Liquid Chromatography (HPLC) according to the method described by Ciriello et al.[33].

All reagents and solvents were HPLC grade (Sigma Aldrich, Milan, Italy). Two mL of 70% aqueous methanol (*v*/*v*) were mixed with 100 mg of freeze-dried basil leaves and then stirred for 1 minute with a Vortex Classic stirrer (Velp® Scientifica, Usmate Velate, Monza Brianza, Italy), sonicated for 20 min with a Q500 ultrasonic sonicator (Qsonica, Newtown, Connecticut, USA) and shaken for 10 minutes with a SSL4 see-saw rocker (Cole-Parmer™, Vernon Hills, IL, USA). The extracts were centrifuged at 6800 rpm for 10 minutes with a R-10M centrifuge (Remi Elektrotechnik Ltd., Mumbai, Maharashtra, In-dia) and filtered through a 0.45 µm Teflon (PTFE) membrane filter (Phenomenex, Torrance, CA, USA) into vials for analysis. Chromatographic separation of free phenolic compounds was performed on an Agilent Technologies Model 1100 chromatograph (Palo Alto, CA, USA) equipped with a G4225A degasser, a G13111A four-channel low-pressure gradient pump, and a G1315B diode-matrix detector, using a 20 µl sample injection volume. A reversed-phase Kinetex® C18 100 Å column (5 µm particle size, 150 × 4.6 mm; Phenomenex, Torrance, CA, USA) was used. The eluents were 0.1% (*v*/*v*) trichloroacetic acid in water (A) and acetonitrile (B). The gradient program was 0–50%

B for 50 min with a constant flow of 1 mL min^{-1}. Detection of the individual free phenolic compounds was performed at 280 nm and shown in the representative chromatogram in **Supplementary Figure S1**.



Supplementary Figure 1. Separation of caffeic acid (1), p-Coumaric acid (2), ferulic acid (3), chicoric acid (4), and rosmarinic acid (5) in Genovese basil extract by HPLC.

The calibration curves were constructed using seven concentration levels (0.15, 0.5, 1, 10, 20, 50, and 100 mg L^{-1}) for each standard. The concentration of each phenolic compound was reported as mean ± SE (standard error) expressed in mg kg^{-1} dw, n = 3.

2.5. Aroma Profile: Extraction and Determination

All The determination of volatile compounds (VOCs) was performed by Gas Chromatography coupled to a Mass Spectrometer (GC/MS) after extraction and concentration by Solid-Phase MicroExtraction (SPME) technique[33].

For SPME, 500 mg of frozen basil leaves (−20 °C) were at, manually crushed, and placed into a 20 mL glass headspace vial with a screw cap and PTFE septum (Supelco®, Bellefonte, PA, USA). The vial was stirred with an ARE® magnetic stirrer (Velp® Scientifica, Usmate Velate, Monza Brianza, Italy) for 10 min at 30 °C to facilitate the VOCs migration into the headspace. A 1 cm long and 50/30 µm thick divinylbenzene/carboxane/polydimethylsiloxane fiber (Supelco®, Bellefonte, PA, USA) was introduced into the vial to absorb VOCs and then introduced into the split-splitless injector of GC 6890N coupled to MS 5973N (Agilent, Santa Clara, CA, USA) at 230 °C for 10 min (desorption phase). The GC/MS was equipped with a 30 m long and 0.250 mm thick capillary column, coated with a 0.25 µm 5% Phenyl/95% dimethylpolysiloxane film (Supelco®, Bellefonte, PA, USA). Splitless injection was used for sample analysis. The oven temperature was maintained at 50 °C for 2 min and increased from 50 °C to 150 °C to 10 °C/min and from 150 °C to 280 °C to 15 °C/min. The injection source and ion source temperatures were 250 °C and 230 °C, respectively, while helium was used as a carrier

gas with a flow rate of 1 mL min^{-1}. The mass spectrometer was set to 70 eV. The identification of VOCs in the headspace was performed comparing the mass spectra and retention times of the different VOCs with the Atomic Spectra Database version 1.6 (U.S. Department of Commerce, Gaithersburg, MD, USA) of the National Institute of Standards and Technology (NIST). Each treatment was analyzed in triplicate, with the corresponding results expressed as % total area normalization.

2.6. Statistical Analysis

A Two-way Analysis of variance (ANOVA) was implemented to assess the significance of the effects and interaction between the two main factors (Cultivar (C) and Cut (CT)). The mean effect of C and CT were compared according to One-way ANOVA and t-test, respectively. Statistical significance was determined at the $p < 0.05$ level using Duncan's Multiple Range Test (DRMT) for C × CT interaction and C factor. All data are presented as mean ± standard error (SE). All statistical analyses were performed using IBM SPSS Statistics.

3. Results

3.1. Production Response

The ample versatility of basil, used both in the culinary and cosmetic fields, has attracted the industry's interest, which requires great amounts of fresh produce available all year round. Inevitably, this pattern has driven growers to select cultivars with higher productivity and to adopt agricultural practices to maximize crop yield.

In the current experiment, the interaction of both factors, cultivar and cut (C × CT), did not result in significant variations in fresh biomass production, in contrast to a significant effect of cultivar and cut factors (**Figure 2**). Concerning the cultivar, the average total fresh biomass was 7.53 kg m^{-2}; this result, in accordance with the typical values of intensive cultivation of basil for industrial processing, which is probably due to the high plant density[34]. However, among the tested cultivars, Italiano Classico had the highest unit production (8.69 kg m^{-2}), higher by 18% and 33% than Aroma 2 and Eleonora, respectively, although it was only statistically significant in respect to the latter (**Figure 2**). The results confirmed the significant genotypic impact on total production, as demonstrated by other recent research on basil[35].

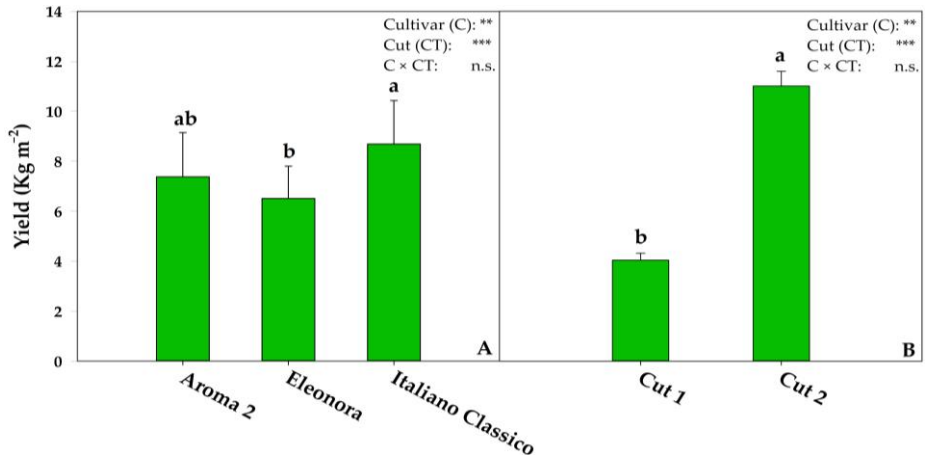


Figure 2. Effect of cultivar (A) and cut (B) on the yield of green Genovese basil. n.s., **, *** non-significant or significant at $p \leq 0.01$ and 0.001, respectively. Cultivar means were compared by ANOVA; according to Duncan's multiple range test ($p = 0.05$), different letters indicate significant differences. Cut means were compared using a t-test. All data are expressed as mean ± standard error (SE) (n = 3).

Similar to other leafy vegetables (e.g., rocket, spinach, coriander), basil is harvested more than once in a crop cycle to ensure the highest yield and to amortize the costs of production[10,34]. In our experiment, the two successive cuts resulted in notable differences in the total production of fresh biomass (**Figure 2**). Specifically, the yield of the second cut was 172% higher than the first, in agreement with previous findings[30,36]. As stated by Zheljazkov et al.[36], the higher production achieved in the second cut could be due to a well-rooted hypogeal system, which limits the competition for nutrients and water uptake, thus helping to reconstitute the epigeal system efficiently. A further study suggested that, in response to the cut, the interruption of apical dominance reduced the auxinic flow, which would promote the lateral shoot emission as occurred in our experiment (data not shown), leading to an increase in production[30]. However, a recent investigation on basil grown in a soilless system does not corroborate the findings presented in the current experiment, as it demonstrated a reduction in fresh biomass at the second cut[10].

3.2. Leaf Colorimetry

Visual quality is undoubtedly of crucial relevance, which can influence the industry and consumer choice[37]. However, the color of plants varies according to genetic and pre-/post-harvest factors, such as agronomic practices, maturation, and storage methods[38]. The characteristic green color of basil leaves is an important industrial requirement for

the preparation of “pesto Genovese”. A more intense color attracts the consumer's interest and reduces the use of artificial colorants.

The color of the leaves was affected by genotype and cut (**Table 1**); however, the interaction of these factors was not significant for color coordinates L*, a*, and b* and for chroma and hue angle. The b* values demonstrated a cultivar-dependent variation, with Italiano Classico and Eleonora recording the highest (21.62) and lowest (18.64) ones, respectively. These results are consistent with the chroma values that indicate a lower color intensity perceived in Eleonora, although statistically not different from Aroma 2. On the other hand, the degree of lightness (L*) and greenness (a*) did not vary, which means that the leaves maintained the same shade of green in each cultivar. These findings are in accordance with those reported in a comparable study on green basil[39], which showed that, under the same growing conditions, plants tended to change mainly the b* value, without affecting the a* value, confirming that this effect depends only on the genotype. Notwithstanding the change in color from green to yellow is usually attributed to tissue senescence[40], in this experiment basil plants were harvested in the pre-flowering stage, without senescence symptoms, as evidenced by the high yield achieved, highlighting the influence of the genotype once again.

Table 1. Effect of cultivar and cut on CIELAB colorimetric coordinates, chroma, and hue angle of basil leaves.

Source of Variance	L *	a *	b *	Chroma	Hue Angle
Cultivar (C)					
Aroma 2	45.80±0.91	−7.31±0.29	20.06±0.66 ab	21.36±0.71 ab	110.10±0.37
Eleonora	45.59±0.73	−6.85±0.35	18.64±0.57 b	19.89±0.60 b	110.32±0.87
Italiano Classico	45.40±0.64	−7.59±0.22	21.62±0.42 a	22.93±0.45 a	109.49±0.46
	n.s.	n.s.	*	*	n.s.
Cut (CT)					
CT 1	46.41±0.66	−6.81±0.23	20.00±0.62	21.14±0.66	108.84±0.25
CT 2	44.79±0.38	−7.69±0.17	20.20±0.61	21.64±0.62	111.09±0.33
t-test	*	**	n.s.	n.s.	***
C × CT					
Aroma 2 × CT1	46.74±1.62	−6.97±0.35	19.96±1.08	21.15±1.13	109.34±0.22
Aroma 2 × CT2	44.86±0.78	−7.65±0.43	20.15±1.00	21.56±1.08	110.86±0.22
Eleonora × CT1	47.02±0.73	−6.21±0.45	18.48±0.95	19.52±1.04	108.65±0.60
Eleonora × CT2	44.16±0.26	−7.49±0.09	18.79±0.82	20.25±0.77	111.98±0.81
Italiano Classico × CT1	45.46±1.16	−7.24±0.09	21.57±0.45	22.77±0.43	108.54±0.39
Italiano Classico × CT2	45.34±0.84	−7.94±0.34	21.67±0.83	23.10±0.89	110.43±0.04
	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. According to Duncan's multiple range test ($p = 0.05$), different letters indicate significant differences. Cut means were compared by a *t*-test. All data are expressed as mean ± SE (n = 3). L*: lightness; a*: greenness; b*: yellowness.

The cut mean effect shows that the second harvest positively impacted the colorimetric parameters (**Table 1**). L* and a* values decreased by 3.5% and 11.4%, respectively. These results are supported by the hue angle, which decreased by 2.1%, showing the change in leaf coloring towards green. The freshly harvested vegetables usually have a glossy and bright surface (higher L* value), which generally varies with post-harvest processing[38]. However, in our experiment, the lower gloss and the greater shade of green could be due to a higher accumulation of antioxidants in response to mechanical stress induced by the cut for protecting the photosynthetic machinery and preserving it from oxidation. In fact, an intense green color indicates a more significant accumulation of compounds with antioxidant function[41], confirmed by the increased build-up of phenolic acids achieved at the second cut, in accordance with our results.

3.3. Nitrate Accumulation

The nitrate content was affected by the cultivar and cut interaction (**Figure 3**). Aroma 2 and Eleonora recorded, on average, a decreased nitrate content of ~33% in the second cut; in contrast, Italiano Classico showed an opposite trend with a higher nitrate concentration of 1130.97 mg kg^{-1} fw (+116%) in the second harvest.

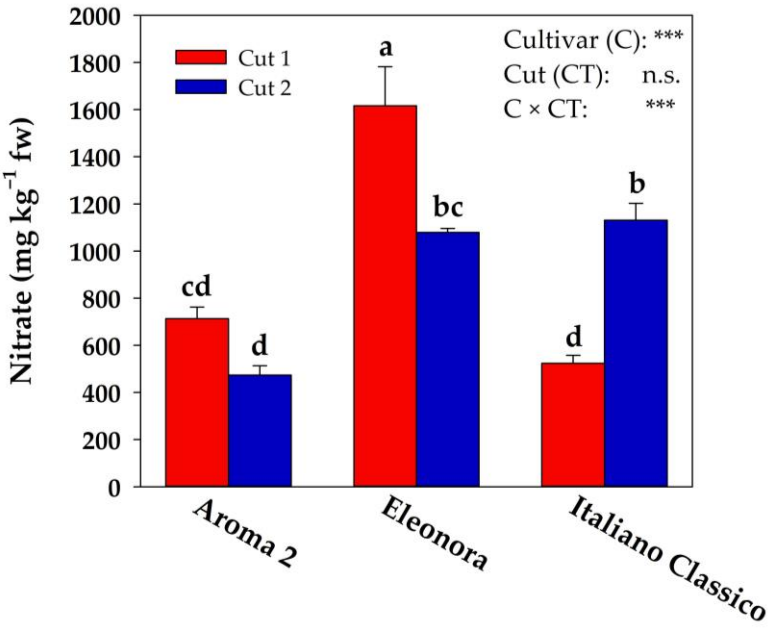


Figure 3. Nitrate content in different cultivars and cuts. n.s. and *** non-significant or significant at $p \leq 0.001$, respectively. According to Duncan's multiple range test ($p = 0.05$), different letters indicate significant differences.

Nitrate is the primary source of nitrogen for plants that are accumulated in considerable amounts in their tissues. However, scientific evidence regarding the correlation of nitrate consumption with chronic disease onset relegates it among the most widely recognized anti-nutritional compounds[25]. Usually, nitrate is safe due to its rapid excretion in the urine[42], but under specific conditions, it can be reduced to nitrite[43], which is acknowledged to play a crucial role in carcinogenesis and the incidence of blood diseases such as methemoglobinemia[27]. Given its well-documented hazardousness, nitrate is a potentially harmful anion that can affect horticultural produce quality since its raw consumption is the principal source of human dietary intake[26].

However, in our experiment, Eleonora recorded, on average, 95% more nitrate than Aroma 2 and Italiano Classico (**Figure 3**), under the same growing conditions (spring-summer and high light intensity), thus evidencing a clear genotypic impact, in accordance with former studies[26,42]. To corroborate our results, it is worth pointing out that only Aroma 2 and Eleonora showed a lower nitrate content at the second harvest (**Figure 3**). This finding was also achieved by Nicoletto et al.[30], underlining further that the effect of cut-induced mechanical stress could be genotype-dependent. The investigation carried out by Corrado et al.[10] gave an additional explanation for the increase in nitrate in reply to the cut, tracing it back to passive plasticity that can determine plant luxuriation. However, contrary to our experiment, the fact that only one cultivar of basil had been used cannot exclude a priori the genotype effect.

Nevertheless, in the present experiment, the tested Genovese basil cultivars accumulated low nitrate values (473.78–1616.41 mg kg^{-1} fw) compared to similar results achieved in a comparable study performed in soilless systems under a protected environment[42]. Apart from the genetic aspect, according to Santamaria[44], the environment plays a vital task in nitrate build-up. Especially, the nitrate reductase enzyme in highlight conditions has higher activity, leading to a lower nitrate accumulation in plant tissues. Despite its potentially high nitrate content, basil is not yet regulated by the European Commission regulation n 1258/2011. However, even the highest value presently achieved by Eleonora at the first cut (**Figure 3**) is below the maximum threshold set for spring-summer leafy vegetables.

3.4. Impact of Cultivar and Cut on Phenolic Acids Accumulation

Due to their sessile nature, plants rely on effective defensive systems to protect themselves against potential environmental threats. Among passive protection mechanisms, specialized metabolites play a relevant role in plant survival and colonization of our planet[45]. Most of the technological and nutritional attributes of medicinal herbs such as basil are indeed associated with their high levels of these metabolites, of which phenolic acids are the most representative[8].

The HPLC assay of the phenolic profile in the experimented basil plants revealed a predominance of rosmarinic, chicoric, and caffeic acids, in accordance with the results achieved by Prinsi et al.[46]. Apart from p-Coumaric acid, the phenolic profile was affected by the interaction of the examined factors (**Table 2**). In the second harvest, there was a considerable rise in phenolic acids concentration in all the assessed cultivars. Notably, Italiano Classico recorded the highest increase of chicoric acid (517%) and rosmarinic acid (1128%), whereas caffeic and ferulic acids increase was higher in Aroma 2, marking 237% and 162%, respectively. Eleonora recorded the highest value of p-Coumaric acid, rising by 160% in the second cut (**Table 2**). Compared with other cultivars, Italiano Classico showed the highest total phenolic acids content (1080.79 mg kg^{-1} dw), mainly due to the high rosmarinic acid (**Table 2**). This finding demonstrates a strong genotypic impact of basil on the biosynthesis of the above-mentioned specialized metabolites[45,47]. It also points out the tendency of Genovese cultivars to metabolize preferably rosmarinic acid[30]. According to Nicoletto et al.[30], rosmarinic acid increased after the cut, independently from the cultivar. This result confirms that the stress induced by the cut can be a valuable tool to increase the antioxidant activity of basil. Chemically, rosmarinic acid is the ester of caffeic acid, belonging to the chemical group of phenylpropanoids, whose biosynthesis occurs via the amino acids L-tyrosine and L-phenylalanine pathway[48,49]. Its molecular structure, characterized by the presence of hydroxyl groups, confers relevant antioxidant activity and a regulating function on tyrosinase enzyme activity and melanin production[50,51], which provide benefits in the prevention of disease, including diabetes and skin melanoma[23]. It could also be a valuable adjuvant in the development of new antibiotic drugs with its strong and recognized antibiotic activity[52]. The amount of rosmarinic acid obtained in this study did not reflect the data in the literature, which could be due to the different growing conditions as well as the analytical method used to assess them, as suggested by Maggini et al.[53]. In our samples, rosmarinic acid ranged from 87.98 to 1185.29 mg kg^{-1} dw, in contrast to the results recorded in previous studies[47,54]. The results achieved by Javanmardi et al.[54] showed rosmarinic acid values up to 100 times higher than those obtained in our investigation. However, it is worth noting that non-domesticated basil plants used by Javanmardi et al.[54] were obtained from unselected seeds from local markets or supplied by farmers. As argued by Vallarino et al.[55], domestication would determine a reduction in secondary metabolic activity in favor of the primary one, supporting the results obtained in our work.

Table 2. Effect of cultivar and cut on free phenolic acids (mg kg^{-1} dw) profile of basil.

Source of Variance	Caffeic Acid	Chicoric Acid	Rosmarinic Acid	*p*-Coumaric Acid	Ferulic Acid	Total Phenolic Acids
Cultivar (C)						
Aroma 2	95.86± 23.35b	226.56±75.18b	420.48±149.27b	6.63±1.32ab	17.24±3.48	766.77±252.23b
Eleonora	111.07±6.73a	304.53±72.07a	366.19±99.41b	7.04±1.41a	16.83±1.67	805.67±179.73b
Italiano Classico	90.62±20.40b	326.47±105.50a	640.91±244.14a	6.24±1.25b	16.54±1.44	1080.79±371.94a
	***	***	***	*	n.s.	***
Cut (CT)						
1	62.13±8.86	98.81 ±12.49	111.64±10.03	3.69±0.11	12.3±0.77	288.55±31.33
2	136.24±4.07	472.90±26.72	840.09±92.19	9.59± 0.21	21.44±1.13	1480.27±112.92
t-test	***	***	***	***	***	***
C × CT						
Aroma 2 × CT1	43.88±1.81d	59.96±3.02d	87.98±3.88d	3.69±0.30	9.51±0.24c	205.03± 4.05d
Aroma 2 × CT2	147.83±4.71a	393.17±22.21b	752.98±28.89b	9.56±0.30	24.96±0.92a	1328.50±50.46b
Eleonora × CT1	97.45±0.38c	145.22±1.12c	150.39±6.34d	3.90±0.11	13.88±0.62c	410.83±5.95c
Eleonora × CT2	124.70±6.37b	463.85±24.31b	582.00±52.93c	10.18±0.10	19.79±2.18ab	1200.51±74.70b
Italiano Classico × CT1	45.05±1.51d	91.24±0.99cd	96.54±2.54d	3.47±0.12	13.50±0.90c	249.79±2.90cd
Italiano Classico × CT2	136.20±1.37ab	561.70±17.92a	1185.29±40.91a	9.02±0.30	19.58±0.56b	1911.79±33.48a
	***	***	***	n.s.	**	***

n.s., *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. According to Duncan's multiple range test (p = 0.05) different letters indicate significant differences. Cut means were compared by a *t*-test. All data are expressed as mean ± SE (n = 3).

The phenolic compounds in aromatic herbs have a high antioxidant activity that imparts health benefits, reinforces the immune system, and improves life expectancy[5]. These bioactive compounds' attributes are a valuable resource for the food industry to replace the widespread synthetic antioxidants (e.g., BHA, butylated hydroxyanisole; BHT, Butylated hydroxytoluene)[8], thus making the food system safer and more sustainable. For example, Genovese basil with high phenolic acid content could be an excellent tool to improve "pesto" quality, extending its shelf-life and reducing oxidation during storage[56]. As the build-up of polyphenols is an adaptive plant response to adverse environmental conditions[57], resulting in higher oxygen reactive species (ROS) evolution[58,59]. The increase in polyphenols in all basil cultivars in response to subsequent cuts, reveals that this agricultural practice is a helpful tool to grow basil with better biochemical features.

Despite the application of eustress that enhances the concentrations of desirable phytochemicals[45] but often results in a slowdown in growth rates[60], our findings showed a surprising increase in unit yield caused by the cut. As suggested by Crozier et al.[61] and Shaw et al.[62], the improvement in production performance and the con-sequent increase in photosynthetic products may have enhanced the phenolic concentrations because of the allocation of excessively fixed carbon to the shikimate pathway.

3.5. Impact of Cultivar and Cut on Aroma Profile

In Genovese basil for "pesto" production, aroma is undoubtedly the critical quality attribute for taste determination[63]. The unmistakable intense aroma of Genovese basil, without mint flavor, is characterized by wide variability in the composition of essential oils, of which monoterpenes and phenylpropanoids are the major constituents[34].

SPME-GC/MS analysis of the volatiles of all tested basil cultivars identified 40 molecules, among which six were above 2% (**Table 3**). The most abundant compound was linalool, followed by eucalyptol, trans-α-bergamotene, eugenol, 1-octen-3-ol, and β-cis-ocimene. The interaction between the factors under investigation showed significant changes in the aroma profile, except for linalool. The eucalyptol content recorded a substantial decrease of 34.4% in Eleonora in response to the cut. Similarly, β-cis-ocimene showed the same trend in Italiano Classico (−24.1%) and Eleonora (−51.6%), while in Aroma 2 it was unchanged. On the contrary, in Eleonora, eugenol, and trans-α-bergamotene increased in the second harvest by 75% and 48.2%, respectively, whereas the highest value of 1-octen-3-ol was achieved in the first harvest. However, in comparison with Aroma 2 and Italiano Classico, the aroma profile of Eleonora was characterized by a reduced linalool content.

Table 3. Effect of cultivar and cut on aroma profile (%) of basil.

Source of Variance	1-octen-3-ol	Eucalyptol	β-cis-ocimene	Linalool	Eugenol	Trans-α-Bergamotene
Cultivar (C)						
Aroma 2	3.64±0.19b	27.11±1.64	2.90±0.13b	39.62±1.65a	2.89±0.42c	3.55±0.54b
Eleonora	4.48±0.29a	27.47±2.86	3.73±0.59a	35.47±2.65b	4.63±0.59b	6.95±0.70a
Italiano Classico	3.13±0.16b	24.17±0.96	3.21±0.22b	39.81±1.94a	6.68±0.59a	6.00±0.46a
	***	n.s.	**	**	***	***
Cut (CT)						
1	3.85±0.35	28.16±1.61	3.84±0.33	33.92±1.27	4.78±0.79	5.55±0.38
2	3.65±0.11	24.34±1.40	2.72±0.13	42.68±0.46	4.68±0.59	5.45±0.88
t-test	n.s.	*	***	***	n.s.	n.s.
C × CT						
Aroma 2 × CT1	3.67±0.39b	25.42±2.09ab	2.83± 0.09bc	36.29±1.49	3.32±0.73bc	4.51±0.47bc
Aroma 2 × CT2	3.61± 0.19b	28.80±2.49ab	2.96± 0.27bc	42.95±0.50	2.45±0.38c	2.59±0.55c
Eleonora × CT1	5.02±0.34a	33.18± 2.62a	5.02± 0.16a	29.66±1.04	3.36± 0.27bc	5.60±0.28b
Eleonora × CT2	3.95± 0.11ab	21.75±1.12b	2.43±0.15c	41.28±0.52	5.89±0.24a	8.30±0.76a
Italiano Classico × CT1	2.86± 0.22b	25.86±0.83ab	3.65± 0.15b	35.80±1.56	7.67± 0.76a	6.55±0.61ab
Italiano Classico × CT2	3.40±0.10b	22.48±1.02b	2.77±0.16c	43.82±0.59	5.69±0.47ab	5.46±0.61b
	*	**	***	n.s.	**	**

n.s., *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. According to Duncan's multiple range test (p = 0.05) different letters indicate significant differences. Cut means were compared by a *t*-test. All data are expressed as mean ± SE (n = 3).

Regarding the average cut impact, linalool increased in the second harvest by 25.8%. The aroma profile results are in accordance with similar research findings, where linalool predominated while estragole was absent; the latter compound is neither appreciated by consumers nor by the food industry[11,34]. It is worth noting that linalool, other than its role in enhancing oil quality, also has a well-documented anxiolytic and anti-depressive properties, which can be exploited as a supplementary therapeutic to alleviate the symptoms of these diseases that afflict a large part of the population[64]. However, each volatile compound's biosynthesis showed significant variation among the sampled cultivars, highlighting how genetic variables impact the aroma profile[65]. Interestingly, eugenol content showed a robust cultivar-dependent response, with the maximum value recorded in Italiano Classico (6.68%), which was also characterized by the highest total phenolic acids' build-up. Indeed, eugenol is a phenylpropanoid that, like other phenolic acids, shares the same metabolic pathway[66] (**Figure 4**). Moreover, several studies pointed to eugenol as the key volatile compound with antioxidant activity in basil[67,68]. Our findings revealed a high impact of the cut on the expression of the oxygenated monoterpenes (**Table 3**), thus supporting that the biosynthesis of these specialized metabolites depends on genetic and stress-related factors[69]. In our study, linalool increased in response to the cut, in agreement with several authors[29,36] compared with the other oxygenated monoterpenes, which showed an opposite trend. Although linalool, eucalyptol, and β-cis-ocimene share the same precursor (GPP, geranyl pyrophosphate), it is still unclear how both environmental and cut factors may affect gene expression of linalool synthase (LIS) enzyme activity, 1,8-cineole synthase, and β-cis-ocimene synthase that respectively catalyze the conversion of GPP to linalool, eucalyptol, and β-cis-ocimene[70].

In aromatic herbs such as basil, the genotype × environment interaction mostly affects the biosynthesis of specialized metabolites and volatile oils concentration[71], in part confirming our results. Specifically, we observed a more robust cultivar-dependent response in Eleonora, which after harvesting, a −34.4%, −51.6%, and +75.3% variation in eucalyptol, β-cis-ocimene, and eugenol content, respectively, was evidenced. The simultaneous decrease of the monoterpenes (eucalyptol and β-cis-ocimene) and the increase of the phenylpropanoid eugenol, which are synthesized via two distinct metabolic pathways (i.e., mevalonate and shikimate pathways)[72], confirm that terpene synthase is negatively correlated with phenylalanine ammonia-lyase (PAL) activity[73].

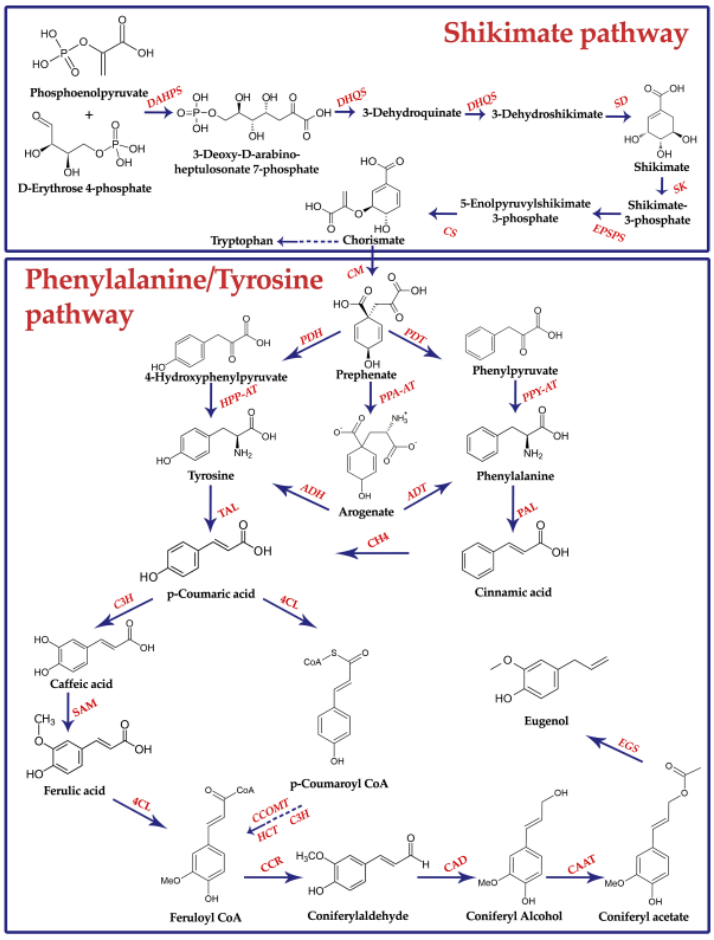


Figure 4. Aromatic amino acid biosynthesis in plants: schematic diagram of the shikimate and phenylalanine/tyrosine pathways. Dashed arrows indicate different enzymatic steps. Abbreviations: DAHPS, 3-Deoxy-d-arabino-2-heptulosonate 7-phosphate synthase; DHQS, 3-Dehydroquinate synthase; DHQD, 3-Dehydroquinate dehydratase; SD, Shikimate dehydrogenase; SK, Shikimate kinase; EPSPS, 5-Enolpyruvylshikimate 3-phosphate synthase; CS, Chorismate synthase; CM, Chorismate mutase; PDH, Prephenate dehydrogenase; PDT, Prephenate dehydratase; HPP-AT, 4-Hydroxyphenylpyruvate aminotransferase; PPY-AT, Phenylpyruvate aminotransferase; PPA-AT, Prephenate aminotransferase; ADH, Arogenate dehydrogenase; ADT, Arogenate dehydratase; TAL, Tyrosine ammonia-lyase; C4H, Cinnamate 4-hydroxylase; PAL, Phenylalanine ammonia-lyase; C3H, p-Coumarate 3-hydroxylase; 4CL, 4-Hydroxycinnamoyl CoA ligase; SAM, S-Adenosylmethionine synthetase; CCOMT, Caffeoyl-CoA O-methyltransferase; HCT, Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; CCR, Cinnamoyl reductase; CAD, Cinnamyl alcohol dehydrogenase; CAAT, Coniferyl alcohol acetyltransferase; EGS, Eugenol synthase.

4. Conclusions

For the food industry, the final product's standardization is the pivotal goal for its commercial success, increasingly focused on quality requirements. Aroma and color constitute outstanding and influential basil quality indicators to consumers. As stated in this study, the cut affected all these aspects, posing a challenge to the processing industry, which must guarantee uniformity in production. Therefore, it must be considered in order to identify the best Genovese basil cultivar for this purpose. The findings achieved for the aromatic profile showed a cultivar-dependent response to the cuts. Specifically, Aroma 2 and Italiano Classico underwent a lower variation in volatiles than Eleonora, in conformity to the agroindustry demands. All cultivars reacted positively to the cuts, resulting in better productive performance (+172%) as well as in the bioaccumulation of specialized metabolites (+413%) to which are attributed beneficial health properties that draw the appreciation of the food, cosmetic and pharmaceutical industries. Finally, for "pesto" basil cultivation, Aroma 2 showed the best performance in response to cuts by achieving high yield, standard sensory profile, and the lowest nitrate content.

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Chapter 5

Successive Harvests Modulate the Productive and Physiological Behavior of Three Genovese Pesto Basil Cultivars

Abstract: In the Italian culinary tradition, young and tender leaves of Genovese basil (*Ocimum basilicum* L.) are used to prepare pesto sauce, a tasty condiment that attracts the interest of the food processing industry. Like other leafy or aromatic vegetables, basil is harvested more than once during the crop cycle to maximize yield. However, the mechanical stress induced by successive cuts can affect crucial parameters associated with pesto processing (leaf/stem ratio, stem diameter, and dry matter). Our research accordingly aimed to evaluate the impact of successive harvests on three field-grown Genovese basil cultivars ('Aroma 2', 'Eleonora' and 'Italiano Classico') in terms of production, physiological behavior, and technological parameters. Between the first and second harvest, marketable fresh yield and shoot dry biomass increased by 148.4 % and 172.9 %, respectively; by contrast, the leaf-to-stem ratio decreased by 22.5 %, while the dry matter content was unchanged. The increased fresh yield and shoot dry biomass at the second harvest derived from improved photosynthetic efficiency, which enabled higher net CO_2 assimilation, F_v/F_m and transpiration as well as reduced stomatal resistance. Our findings suggest that, under the Mediterranean environment, 'Italiano Classico' carries superior productive performance and optimal technological characteristics in line with industrial requirements. These promising results warrant further investigation of the impact successive harvests may have on the qualitative components of high-yielding basil genotypes with respect to consumer expectations of the final product.

Keywords: *Ocimum basilicum* L.; Italiano classico; Marketable yield; Mineral composition; Organic acids; F_v/F_m; Photosynthetic activity

1. Introduction

When attempting to classify the most popular aromatic herbs, sweet basil (*Ocimum basilicum* L.) easily outranks the rest; its versatile use as an ornamental plant for sauce production, for flavoring and garnishing dishes, and as a cosmetic fragrance, justifiably bestowed it the title "king of herbs"[1]. Universally recognized for its aroma, basil is an annual herbaceous plant belonging to the Lamiaceae family[2]. Due to its intrinsic genetic variability, the myriad species of basil identified by researchers and botanists, differ in morphological traits (e.g., color and shape) and in chemical and aromatic composition[1,3]. Hence, making it a multifaceted vegetable highly demanded by the pharmaceutical, cosmetic, and food processing industries[4]. Native to the Asian continent (India, Pakistan, Iran, and Thailand), nowadays basil is widespread and cultivated worldwide[5,6]. Its dispersal to the European continent is mainly ascribed to its gastronomic value as fresh and dried herb in typical regional recipes[7]. Nutritionally, basil has remarkable health-promoting properties owing to its good content of minerals, vitamins, antioxidants, and low caloric value, which attract the growing consumers' interest[8–10]. In Italy, basil cultivation was in the 1990s mainly located in the Liguria region with an overall production of 170 tons per year, mostly in protected environment[11]. However, the ongoing high demand for fresh products all year round and the growing interest of the food industry for this aromatic plant has led to a 60% increase in the area dedicated to its cultivation in the last two decades[12]. In Italy, basil is mainly processed into "pesto", a typical green sauce of the Italian gastronomic tradition, appreciated worldwide[13]. The sensory attributes of Genovese basil used to produce this tasty sauce and the impact of pedoclimatic conditions in the Mediterranean area, unequivocally characterize pesto's organoleptic properties, mainly characterized by phenylpropanoids and monoterpenes produced by specialized glandular trichomes[14,15].

Relevant in basil cultivation is temperature, representing a limiting factor for its development[16]. Walters[17] confirmed that an increase in air temperature up to 29 °C led to an increment in fresh and dry weight, number of nodes, internode length, per-centage of flower buds, and plant height. Basil is a long-day plant that blooms in summer and grows well in the full sun[1]. Beaman et al.[18] achieved an increase in edible biomass in basil Genovese cultivars exposed to a photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}. Further studies revealed that increased daily light raised the number of branches per plant and plant height[19]. Like other leafy vegetables, basil for agroindustry is harvested several times during its crop cycle, in the pre-flowering phase (2–3 times depending on the latitude)[20].

In line with the needs of the industrial supply chain, the agri-food practice of successive harvests, other than ensuring earlier production, leads to reduced labor costs by extending the crop cycle, thus circumventing multiple sowings during the growing season[7], and avoiding tanks and greenhouse disinfection. The mechanical stress induced

by successive cuts can trigger productive and qualitative responses in the basil crop[7,20,21], warranting more substantial understanding of the impact this agronomic practice may have on parameters (e.g., leaf number, leaf-to-stem ratio, stem diameter, and dry matter) that are critical for industrial processing. In line with the above, our investigation aimed to assess the productive and eco-physiological responses of Genovese basil grown in the Campania region to successive harvests, using three commercial cultivars ordinarily employed for intensive open-field cultivation, which are characterized by an aromatic profile rich in linalool and poor in estragole that is responsible for the undesirable hint of mint[20].

2. Materials and Methods

2.1. Plant Material Tested, Experimental Design, and Growth Conditions

The A field experiment was conducted in spring-summer 2019 at the Department of Agriculture of the University of Naples "Federico II", at the experimental farm "Torre Lama", located in Bellizzi (Salerno, Italy; 43°31′ N, 14°58′ E; 60 m a.s.l). The clay loam soil (46% sand, 24% silt, and 30% clay) had the following chemical and physical characteristics: pH 7.7, electrical conductivity 0.16 dS m^{-1}, organic matter (*w*/*w*) 1.21%, total N 0.11 %, extractable phosphorus 88 mg kg^{-1}, and exchangeable potassium 980 mg kg^{-1} in the first 0–30 cm soil layer. Before transplanting, the soil was plowed and simultaneously manured with 2 kg m^{-2} of mature manure, while during the experiment seedlings were fertigated with soluble 8-12-24 (10) NPK (SO_3) complex fertilizer. The experiment was set up as a factorial combination of three basil cultivars ('Aroma 2', Fenix, Belpasso, Italy; 'Eleonora', Enza Zaden, Enkhuizen, The Netherlands; 'Italiano Classico', La Semiorto, Sarno, Salerno, Italy) and two successive harvests (first and second corresponding to CT1 and CT2, respectively). The three cultivars were arranged in a randomized complete block design (RCB), with three replicates accounting for nine experimental units (2 square meters each). Basil plants were sown in a mixture of peat and vermiculite on 15 May 2019 and were transplanted on 6 June with a density of 250 plants m^{-2}. During the experiment, the mean air temperature was 28 °C (min: 17 °C; max: 33 °C), while the mean relative humidity was 57.0% (min: 36 %; max: 78%). At 1, 25, and 50 days after transplant (DAT), fifteen measurements of PPFD were recorded between 11:00 and 13:00 h with a MSC15 spectral radiometer (Gigahertz Optik, Turkenfeld, Germany). The mean PPFD was 2012 μmol m^{-2} s^{-1}. Precipitation was insufficient during the 2019 growing season and irrigation was provided by a drip irrigation system consisting of a main 32 mm polyethylene pipeline equipped with a series of semi-compensating dripping laterals (t-tape, 16 mm diameter, 200 μm thickness, with 1.5 L h^{-1} drippers and 10 cm spacing). The ion concentration of the irrigation water (mg L^{-1}) was: HCO_3^- (285); Ca^{2+} (86); Cl^- (9); Mg^{2+} (20); Na^{2+} (7); NO_3^- (4.5); K^+ (und); SO_4^{2-} (9). The irrigation water electrical conductivity and pH were 0.43 dS m^{-1} and 7.5, respectively.

2.2. Plant Collection, Yield, and Growth Analysis

At the preflowering stage, basil plants were harvested leaving two internodes to promote regrowth for the second harvest. The experimental trial lasted two months, in which two successive harvests (33 and 54 DAT) were made. For each experimental unit, 100 plants were sampled. At each harvest, plants were separated into leaves and stems to determine the number of leaves per plant, stem diameter (cm), fresh marketable yield (kg of fresh leaves m^{-2}), and leaf-to-stem ratio. The sampled material was dried in a ventilated oven at 70 °C to constant weight (72 h) in order to determine total dry shoot biomass (g m^{-2}), dry weight (dw) of leaves and stem (g $plant^{-1}$), and dry matter percentage (%). Samples of dry plants were stored for mineral and organic acids analyses.

2.3. Leaf Gas Exchange, Chlorophyll Fluorescence, and SPAD Index Determination

At both harvests: 33 and 54 DAT, an LCA-4 leaf Chamber Analyser (ADC Bio Scientific Ltd., Hoddesdon, UK) was used to measure net photosynthesis (μmol CO_2 m^{-2} s^{-1}), transpiration (mol H_2O m^{-2} s^{-1}), stomatal resistance (m^2 s mol^{-1} H_2O). As for intrinsic water use efficiency (WUEi), it was calculated as net photosynthesis/transpiration. Leaf gas exchange parameters were measured between 11:00 and 13:00 at a temperature range of 28–30 °C, on the uppermost fully expanded terminal leaflets. Environmental parameters such as PPFD, relative humidity (RH), and CO_2 concentration were set according to the ambient values (700 ± 50 μmol m^{-2} s^{-1}, RH 55 ± 5 %, and 390 ± 10 ppm, respectively) while the airflow rate was 400 mL s^{-1}. Eight measurements were made for each replicate.

On the same date, chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA) on the same leaf used for the gas exchange determination, after a dark adaptation time for 10 min[22], the chlorophyll fluorescence ratio (F_v/F_m) was recorded.

At 54 DAT, measurements of the leaf chlorophyll index (SPAD) were made on adaxial side of fully expanded leaves from 8 randomly selected plants per each experimental unit with a chlorophyll meter SPAD 502 (Minolta Camera Co., Osaka, Japan). Measurements were taken by avoiding the leaf midrib, and a single average SPAD value for each replicate was calculated.

2.4. Minerals and Organic Acids Determination

Dried tissues of sampled basil plants were finely ground (MF10.1 Wiley laboratory mill, IKA®, Staufen im Breisgau, Baden-Württemberg, Germany) and analyzed with a gas chromatograph coupled with a conductivity detector (ICS3000, Dionex, Sunnyvale, CA, USA) for determination of mineral composition and organic acids according to the protocol described by Rouphael et al.[23]. Briefly, 250 mg of the dried material were

suspended in 50 mL of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany), subjected to three freeze–thaw cycles in liquid nitrogen, centrifuged for 10 min at 6,000 rpm (R-10 M, Remi Elektrotechnik Limited, India) and filtered through a 0.20 μm filter Whatman paper (Whatman International Ltd., Maidstone, U.K.). The clear supernatant was assayed by ion exchange chromatography. Concentrations of anions, cations, and organic acids were expressed as g kg^{-1} dry matter.

2.5. Statistical Analysis

All data are presented as the mean ± Standard Error (SE). A two-way analysis of variance (ANOVA) was conducted with cultivar (CV) and cut (CT) as the main effects. In the absence of significant CV × CT interaction, mean comparisons for the main effects were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables that were subject to significant CV × CT interaction, one-way ANOVA was performed separately for each cultivar and where CT effect was significant, means were compared by *t*-Test. All experimental data were analyzed using the software IBM SPSS Statistics ver. 10 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Production Response

The production parameters of Genovese basil cultivars in response to successive harvests are shown in **Table 1**. For all the measured production parameters, no significant interaction between the two tested factors (Cultivar-CV and Cut-CT) was observed. Irrespective of cut order, 'Italiano Classico' revealed a significant higher dry shoot biomass of 39.4% compared to "Eleonora". However, "Eleonora" exhibited a 6.5% higher dry matter when compared to "Aroma 2". Neither the number of leaves per plant nor the fresh marketable yield differed among cultivars. When averaged over basil cultivars, the cut resulted in significant differences in all investigated parameters, except for dry matter %. Specifically, the number of leaves per plant, fresh marketable yield (kg m^{-2} of fresh leaves), and dry shoot biomass increased at the second cut by 100.0%, 148.4%, and 172.9%, respectively.

Table 1. Effect of cultivar and cut on leaf number, fresh marketable yield, dry shoot biomass, and dry matter of basil.

Source of Variance	Leaf Number	Fresh Marketable Yield	Dry Shoot Biomass	Dry Matter
	(No. Plant^{-1})	(kg m^{-2})	(g m^{-2})	(%)
Cultivar (CV)				
'Aroma 2'	69.58 ± 10.88	3.56 ± 0.75	889.38 ± 219.59 ab	11.92 ± 0.15 a
'Eleonora'	65.92 ± 10.23	3.23 ± 0.58	727.17 ± 144.22 b	11.14 ± 0.27 b
'Italiano Classico'	69.22 ± 9.85	4.86 ± 1.13	1013.75 ± 194.35 a	11.78 ± 0.16 ab
Cut (CT)				
1	45.50 ± 1.24 b	2.23 ± 0.14 b	470.22 ± 37.13 b	11.57 ± 0.19
2	90.98 ± 1.80 a	5.54 ± 0.57 a	1283.31 ± 72.38 a	11.66 ± 0.21
ANOVA				
Cultivar	n.s.	n.s.	**	*
Cut	***	***	***	n.s.
Cultivar × Cut	n.s.	n.s.	n.s.	n.s.

Data are mean values ± standard error, n = 3. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by t-Test for C. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

3.2. Biometric Parameters

Similarly, to the crop production parameters, no significant interaction was observed among the two factors examined (Cultivar-CV and Cut-CT) for the tested morphometric parameters (i.e., leaf Dw, stem Dw, leaf-to-stem ratio, and stem diameter) (**Table 2**). Irrespective of the cut order, the cultivar effect was evident only for leaf Dw and stem diameter. Interestingly, 'Italiano Classico' resulted 60.8% greater in leaf Dw and 9.4% greater in stem diameter than 'Eleonora' and 'Aroma 2', respectively (**Table 2**). Successive cuts performed on basil plants led to significant changes in all morphometric parameters mentioned below in **Table 2**. Specifically, when averaged over cultivars the second cut recorded a decrease in leaf-to-stem ratio (−22.6%). In contrast, leaf Dw, stem Dw, and stem diameter increased by 134.5%, 261.1%, and 31.9%, respectively.

Table 2. Effect of cultivar and cut on leaf dry weight, stem dry weight, leaf-to-stem ratio, and stem diameter.

Source of Variance	Leaf Dw (g Plant^{-1})	Stem Dw (g Plant^{-1})	Leaf-to-Stem Ratio	Stem Diameter (cm)
Cultivar (CV)				
'Aroma 2'	1.82 ± 0.39 ab	1.57 ± 0.46	1.16 ± 0.09	0.51 ± 0.04 b
'Eleonora'	1.53 ± 0.24 b	1.38 ± 0.34	1.05 ± 0.07	0.55 ± 0.03 ab
'Italiano Classico'	2.46 ± 0.54 a	2.03 ± 0.55	1.10 ± 0.06	0.58 ± 0.04 a
Cut (CT)				
1	1.16 ± 0.10 b	0.72 ± 0.05 b	1.24 ± 0.04 a	0.47 ± 0.01 b
2	2.72 ± 0.29 a	2.60 ± 0.24 a	0.96 ± 0.02 b	0.62 ± 0.01 a
ANOVA				
Cultivar	*	n.s.	n.s.	**
Cut	***	***	***	***
Cultivar × Cut	n.s.	n.s.	n.s.	n.s.

Data are mean values ± standard error, $n = 3$. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

3.3. Leaf Chlorophyll Index (SPAD), Physiological, and Biochemical Parameters

Significant interaction was observed between cultivar and cut for net photosynthesis, intrinsic water efficiency (WUEi), and maximum quantum yield of photosystem II (F_v/F_m) (**Table 3**). For net photosynthesis, 'Aroma 2' was the source of the interaction since its behavior was different than the other two cultivars during the two cuts, while for WUEi the interaction was observed because the three cultivars behaved differently during the two cuts. For 'Aroma 2', at the second cut (CT2), net photosynthesis and WUEi increased by 61.8% and 41.0 %, respectively. In contrast, WUEi decreased in 'Italiano Classico' by 25.5 % while no significant difference was observed for net photosynthesis (**Table 3**). The F_v/F_m increased significantly from the first to the second cut by 10.0%, 5.5%, and 3.9% in 'Aroma 2', 'Eleonora', and 'Italiano Classico', respectively.

The leaf chlorophyll index (SPAD), stomatal resistance, and transpiration were influenced by cultivar and/or cut factors and no interaction was observed among the two factors. When averaged over basil cultivars, the cut order (from CT1 to CT2), elicited an increase in transpiration (+ 22.1%) and a consequent decrease in stomatal resistance (by 44.8%). Finally, irrespective of cut order the highest leaf chlorophyl index (SPAD) (42.7) and stomatal resistance (9.3 m^2 s mol^{-1}) were both obtained in 'Aroma 2'.

Table 3. Effect of cultivar and cut on leaf chlorophyll index (SPAD), net photosynthesis (Pn), stomatal resistance (r_s), transpiration (E), intrinsic water use efficiency (WUEi), and maximum chlorophyll fluorescence ratio (F_v/F_m).

Source of Variance	SPAD Index	Pn	r_s	E	WUEi	Fv/Fm
		μmol CO_2 m^{-2} s^{-1} m^2 s mol^{-1} H_2O		mol H_2O m^{-2} s^{-1}	μmol CO_2 mol^{-1} H_2O	
Cultivar (CV)						
'Aroma 2'	42.74 ± 0.21 a	17.18 ± 1.86 b	9.27 ± 1.17 a	4.34 ± 0.25	3.96 ± 0.37	0.74 ± 0.02 c
'Eleonora'	41.08 ± 0.19 b	18.01 ± 0.78 b	6.82 ± 1.15 b	4.54 ± 0.36	4.04 ± 0.19	0.75 ± 0.01 b
'Italiano Classico'	39.96 ± 0.19 c	19.87 ± 0.59 a	5.54 ± 0.75 b	4.55 ± 0.23	4.44 ± 0.31	0.77 ± 0.01 a
Cut (CT)						
1	41.24 ± 0.39	16.91 ± 1.18 b	9.29 ± 0.79 a	4.03 ± 0.18 b	4.23 ± 0.28	0.73 ± 0.01 b
2	41.28 ± 0.48	19.79 ± 0.49 a	5.13 ± 0.51 b	4.92 ± 0.15 a	4.07 ± 0.21	0.78 ± 0.01 a
CV × CT interaction						
"Aroma 2"						
CT1	42.42 ± 0.32	13.13 ± 0.36 b	11.74 ± 0.66	4.04 ± 0.39	3.29 ± 0.21 b	0.70 ± 0.01 b
CT2	43.06 ± 0.15	21.24 ± 0.84 a	6.81 ± 0.62	4.63 ± 0.26	4.64 ± 0.44 a	0.77 ± 0.01 a
"Eleonora"						
CT1	41.33 ± 0.22	16.71 ± 0.93 a	9.09 ± 1.07	3.93 ± 0.44	4.31 ± 0.24 a	0.73 ± 0.01 b
CT2	40.84 ± 0.26	19.30 ± 0.69 a	4.56 ± 0.58	5.15 ± 0.30	3.77 ± 0.20 a	0.77 ± 0.01 a
"Italiano Classico"						
CT1	39.96 ± 0.35	20.90 ± 0.77 a	7.04 ± 0.53	4.12 ± 0.20	5.09 ± 0.18 a	0.76 ± 0.01 b
CT2	39.95 ± 0.23	18.83 ± 0.29 a	4.04 ± 0.51	4.99 ± 0.22	3.79 ± 0.18 b	0.79 ± 0.01 a
ANOVA						
Cultivar	***	**	***	n.s.	n.s.	***
Cut	n.s.	***	***	**	n.s.	***
Cultivar × Cut	n.s.	***	n.s.	n.s.	***	**

Data are mean values ± standard error, $n = 3$. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables subject to significant CV × CT interaction, CT means within cultivars were compared by *t*-Test. Different letters within columns indicate significant mean differences. n.s., ** and *** denote non-significant or significant effects at $p \leq 0.01$ and 0.001, respectively.

3.4. Minerals and Organic Acids

Regardless of the cultivar and cut effect, potassium was found in higher concentrations than the other minerals, with values ranging from 22.4 to 46.4 g kg^{-1} Dw, obtained in 'Italiano Classico' and 'Eleonora' at CT1, respectively (**Table 4**). As shown in **Table 4**, the concentrations of potassium, phosphorus, and magnesium were significantly influenced by CV × CT interaction (**Table 4**). For P "Aroma" was the source of interaction since it behaved differently from the other two cultivars during the two cuts, whereas for K and Mg the three cultivars behaved differently during the 2 cuts which caused the interaction. Moreover, at the second cut, K and Mg content in 'Italiano Classico' showed an increase by 40.2% and 11.6%, respectively. The opposite trend was observed in 'Eleonora', in which K and Mg decreased by 38.5% and 26.0%, respectively. Furthermore, no significant differences occurred in 'Aroma 2' for potassium and

magnesium after the cuts, while a significant increase in phosphorus (19.4%) from CT1 to CT2 was observed.

As for the citric acid the interaction observed was an interaction in scale. 'Italiano Classico' citric acid was almost doubled during the second cut when compared to the other two cultivars. When averaged over basil cultivars, the cut order (from CT1 to CT2), elicited a decrease in tartaric acid (−14.8%). Malic acid showed significant cultivar-dependent variation, with the lowest value obtained in "Eleonora" (13.63 g kg^{-1}).

Table 4. Effect of cultivar and cut on minerals and organic acids accumulation of basil.

Source of Variance	P	K	Mg	Malic Acid	Tartaric Acid	Citric Acid
	(g kg^{-1} Dw)	(g kg^{-1} Dw)	(g kg^{-1} Dw)	(g kg^{-1} Dw)	(g kg^{-1} Dw)	(g kg^{-1} Dw)
Cultivar (CV)						
"Aroma 2"	2.77 ± 0.12 a	33.31 ± 0.97 b	2.88 ± 0.04 a	14.96 ± 0.39 a	14.20 ± 0.57	3.99 ± 0.36 a
"Eleonora"	2.58 ± 0.02 b	37.49 ± 4.25 a	2.71 ± 0.18 b	13.63 ± 0.22 b	14.30 ± 0.88	3.26 ± 0.22 b
"Italiano Classico"	2.67 ± 0.04 ab	26.92 ± 2.06 c	2.82 ± 0.08ab	15.19 ± 0.38 a	13.74 ± 0.47	3.85 ± 0.55 a
Cut (CT)						
1	2.62 ± 0.05 b	33.56 ± 3.64	2.84 ± 0.07 a	14.64 ± 0.33	15.20 ± 0.40 a	2.90 ± 0.11 b
2	2.73 ± 0.07 a	31.58 ± 0.97	2.73 ± 0.11 b	14.55 ± 0.39	12.95 ± 0.29 b	4.50 ± 0.23 a
CV × CT interaction						
"Aroma 2"						
CT1	2.53 ± 0.09 b	31.84 ± 1.34 a	2.73 ± 0.05 a	14.38 ± 0.08	15.16 ± 0.76	3.20 ± 0.12 b
CT2	3.02 ± 0.01 a	34.78 ± 0.86 a	2.92 ± 0.04 a	15.55 ± 0.64	13.23 ± 0.34	4.78 ± 0.12 a
"Eleonora"						
CT1	2.57 ± 0.03 a	46.43 ± 3.15 a	3.11 ± 0.03 a	13.89 ± 0.27	16.09 ± 0.20	2.85 ± 0.16 b
CT2	2.59 ± 0.01 a	28.55 ± 0.60 b	2.30 ± 0.05 b	13.37 ± 0.31	12.51 ± 0.78	3.67 ± 0.22 a
"Italiano Classico"						
CT1	2.75 ± 0.04 a	22.41 ± 0.73 b	2.66 ± 0.06 b	15.65 ± 0.61	14.36 ± 0.78	2.66 ± 0.15 b
CT2	2.59 ±0.04 a	31.43 ± 0.68 a	2.97 ± 0.05 a	14.73 ± 0.34	13.11 ± 0.32	5.04 ± 0.22 a
ANOVA						
Cultivar	**	***	*	**	n.s.	**
Cut	**	n.s.	**	n.s.	***	***
Cultivar × Cut	***	***	***	n.s.	n.s.	**

Data are mean values ± standard error, $n = 3$. Mean comparisons were performed by Duncan's Multiple Range Test for CV and by *t*-Test for CT. For variables subject to significant CV × CT interaction, CT means within cultivars were compared by *t*-Test. Different letters within columns indicate significant mean differences. n.s., *, ** and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

4. Discussion

Over the years, basil has gained a prestigious role in the national horticultural markets, mainly for producing the pesto sauce, a condiment with a strong territorial connotation and linked to the Italian gastronomic tradition. On the other hand, the need to supply the industry with fresh leaves all year round[13] has motivated producers to

broaden their horizons about traditional cultivation practices, encouraging them to research and develop alternative agronomic strategies. However, careful selection of the most suitable genotype for a specific environment and possible abiotic interferences to which plants are unavoidably exposed is mandatory for maximizing marketable fresh production.

Our research's goal was to assess three Genovese basil cultivars' productive performance for pesto production after two successive cuts in a spring-summer open field cycle. Successive harvests usually performed on basil can elicit different morpho-physiological and biochemical responses, affecting technological properties mandatory for the food processing industry (e.g., leaf-to-stem ratio and dry matter percentage)[20]. Our results confirm a positive correlation between the tested cultivars and the successive cuts for physiological and growth responses.

The present experiment's total production was lower than previously obtained by De Masi et al.[24] on Genovese basil cultivars, although an additional harvest was performed. Regardless of the cultivars, our findings showed increased marketable fresh production (kg of fresh leaves m^{-2}) due to successive harvests, in agreement with the results of a comparable study[21]. The increased leaf number at the second harvest could be attributable to cut-induced mechanical distress or due to the increased number of stems after the cut. Probably, as outlined by Wang et al.[25], the cut would induce higher cytokinin production, which stimulates cell division, regulates leaf primordia number, and reduces stomatal resistance[7,26,27], as supported by our results (**Tables 1 and 3**).

The increased dry shoot biomass at the second harvest resulted from improved photosynthetic efficiency, which led to higher net photosynthesis and transpiration, reduced stomatal resistance. Although the cut decreased leaf-to-stem ratio and increased the number of leaves per plant, there was no change in the leaf chlorophyll index. Among the tested cultivars, the highest leaf chlorophyll index was obtained in 'Aroma 2' (42.7). This index other than giving an indirect measure of the chlorophyll content, is a useful nondestructive tool for leaf greenness measurement, a quality attribute that can influence consumer and industry choices[28,29]. The increased dry shoot biomass could probably result from more root growth in response to the cut, which would have improved the allocation of photosynthates to the plants' epigeal portion, thus fostering the emergence of more leaves and stems[21]. Furthermore, the F_v/F_m increase in all cultivars confirmed that the cut did not pose a damage to the photosynthetic apparatus, specifically to Photosystem II[30-32]. On the contrary, F_v/F_m values at the second harvest were typical of healthy plants with an efficient photosynthetic system[31,32].

As observed by Nicoletto et al.[20] the leaf-to-stem ratio, a key parameter for pesto's industrial processing, did not show significant differences among the tested cultivars due to their genetic suitability for industrial needs. On the other hand, even though the plants at the second harvest had more leaves, the cut resulted in a reduced leaf-to-stem

ratio, due to the increased stem percentage and its diameter, results in accordance with Nicoletto et al.[20]. It is necessary for industrial pesto processing to ensure low fiber levels because excessive lignification of tissues, therefore higher dry matter, would extend the processing time and promote oxidation, leading to pesto blackening. Our results showed significant differences in dry matter percentage among the cultivars whose values (11.1–11.9%) were well below those obtained by Khalid[33] in a previous study. In contrast, the cut effect did not result in any differences in dry matter accumulation, compared to a recent investigation on basil[34]. However, the dissimilar result achieved by Corrado et al.[34]. could be related to the different genetic material and the different growth conditions and crop management techniques.

The availability of essential minerals and light helps plants to synthesize all compounds they need in order to grow. Phosphorus is a limiting factor for plant development, playing a crucial role in the photosynthetic process[35]. The lack of phosphorus induces a decrease in the ATP/ADP ratio leading to impaired phosphorylation and subsequent photosynthesis carbon depletion[36]. However, the average phosphorus content in the tested cultivars, independent of cut, was in line with the standard values for basil[8]. In light of the above, our experimental results indicate a significant correlation between phosphorus and CO_2 fixation increases after cut, independently of the cultivar. By contrast, all cultivars exhibited no unique behavior for potassium and magnesium buildup after the cut. Nicoletto et al.[20] suggested that this result could be due to the different environmental conditions characterizing the first and second harvests.

Additionally, organic acids are the primary metabolites involved in different biochemical pathways and play a key role in taste definition of horticultural products[37]. Our results showed a lower citric acid level, in accordance with a previous study performed on different basil cultivars[38]. However, organic acids are transported by roots through xylem tissues[39], hence, the increase in citric acid could be justified by the increased transpiration at the second cut. In contrast, the reduction in tartaric acid level at the second harvest could be derived from its role as a precursor in chicoric acid's biosynthesis[40]. Indeed, chicoric acid and most phenolic compounds increase in response to cut-induced stress[41] would lead to a reduction in tartaric acid.

5. Conclusions

The high demand for premium quality basil products poses a challenge for growers and the food industry alike, which must evaluate a plethora of agronomic and technological traits among genotypes that differ in habit, color, and productivity. However, potential environmental interaction with cultivation techniques necessitates careful assessment of suitable cultivars to ensure high yield outputs. Our findings suggest that successive harvests are a useful tool for enhancing productivity. The second harvest resulted in an improved fresh marketable yield (+148.4%) and dry shoot biomass

(+172.9%) while not altering the key technological attributes desired by the food industry (i.e., dry matter percentage and color). Among the tested cultivars, 'Italiano Classico' performed better under Mediterranean pedoclimatic conditions and successive cuts, resulting in increased productivity (+43.1%). The promising results achieved in this study can pave the way for future investigations to evaluate the qualitative responses of 'Italiano Classico' to successive cuts under Mediterranean environmental conditions both in the open field and greenhouse modules.

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Chapter 6
Morpho-physiological Responses and Secondary Metabolites Modulation by Preharvest Factors of Three Hydroponically Grown Genovese Basil Cultivars

Abstract: Sweet basil (*Ocimum basilicum* L.) is an economically important leafy vegetable especially in Mediterranean countries. In Italian gastronomy, the large elliptical leaves of the Genovese type are mostly used for the well-known pesto sauce, and almost all (> 90%) professional production is for the food industry. The growing demand for fresh leaves with standardized technological and sensory characteristics has prompted basil producers to adopt advanced cultivation methods such as the floating raft system. The aim of this study was to evaluate the productive, qualitative, and physiological performance of three Genovese basil cultivars ('Aroma 2', 'Eleonora', and 'Italiano Classico') in two successive harvests and at two densities (159 and 317 plants m^{-2}). Caffeic, chicoric, rosmarinic, and ferulic acid were determined through High Performance Liquid Chromatography (HPLC) system, whereas the extraction and quantification of the volatile organic compounds (VOCs) was performed by Solid-Phase MicroExtraction (SPME) and Gas Chromatography coupled to a Mass Spectrometer (GC/MS). 'Aroma 2' showed the highest fresh yield and photosynthetic rate together with the lowest nitrate content. For all the tested cultivars, the higher density, while reducing the number of leaves per plant, resulted in higher fresh and dry production per unit area, without altering the aroma profile. Successive harvests resulted in a significant increase in both the yield (37.5%) and the total phenolic acids (75.1%) and favored Eucalyptol and 1-octen-3-ol accumulation (+25.9% and +15.1%, respectively). The here presented comprehensive and multifactorial assessment of the productive and qualitative response of basil provides evidence of the positive effects (from biomass to specialized metabolites) that can be obtained from the management of the pre-harvest factors in soilless cultivation. In addition, it also highlights the role and constraints of the genetic factor in the observed response. We also discuss the implications of our work considering the impact for the food processing industry. Future research may explore the phenolic acids accumulation as a possible fortification means to extend the pesto sauce shelf-life, reducing the need of added antioxidants and thermal processing.

Keywords: *Ocimum basilicum* L.; Floating raft system; Cut; Specialized metabolites; Phenolic acids; Volatile compounds

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an annual herbaceous species of the Lamiaceae family considered among the most popular Mediterranean aromatic and edible herbs[1]. The genetic and morphological variability of the *Ocimum* genus has led to the classification of over sixty species[2], which differ in growth habits, leaf morphology, pigmentation, and aromatic content[3]. Furthermore, the recent intense plant breeding has made taxonomic classification more challenging by fixing morphological natural variation in a number of different horticultural types[4]. Basil has also historically been used in folk medicine as a soothing agent for stomach and intestinal discomforts. Nowadays, *O. basilicum* is used for its distinctive aroma in the food processing, cosmetic, and pharmaceutical industries[5]. In Italian cuisine, freshly picked leaves are a popular food garnish (e.g., the real pizza Margherita, Caprese salad). Specifically, the 'Basilico Genovese', which has obtained the European Union (EU) Protected Designation of Origin label (EU Reg. 611/2010), is the central ingredient of the famous green sauce worldwide known as "pesto."[6-7] Over the last decades, the total area used for the cultivation of Genovese basil in Italy has increased by over 66%, with a 25% increase in the protected environment (ISTAT, Italian National Institute of Statistics, 2019)[8], driven mainly by the growing demand of the food industry[9].

In aromatic plants, composition of the essential oil is a relevant qualitative feature, which can influence consumer choice[3]. In sweet basil, most of the aromatic molecules are stored in trichomes and belong to (mono-)terpenes and phenylpropanoids[10]. Among the latter, linalool, and methyl chavicol characterize the fine aroma of this herb[2,3,11]. Nowadays, consumer's choice is increasingly oriented towards high quality foods with nutritional properties[9,12]. Recently, there has been a strong interest in the biochemical characterization of minor species that could represent a relevant source of antioxidants beneficial to human health[13]. Basil's high antioxidant capacity is mainly attributable to rosmarinic acid, a characteristic metabolite of several medicinal plants along with other phenolic acids (e.g., caffeic, chicoric, and ferulic acids)[3,14-16]. The phenolic composition and the aromatic bouquet of basil are also strongly affected by the genetic factor and its interaction with the environment, including agronomic practices[3,12,17,18].

The necessity to meet the growing demands of the processing industry for a clean, crunchy, uniform, tasty, and aromatic product represents a challenge for producers considering the strong effect of year-to-year variability for aromatic plants. This challenge has led the scientific community and growers to focus on alternative growing methods with controlled environmental and nutrient conditions such as hydroponics[16,19]. These systems can guarantee higher yields, improve nutritional quality, reduce the incidence of pests and pathogens[20-23] allow the seasonal adjustment of production and shorten production cycle[24]. Among hydroponic techniques, the floating raft system (FRS) is well suited to the large-scale cultivation of relatively small

medicinal and aromatic plants such as basil, due to simplicity of management and cost-effectiveness[20,25-27]. Hydroponics also represents a useful method to produce leafy vegetables with a low nitrate content due to the possibility of constant monitoring of the nutrient solution[25]. The reduction of nitrates has become an important quality prerogative for the production and marketability of leafy vegetables[28]. The European Commission (EC) regulations n. 1881/2006 and 1258/2011 did not set threshold for nitrate pertaining the *Lamiaceae*. However, sweet basil can accumulate nitrate at levels higher than those permitted by the EC legislation [5000 mg kg^{-1} of fresh weight (fw)], thus entering the hyper accumulative species[29].

In basil, different pre-harvest factors can be manipulated to improve yield and quality, leading to the conclusion that pre-harvest factors should be simultaneously analyzed to uncover their translational value and significant interactions in cultivation[30-33]. For instance, plant density plays a key role in shaping growth and development of different plant organs[9,22]. Likewise, in the ordinary cultivation of basil, plants are cut more than once during the crop cycle, with harvests having a cut-specific leaf quality profile[32,33]. To the authors' knowledge, the scientific literature has mainly focused on the manipulation of the nutrient solution to vary the qualitative and quantitative characteristics of basil in soilless systems, while evidence regarding the impact of plant density and cut, and their interaction with the genotypes, is very scarce. To fill this gap in crop science, a fully factorial experiment was conducted in hydroponics with the aim of evaluating (i) the adaptability of three Genovese basil cultivars, (ii) the impact of two densities and (iii) the influence of two cuts on yield and quality attributes, in order to characterize and standardize production during spring season. The specific and significant morpho-physiological, phytochemicals and aroma variations revealed the strong impact of the analyzed factors and the complexity of their interaction, whose implication are of interest also for the production of basil for the food industry.

2. Materials and Methods

2.1. Plant material, Experimental Design, and Harvest

The experiment was conducted at the pilot farm "Torre Lama" (Department of Agricultural Sciences, University of Naples Federico II) located in Bellizzi (SA, Italy; latitude 43°10′ N, longitude 14°58′ E, altitude 60 m a.s.l.) in a glass-greenhouse with passive ventilation (10 m wide, 30 m long, 3 and 4.5 m high at the eaves and ridge, respectively) from April 11th to May 13th 2019. The mean air temperature was 25 °C (min: 15 °C; max: 32 °C), while relative humidity was 55% during day and 79% during night. Fifteen days after sowing, seedlings of three Genovese basil (*Ocimum basilicum* L. var. *basilicum*) cultivars 'Eleonora' (Enza Zaden, Enkhuizen, The Netherlands), 'Aroma 2' (Fenix, Belpasso, Italy), and 'Italiano Classico' (La Semiorto, Sarno, Italy), were grown in a floating raft system. The nutrient solution (NS) was a modified Hoagland

formulation prepared with reverse osmosis-water and the following nutrients: 14 mM N-NO_3^-, 1.75 mM S, 1.5 mM P, 3.0 mM K, 4.5 mM Ca, 1.5 mM Mg, 1.0 mM NH_4^+, 15 μM Fe, 9 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo. As recommended by Singh and Dunn[34], the electrical conductivity (EC) of the NS was 2.0 ± 0.1 dS m^{-1}. The pH was monitored daily and maintained at 6.0 ± 0.3 using a portable pH/EC/TDS/Temperature Meter HI991301 with HI1288 probe (Hanna instruments, Woonsocket, RI, USA). The instrument was calibrated according to manufacturer's recommendations with calibration solutions (2-points calibration at pH 4.01 and 7.01; EC: 1-point calibration at 12.88 dS m^{-1}). The experimental design was full factorial, with three factors: cultivar (CV) with three levels ('Aroma 2', 'Eleonora', and 'Italiano Classico'), density (D) with two levels (D_{High} and D_{Low}) and cut (CT) with two levels (first, CT1 and second, CT2). Each experimental unit consisted of a single plastic tank filled with 35 l of NS, containing a 54-hole polystyrene tray (52 × 32 × 6 cm; upper hole diameter: 4.5; bottom hole diameter: 3 cm; volume: 0.06 l) and an immersion pump Aquaball 60 (Eheim, Stuttgart, Germany) to maintain a constant dissolved oxygen level above the threshold limit of 6 mg l^{-1}. The planting densities were 317 plant (pl) m^{-2} (54 plants/tray; D_{High}) and 159 pl m^{-2} (27 plants/tray; D_{Low}) (**Figure 1**). During the trial, basil plants were harvested twice (18 days, CT1, and 32 days after transplanting (DAT), CT2), when they reached the phenological phase of pre-flowering, leaving two internodes at CT1. Soon after CT1, the NS was replaced to guarantee the same initial mineral nutrient conditions.

Figure 1. Fresh biomasses of Genovese basil plants at the end of the first harvest at different densities. (**A-B**) Illustrative pictures of Genovese basil cv. 'Aroma 2' at D_{High} and D_{Low} densities. (**C-D**) Illustrative pictures of Genovese basil cv. 'Eleonora' at D_{High} and D_{Low} densities. (**E-F**) Illustrative pictures of Genovese basil cv. 'Italiano Classico' at D_{High} and D_{Low} densities.

2.2. Yield, Growth and Analysis Sampling

From each experimental unit (54 pl for D_{High} and 27 pl for D_{Low}), fifteen basil plants (observational unit) were sampled at each cut, separated into leaves, side branches, and stems, that were weighed and counted. Stem diameter, total fw, and leaf to stem ratio were recorded. A subsample of the plant was stored in paper bags and dried in a forced-air oven at 70 °C until constant weight (72 hours) to determine the dry weight (dw). Dry matter content was calculated as follows: dw/fw × 100. A sample of plants was collected and immediately frozen in liquid nitrogen and stored at −80 °C before being freeze-dried for further qualitative analysis (i.e., phenolics and volatiles determination). For mineral determination, the dry plant material was milled and sieved with a MF10.1 Wiley laboratory mill equipped with a MF0.5 sieve (IKA®, Staufen im Breisgau, Baden-Württemberg, Germany).

2.3. CIELAB Leaf Colorimetry and Soil Plant Analysis Development (SPAD) Index

Ten colorimetric coordinates were recorded on ten representative leaves of each experimental unit at each harvest date, using a Chroma Meter Minolta CR–300 (Minolta Co. Ltd, Osaka, Japan) calibrated with a correspondent Minolta standard. The color spaces were expressed with L*, a*, and b* values, Hue angle, and Chroma, as described by the International Commission of Illumination (CIE) where L^* is degree of lightness (100) to darkness (0), a^* is degree of greenness (–) to redness (+), b^* is degree of blueness (–) to yellowness (+).

Chroma and Hue angle were calculated based on the following equations:

$$Chroma = [(a*)^2 + (b*)^2]^{0.5}$$

$$Hue\ angle = tan^{-1}\frac{b*}{a*}$$

Chroma is the "colorfulness" quantitative attribute, the degree of visual difference from neutral grey of the same lightness. A higher color intensity perceived by humans is indicated by high chroma values. The hue angle describes the qualitative color attribute in the relative amounts of redness and yellowness (i.e., the difference of certain color in reference to the gray color with the same lightness).

At 17 and 31 DAT, the SPAD (Soil-plant analyses development) index measurements as indicator of greenness, were performed on twenty young fully expanded leaves of ten representative plants per experimental unit using a portable chlorophyll meter SPAD-502 (Minolta Co. Ltd, Osaka, Japan), as described by Singh et al.[35].

2.4. Leaf Gas Exchange and Chlorophyll Fluorescence

At 17 and 31 DAT, between 11:00 and 13:00, gas exchange and chlorophyll fluorescence emission measurements were carried out. The measurements were performed on young fully expanded basil leaves, avoiding the central rib, using nine plants per experimental unit. The net carbon dioxide (CO_2), assimilation rate (A_{CO2}), transpiration rate (E), and stomatal resistance (r_s) were determined through a portable gas exchange analyzer (LCA 4; ADC BioScientific Ltd., Hoddesdon, UK), equipped with a broad-leaf chamber (window cuvette area of 6.25 cm^2). The CO_2 concentration, photosynthetically active radiation (PAR), as well as relative humidity (RH), were set to ambient values (365 ± 5 ppm, 700 ± 50 µmol photons m^{-2} s^{-1}, 55 ± 5%, respectively) and the airflow rate to 400 mL s^{-1}. The instantaneous water use efficiency was calculated as A_{CO2}/E.

On the same day of leaf gas exchange measurements (17 and 31 DAT), a portable fluorometer F_v/F_m Meter (Opti-Sciences Inc, Hudson, USA) was used for chlorophyll fluorescence determination. Chlorophyll fluorescence was performed on the leaves of nine plants per experimental unit after their dark adaptation (for at least 10 min) by leaf clips. According to Kitajima and Butler[36], the maximum quantum efficiency of Photosystem II (PSII) F_v/F_m was calculated as $(F_m - F_0)/F_m$, where F_0 and F_m were the

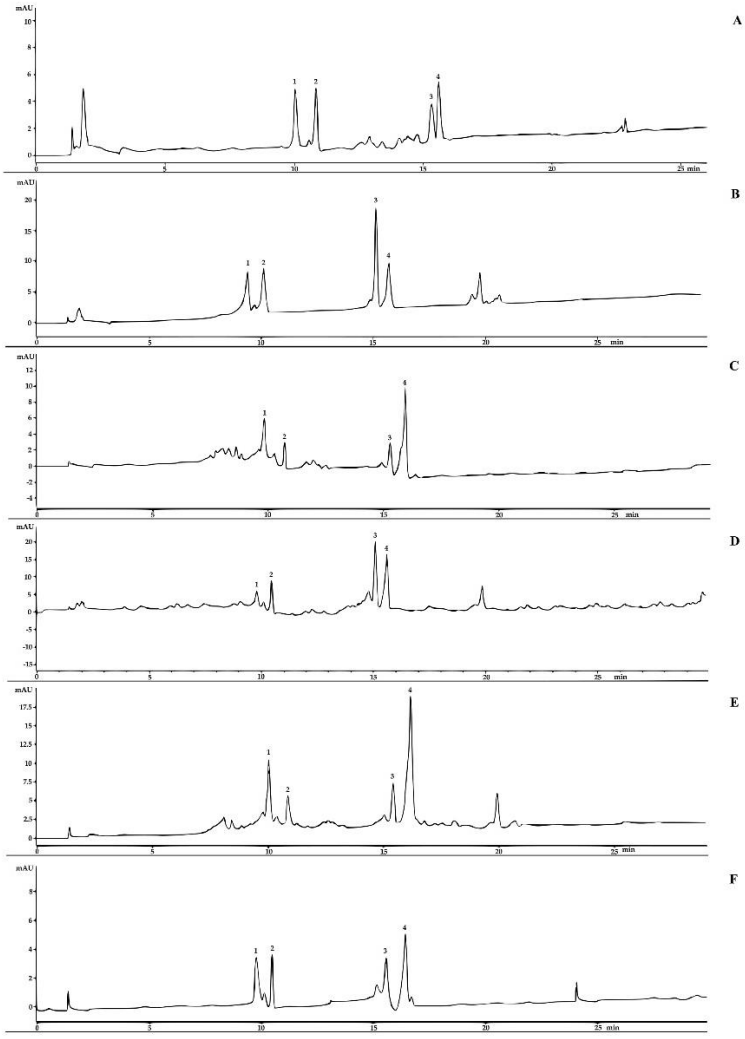
ground fluorescence signal and the maximal fluorescence intensities in the dark-adapted state, respectively.

2.5. Mineral Determination

The ion chromatography system ICS 3000 (Thermo Scientific Dionex, Sunnyvale, California, USA) was used to determine the cationic (K^+, Ca^{2+}, and Mg^{2+}) and anionic (NO_3^- and PO_4^{3-}) profile of basil, following the protocol described by Rouphael et al.[37]. For the determination of the cations, the IonPac CG12A guard column (4 × 250mm) and the IonPac CS12A analytical column (4 × 250 mm) were used, whereas the IonPac AG11−HC guard column (4 × 50 mm) and the IonPac AS11−HC analytical column (4 × 250 mm) were used for anions determination. The ion concentrations of the tested samples were calculated based on the standard curves of cations and anions. All chemicals were purchased from Sigma Aldrich (Milan, Italy). The detected minerals were expressed in g kg^{-1} dw, except for nitrate that was expressed in mg kg^{-1} fw by taking into consideration the dry matter percentage of each sample.

2.6. Phenolics Determination

Phenolic extracts for High Performance Liquid Chromatography (HPLC) analysis were obtained following the method described by Ciriello et al.[38], with some modifications. Briefly, 100 mg of freeze-dried basil samples were added to 2 ml of 70% aqueous methanol (*v*/*v*). The mixture was thoroughly mixed for 1 min (Vortex Classic stirrer; Velp Scientifica, Usmate Velate, Monza Brianza, Italy), sonicated for 20 min (Q500 ultrasonic sonicator; Qsonica, Newtown, Connecticut, USA), stirred by tilting shaker for 10 min (SSL4 see-saw rocker; Cole-Parmer, Vernon Hills, Illinois, USA), centrifuged at 6800 rpm for 10 min (R10M, Remi Elektrotechnik Limited, Mumbai, India), and finally filtered through 0.45 μm Teflon membrane (Phenomenex, Torrance, CA, USA). The supernatant was pipetted into a vial and analyzed by HPLC to quantify the following phenolic acids: caffeic, rosmarinic, chicoric, and ferulic acids. The chromatographic separation of phenolic acids in the extract was performed on an Agilent Technologies 1100 Series HPLC system (Palo Alto, CA, USA) equipped with a degasser (G4225A), a quaternary pump (G13111A) and a diode matrix detector (G1315B) using a 20 μl sample injection loop. A reversed-phase Kinetex C18 100 Å column (5 μm particle size, 150 × 4.6 mm; Phenomenex, Torrance, California, USA) was used. The eluents were 0.1% (*v*/*v*) trichloroacetic acid in water (eluent A) and acetonitrile (eluent B). The gradient schedule was 0-50% B in 50 min at a constant flow rate of 1 mL min^{-1}. Identification was made by comparing the retention times with those of commercially available standards. Calibration curves were built using seven concentration levels for each standard (0.15, 0.5, 1, 10, 20, 50, and 100 mg l^{-1}). The detection of each of the phenolic acids was performed at 280 nm and illustrated in **Supplementary Figure S1**. All HPLC grade reagents and solvents were purchased from Sigma Aldrich (Milan, Italy).



Supplementary Figure 1. Chromatograms of phenolic acids in Genovese basil extract by HPLC at density D2 with separation of caffeic acid (1), ferulic acid (2), chicoric acid (3), and rosmarinic acid (4). (**A,B**) Aroma 2 at first and second cut. (**C,D**) Eleonora at first and second cut. (**E,F**) Italiano Classico at first and second cut.

2.7. Volatiles Determination

The extraction and quantification of volatile organic compounds (VOCs) was performed by Solid-Phase MicroExtraction (SPME) and Gas Chromatography coupled to a Mass Spectrometer (GC/MS) following the protocol described by Ciriello et al.[38]. Briefly, 500 mg of fresh frozen basil were transferred into a 20 ml glass headspace vial with a Teflon septum screw cap (Supelco, Bellefonte, Pennsylvania, USA) and stirred for 10 min at 30 °C (ARE magnetic stirrer; Velp Scientifica, Usmate Velate, Monza, Italy) to promote the VOCs migration into the headspace. A 1 cm long and 50/30 μm thick divinylbenzene/carboxane/polydimethylsiloxane SPME fiber (Supelco, Bellefonte, Pennsylvania, USA) was introduced into the vials for VOCs adsorption. The SPME fiber was introduced into the split-splitless injector of GC 6890N coupled to MS 5973N (Agilent, Santa Clara, California, USA), where thermal desorption of the analytes was performed at 250 °C for 10 minutes. The VOCs were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 μm 5% Diphenyl/95% dimethylpolysiloxane film (Supelco, Bellefonte, Pennsylvania, USA). A splitless injection was used for the samples. The temperature was maintained at 50 °C for 2 min and increased from 50 °C to 150 °C to 10 °C/min and from 150 °C to 280 °C to 15 °C/min. The injection source and ion source temperatures were 250 °C and 230 °C, respectively. Helium (99.999%) was used as the carrier gas at a 1 ml min^{-1} flow rate. The mass spectrometer was set to 70 eV. The compounds were identified using the National Institute of Standards and Technology (NIST) Atomic Spectra Database version 1.6 (U.S. Department of Commerce, Gaithersburg, Maryland, USA) and verified by retention indexes.

2.8. Statistical Analysis

The experiment consisted of a randomized block design with three factors: Cultivar-CV, Cut-CT, and density-D. A two-way analysis of variance (ANOVA) was implemented to assess the significance of the effects and interaction between the factor pairs: CV × D, D × CT, and CV × CT. One-way ANOVA was used to compare the mean effect of CV, while CT and D were compared according to the Student's t-test. Statistical significance was determined at $p < 0.05$ level using Duncan's Multiple Range Test (DRMT) for CV × D, D × CT, and CV × CT interactions and for CV factor. All data are presented as mean ± standard error. All statistical analyses were performed using IBM SPSS 20 (Armonkn, NY, USA) package for Microsoft Windows 10. PCA was performed as described by Kassambara[39].

3. Results

3.1. Morphological Traits and Production Response

The cultivar factor had a highly significant main effect on all the measured biometric variables, which were also strongly affected by the cut (**Table 1**). The lower density

(D_{Low}) resulted in a significant increase in the number of leaves, stem diameter, and number of nodes. On the other hand, the higher density (D_{High}) led to higher fresh yield and dry biomass. The cut significantly influenced all biometric variables and, differently from the cultivar factors, there was a significant interaction effect with the density for all (but dry matter percentage) biometric variables (**Table 1**). For instance, a specific density × cut interaction was observed for dry biomass and leaves/stem ratio, while leaf number and fresh yield were also affected by the cultivar × density (CV × D) interaction. When the density was reduced, the leaf number increased (38.5%) while fresh yield decreased (24.1%) in all tested cultivars. Fresh yield and leaves/stem ratio were the most sensitive parameters because the three-way interaction was highly significantly. Overall, as opposed to stem diameter and leaf to stem ratio, the CT1 resulted in a decrease in leaf number and dry biomass for both densities. Specifically, the most significant increase in the leaf number and nodes per plant was at D_{Low} × CT2, which recorded the lowest stem diameter value (0.43 cm) (**Table 1**).

Table 1. Leaf number, stem diameter, node number, fresh yield, dry biomass, leaf to stem ratio, and dry matter of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	Leaf number (no. plant^{-1})	Stem diameter (cm)	Node number (no. plant^{-1})	Fresh yield (kg m^{-2})	Dry biomass (kg m^{-2})	Leaf to stem ratio	Dry matter (%)
Cultivar (CV)							
Eleonora	36.02 ± 3.65 b	0.44 ± 0.03 ab	3.10 ± 0.11 b	3.74 ± 0.19 c	0.43 ± 0.03 c	1.54 ± 0.03 b	11.42 ± 0.73 b
Aroma 2	43.28 ± 5.11 a	0.42 ± 0.02 b	3.43 ± 0.12 a	4.57 ± 0.35 a	0.62 ± 0.08 a	1.48 ± 0.05 c	13.13 ± 0.82 a
Italiano Classico	30.54 ± 3.40 c	0.46 ± 0.02 a	2.72 ± 0.10 c	4.40 ± 0.41 b	0.54 ± 0.06 b	1.74 ± 0.09 a	11.90 ± 0.38 b
	***	**	***	***	***	***	***
Density (D)							
D_{High}	30.70 ± 2.39	0.41 ± 0.02	2.88 ± 0.09	4.81 ± 0.22	0.60 ± 0.05	1.59 ± 0.04	12.38 ± 0.58
D_{Low}	42.52 ± 3.93	0.47 ± 0.01	3.28 ± 0.12	3.66 ± 0.26	0.46 ± 0.05	1.58 ± 0.07	11.92 ± 0.55
t-test	**	**	**	**	*	ns	ns
Cut (CT)							
CT1	25.28 ± 1.07	0.50 ± 0.01	2.92 ± 0.09	3.57 ± 0.22	0.36 ± 0.02	1.75 ± 0.05	10.14 ± 0.19
CT2	47.94 ± 2.99	0.39 ± 0.01	3.25 ± 0.12	4.91 ± 0.24	0.70 ± 0.04	1.42 ± 0.03	14.16 ± 0.37
t-test	***	***	*	***	***	***	***
CV × D							
Eleonora × D_{High}	28.63 ± 2.20 b	0.41 ± 0.04	2.91 ± 0.14	4.15 ± 0.24 abc	0.48 ± 0.02	1.57 ± 0.05	12.03 ± 1.28
Aroma 2 × D_{High}	37.37 ± 5.12 ab	0.39 ± 0.03	3.19 ± 0.07	5.27 ± 0.31 a	0.70 ± 0.10	1.46 ± 0.04	12.93 ± 1.20
Italiano Classico × D_{High}	26.10 ± 3.61 b	0.42 ± 0.03	2.56 ± 0.10	5.03 ± 0.47 ab	0.62 ± 0.08	1.75 ± 0.06	12.19 ± 0.49
Eleonora × D_{Low}	43.40 ± 5.67 ab	0.47 ± 0.04	3.30 ± 0.13	3.34 ± 0.18 c	0.37 ± 0.04	1.50 ± 0.04	10.81 ± 0.76
Aroma 2 × D_{Low}	49.18 ± 8.64 a	0.46 ± 0.02	3.67 ± 0.20	3.88 ± 0.51 bc	0.55 ± 0.12	1.49 ± 0.10	13.34 ± 1.22
Italiano Classico × D_{Low}	34.98 ± 5.48 ab	0.49 ± 0.01	2.88 ± 0.15	3.77 ± 0.59 bc	0.46 ± 0.09	1.74 ± 0.17	11.61 ± 0.60
	**	ns	ns	***	ns	ns	ns

Cont. **Table 1**

Source of variance	Leaf number (no. plant^{-1})	Stem diameter (cm)	Node number (no. plant^{-1})	Fresh yield (kg m^{-2})	Dry biomass (kg m^{-2})	Leaf to stem ratio	Dry matter (%)
D × CT							
D_{High} × CT1	22.63 ± 1.21 c	0.48 ± 0.01 b	2.83 ± 0.14 b	4.41 ± 0.12 b	0.45 ± 0.01 c	1.70 ± 0.05 a	10.20 ± 0.30
D_{Low} × CT1	27.92 ± 1.31 c	0.51 ± 0.01 a	3.00 ± 0.13 b	2.72 ± 0.07 c	0.27 ± 0.01 d	1.80 ± 0.08 a	10.09 ± 0.24
D_{High} × CT2	38.77 ± 2.54 b	0.34 ± 0.01 d	2.93 ± 0.10 b	5.21 ± 0.40 a	0.76 ± 0.06 a	1.48 ± 0.04 b	14.56 ± 0.37
D_{Low} × CT2	57.12 ± 3.24 a	0.43 ± 0.01 c	3.57 ± 0.15 a	4.60 ± 0.23 ab	0.64 ± 0.05 b	1.36 ± 0.02 b	13.75 ± 0.63
	***	**	**	***	*	***	ns
CV × CT							
Eleonora × CT1	27.35 ± 1.64 c	0.53 ± 0.01 a	3.10 ± 0.15 b	3.82 ± 0.39 b	0.35 ± 0.04 d	1.63 ± 0.03 b	9.23 ± 0.14 d
Aroma 2 × CT1	27.98 ± 0.90 c	0.47 ± 0.01 bc	3.17 ± 0.07 b	3.67 ± 0.41 b	0.38 ± 0.04 d	1.63 ± 0.04 b	10.50 ± 0.16 c
Italiano Classico × CT1	20.50 ± 1.18 c	0.49 ± 0.01 ab	2.48 ± 0.07 c	3.21 ± 0.34 b	0.35 ± 0.04 d	1.99 ± 0.06 a	10.71 ± 0.23 c
Eleonora × CT2	44.68 ± 5.10 b	0.36 ± 0.02 e	3.11 ± 0.16 b	3.67 ± 0.05 b	0.50 ± 0.02 c	1.44 ± 0.01 c	13.62 ± 0.64 b
Aroma 2 × CT2	58.57 ± 4.52 a	0.38 ± 0.02 de	3.69 ± 0.19 a	5.47 ± 0.23 a	0.86 ± 0.04 a	1.32 ± 0.02 d	15.76 ± 0.41 a
Italiano Classico × CT2	40.58 ± 3.04 b	0.42 ± 0.03 cd	2.96 ± 0.13 b	5.58 ± 0.22 a	0.73 ± 0.03 b	1.49 ± 0.06 c	13.09 ± 0.11 b
	***	***	*	***	***	***	***

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.2. Soil Plant Analysis Development (SPAD) Index and Color Leaf Measurement

Significant differences were not observed among cultivars for the principal CIELAB colorimetric parameters, as opposed to SPAD index values, which were higher in 'Aroma 2' and lower in 'Italiano Classico' (**Table 2**). Both Lightness (L*) and SPAD index showed significant variations in relation to density. D_{Low} density resulted in a decrease in L* (2.7%), in contrast to the SPAD index (+4.3%). The cut significantly influenced b*, Chroma, and SPAD index that were reduced at CT1, in contrast to a* that showed an opposite trend. Significant differences were found in the interactions (CV × D, D × CT, and CV × CT) between the considered factors under investigation exclusively for SPAD index. The latter parameter increased from higher to lower density and from the first to the second cut, respectively, for CV × D and CV × CT. Specifically, the highest SPAD values were shown for D_{Low} × CT2 (41.96) and Aroma 2 × CT2 (43.34) (**Table 2**). The data indicated that the colorimetric indexes of the cultivars are fixed, as the varieties have been selected to adhere to the Genovese type standard, and little altered by the density and factors interactions.

Table 2. Soil Plant Analysis Development Index (SPAD index), CIELAB color space parameters, Chroma, and Hue angle of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	SPAD index	L*	a*	b*	Chroma	Hue angle
Cultivar (CV)						
Eleonora	39.82 ± 0.53 b	41.61 ± 0.35	−7.06 ± 0.56	14.84 ± 1.22	16.44 ± 1.33	115.60 ± 0.33
Aroma 2	41.62 ± 0.60 a	41.74 ± 0.32	−7.38 ± 0.50	15.29 ± 1.19	16.98 ± 1.29	116.07 ± 0.36
Italiano Classico	38.20 ± 0.21 c	41.91 ± 0.62	−7.35 ± 0.44	16.32 ± 1.07	18.01 ± 1.14	116.99 ± 1.90
	***	ns	ns	ns	ns	ns
Density (D)						
D_{High}	39.05 ± 0.40	42.32 ± 0.30	−7.32 ± 0.40	15.62 ± 0.95	17.25 ± 1.03	115.33 ± 0.27
D_{Low}	40.71 ± 0.54	41.19 ± 0.37	−7.21 ± 0.41	15.35 ± 0.94	17.03 ± 1.02	117.11 ± 1.24
t-test	*	*	ns	ns	ns	ns
Cut (CT)						
CT1	38.88 ± 0.27	41.82 ± 0.24	−8.62 ± 0.24	18.81 ± 0.42	20.76 ± 0.43	116.31 ± 1.27
CT2	40.88 ± 0.58	41.69 ± 0.46	−5.91 ± 0.24	12.16 ± 0.56	13.53 ± 0.61	116.14 ± 0.29
t-test	**	ns	***	***	***	ns
CV × D						
Eleonora × D_{High}	38.53 ± 0.25 b	42.02 ± 0.32	−6.74 ± 0.69	14.28 ± 1.46	15.80 ± 1.61	115.35 ± 0.51
Aroma 2 × D_{High}	40.84 ± 0.68 a	41.84 ± 0.45	−7.35 ± 0.77	15.34 ± 1.75	17.01 ± 1.91	115.80 ± 0.32
Italiano Classico × D_{High}	37.79 ± 0.23 b	43.09 ± 0.64	−7.87 ± 0.69	17.23 ± 1.77	18.95 ± 1.89	114.85 ± 0.55
Eleonora × D_{Low}	41.11 ± 0.72 a	41.20 ± 0.59	−7.38 ± 0.93	15.39 ± 2.06	17.08 ± 2.26	115.86 ± 0.44
Aroma 2 × D_{Low}	42.39 ± 0.93 a	41.64 ± 0.50	−7.41 ± 0.73	15.24 ± 1.78	16.95 ± 1.92	116.35 ± 0.65
Italiano Classico × D_{Low}	38.61 ± 0.26 b	40.73 ± 0.86	−6.84 ± 0.54	15.42 ± 1.25	17.07 ± 1.33	119.12 ± 3.71
	**	ns	ns	ns	ns	ns

Cont. **Table 2**

Source of variance	SPAD index	L*	a*	b*	Chroma	Hue angle
D × CT						
D_{High} × CT1	38.31 ± 0.31 b	42.21 ± 0.27	−8.80 ± 0.21	19.06 ± 0.61	21.00 ± 0.64	114.86 ± 0.30
D_{Low} × CT1	39.45 ± 0.36 b	41.43 ± 0.36	−8.45 ± 0.43	18.55 ± 0.59	20.51 ± 0.61	117.75 ± 2.50
D_{High} × CT2	39.79 ± 0.66 b	42.43 ± 0.55	−5.84 ± 0.32	12.17 ± 0.71	13.51 ± 0.77	115.80 ± 0.41
D_{Low} × CT2	41.96 ± 0.84 a	40.95 ± 0.67	−5.97 ± 0.38	12.15 ± 0.92	13.55 ± 0.99	116.47 ± 0.39
	*	ns	ns	ns	ns	ns
CV × CT						
Eleonora × CT1	38.94 ± 0.39 cd	41.51 ± 0.39	−8.77 ± 0.34	18.34 ± 0.83	20.34 ± 0.89	115.62 ± 0.24
Aroma 2 × CT1	39.90 ± 0.30 bc	41.60 ± 0.50	−8.81 ± 0.14	18.66 ± 0.47	20.64 ± 0.48	115.37 ± 0.39
Italiano Classico × CT1	37.81 ± 0.24 d	42.34 ± 0.29	−8.29 ± 0.64	19.41 ± 0.85	21.29 ± 0.88	117.93 ± 3.93
Eleonora × CT2	40.70 ± 0.89 b	41.71 ± 0.61	−5.35 ± 0.31	11.33 ± 0.95	12.54 ± 0.98	115.59 ± 0.65
Aroma 2 × CT2	43.34 ± 0.54 a	41.88 ± 0.45	−5.95 ± 0.52	11.91 ± 1.21	13.32 ± 1.32	116.78 ± 0.46
Italiano Classico × CT2	38.59 ± 0.26 cd	41.48 ± 1.25	−6.42 ± 0.32	13.24 ± 0.70	14.72 ± 0.77	116.04 ± 0.28
	***	ns	ns	ns	ns	ns

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.3. Physiological and Biochemical Performance

The net CO_2 assimilation rate (A_{CO2}) and the maximum quantum efficiency of open Photosystem II (Fv/Fm) were both affected by the cultivar (**Table 3**). The density choice did not affect the gas exchange parameters nor the instantaneous water use efficiency (WUEi), but the higher density reduced Fv/Fm. On the other hand, the cut significantly affected all physiological measurements performed, except for WUEi. Specifically, plants harvested at CT1 showed an increase of transpiration (E) (17.2%) compared with CT2 and, conversely, stomatal resistance (rs) decreased by 24.5%. All physiological parameters were affected by the interaction between cultivar and density, revealing a robust cultivar-dependent response to the densities under investigation (**Table 3**). Except for Fv/Fm, where the lowest value was obtained at CT2 with density DHigh, the density × cut combination showed no difference for the physiological parameters. With respect to CV × CT, A_{CO2} and Fv/Fm showed significant differences. Particularly, 'Eleonora' and 'Aroma 2' recorded the highest A_{CO2} values at CT1, while 'Eleonora' × CT2 showed the lowest Fv/Fm value.

Table 3. Net photosynthesis (A_{CO2}), stomatal resistance (r_s), transpiration (E), instantaneous water use efficiency (WUEi), and chlorophyll fluorescence of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	A_{CO2} (μmol CO_2 m^{-2} s^{-1})	rs (m^2 s^{-1} mol^{-1})	E (mol H_2O m^{-2} s^{-1})	WUEi (μmol CO_2 mol^{-1} H_2O)	Fluorescence Fv/Fm
Cultivar (CV)					
Eleonora	17.99 ± 0.58 b	7.44 ± 0.63	3.71 ± 0.19	4.97 ± 0.26	0.79 ± 0.01 b
Aroma 2	18.74 ± 0.45 a	6.06 ± 0.51	4.02 ± 0.21	4.79 ± 0.25	0.80 ± 0.00 a
Italiano Classico	17.60 ± 0.25 b	5.86 ± 0.86	4.15 ± 0.26	4.44 ± 0.29	0.78 ± 0.01 b
	***	ns	ns	ns	***
Density (D)					
D_{High}	18.52 ± 0.35	6.13 ± 0.58	4.05 ± 0.17	4.70 ± 0.20	0.78 ± 0.01
D_{Low}	17.70 ± 0.38	6.77 ± 0.56	3.87 ± 0.20	4.76 ± 0.25	0.80 ± 0.00
t-test	ns	ns	ns	ns	*
Cut (CT)					
CT1	19.05 ± 0.34	5.55 ± 0.25	4.28 ± 0.15	4.54 ± 0.16	0.80 ± 0.00
CT2	17.17 ± 0.25	7.35 ± 0.71	3.65 ± 0.19	4.93 ± 0.26	0.78 ± 0.01
t-test	***	*	**	ns	**
CV × D					
Eleonora × D_{High}	19.30 ± 0.62 a	6.81 ± 0.27 ab	3.75 ± 0.08 ab	5.15 ± 0.12 ab	0.77 ± 0.01 b
Aroma 2 × D_{High}	18.71 ± 0.63 a	4.77 ± 0.26 b	4.55 ± 0.12 a	4.11 ± 0.12 bc	0.80 ± 0.01 a
Italiano Classico × D_{High}	17.56 ± 0.36 ab	6.80 ± 1.65 ab	3.86 ± 0.45 ab	4.83 ± 0.50 abc	0.77 ± 0.01 b
Eleonora × D_{Low}	16.69 ± 0.64 b	8.07 ± 1.24 a	3.68 ± 0.39 ab	4.79 ± 0.52 abc	0.80 ± 0.01 a
Aroma 2 × D_{Low}	18.76 ± 0.70 a	7.34 ± 0.64 ab	3.49 ± 0.26 b	5.47 ± 0.29 a	0.81 ± 0.00 a
Italiano Classico × D_{Low}	17.65 ± 0.37 ab	4.91 ± 0.43 b	4.44 ± 0.26 a	4.04 ± 0.24 c	0.80 ± 0.00 a
	***	*	**	***	*

Cont. **Table 3**

Source of variance	A_{CO_2} (μmol CO_2 m^{-2} s^{-1})	rs (m^2 s^{-1} mol^{-1})	E (mol H_2O m^{-2} s^{-1})	WUEi (μmol CO_2 mol^{-1} H_2O)	Fluorescence Fv/Fm
D × CT					
D_{High} × CT1	19.43 ± 0.50	5.50 ± 0.39	4.33 ± 0.23	4.58 ± 0.26	0.80 ± 0.01 a
D_{Low} × CT1	18.68 ± 0.47	5.60 ± 0.34	4.23 ± 0.19	4.49 ± 0.22	0.81 ± 0.00 a
D_{High} × CT2	17.61 ± 0.23	6.75 ± 1.08	3.77 ± 0.23	4.81 ± 0.30	0.77 ± 0.01 b
D_{Low} × CT2	16.73 ± 0.39	7.95 ± 0.94	3.52 ± 0.31	5.04 ± 0.44	0.80 ± 0.00 a
	ns	ns	ns	ns	*
CV × CT					
Eleonora × CT1	19.31 ± 0.62 a	6.03 ± 0.47	4.07 ± 0.20	4.82 ± 0.31	0.81 ± 0.00 a
Aroma 2 × CT1	20.16 ± 0.17 a	5.41 ± 0.45	4.38 ± 0.17	4.64 ± 0.19	0.81 ± 0.00 a
Italiano Classico × CT1	17.69 ± 0.39 b	5.21 ± 0.38	4.40 ± 0.37	4.15 ± 0.30	0.78 ± 0.01 b
Eleonora × CT2	16.68 ± 0.62 b	8.85 ± 0.86	3.36 ± 0.27	5.12 ± 0.44	0.76 ± 0.01 c
Aroma 2 × CT2	17.31 ± 0.21 b	6.70 ± 0.88	3.67 ± 0.34	4.94 ± 0.49	0.80 ± 0.00 ab
Italiano Classico × CT2	17.52 ± 0.34 b	6.50 ± 1.72	3.90 ± 0.38	4.73 ± 0.49	0.78 ± 0.01 b
	***	ns	ns	ns	***

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.4. Mineral Accumulation

The effects on the mineral composition and nitrate content due to the cultivar, density, and cut are presented in **Table 4**. Basil cultivars affected both nitrate and assayed minerals, except for sodium. 'Aroma 2' showed lower average of nitrate (-33%) compared with the other cultivars. The lowest P and Ca content were obtained in 'Eleonora' while K concentration was lower in 'Italiano Classico'. Neither nitrate nor mineral composition was influenced by the density. By contrast, CT2 significantly decreased the nitrate, P, K, Ca, and Mg concentrations. Concerning the interaction between the factors under investigation, the values of nitrate and Mg were influenced by the cultivar and density. In contrast, K values were affected by the interaction between density and cut, with the lowest value obtained in D_{Low} × CT2 (31.13 g kg^{-1} dw). The CV × CT interaction affected Ca content, where the minimum value was obtained in 'Eleonora' × CT1 (0.75 g kg^{-1} dw). However, in response to the interactions between the studied factors P did not show substantial changes.

Table 4. Nitrate and mineral content of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	Nitrate (mg kg^{-1} fw)	P (g kg^{-1} dw)	K (g kg^{-1} dw)	Ca (g kg^{-1} dw)	Mg (g kg^{-1} dw)
Cultivar (CV)					
Eleonora	3590 ± 273 a	3.39 ± 0.37 b	41.67 ± 2.29 a	8.34 ± 0.29 b	2.51 ± 0.08 b
Aroma 2	2332 ± 238 b	4.05 ± 0.42 a	39.31 ± 2.37 ab	9.92 ± 0.32 a	2.83 ± 0.07 a
Italiano Classico	3418 ± 234 a	3.96 ± 0.43 a	37.28 ± 1.65 c	9.37 ± 0.44 a	2.45 ± 0.13 b
	***	***	**	***	***
Density (D)					
D_{High}	2872 ± 189	3.73 ± 0.35	39.84 ± 1.20	9.00 ± 0.34	2.66 ± 0.07
D_{Low}	3354 ± 272	3.87 ± 0.32	39.00 ± 2.19	9.42 ± 0.30	2.54 ± 0.10
t-test	ns	ns	ns	ns	ns
Cut (CT)					
CT 1	3785 ± 174	5.12 ± 0.11	45.30 ± 1.05	10.08 ± 0.29	2.78 ± 0.07
CT 2	2442 ± 182	2.48 ± 0.09	33.54 ± 1.04	8.34 ± 0.21	2.41 ± 0.08
t-test	***	***	***	***	***
CV × D					
Eleonora × D_{High}	3156 ± 410 ab	3.31 ± 0.57	40.13 ± 2.69	7.76 ± 0.27	2.40 ± 0.06 bc
Aroma 2 × D_{High}	2339 ± 100 b	4.08 ± 0.62	40.65 ± 1.26	9.98 ± 0.57	2.93 ± 0.09 a
Italiano Classico × D_{High}	3122 ± 317 ab	3.79 ± 0.68	38.73 ± 2.31	9.25 ± 0.54	2.64 ± 0.12 abc
Eleonora × D_{Low}	4025 ± 289 a	3.47 ± 0.52	43.20 ± 3.87	8.91 ± 0.40	2.63 ± 0.13 abc
Aroma 2 × D_{Low}	2324 ± 489 b	4.02 ± 0.62	37.97 ± 4.74	9.85 ± 0.36	2.72 ± 0.08 ab
Italiano Classico × D_{Low}	3714 ± 323 a	4.13 ± 0.60	35.84 ± 2.42	9.48 ± 0.74	2.27 ± 0.21 c
	**	ns	ns	ns	**

Cont. **Table 4**

Source of variance	Nitrate (mg kg^{-1} fw)	P (g kg^{-1} dw)	K (g kg^{-1} dw)	Ca (g kg^{-1} dw)	Mg (g kg^{-1} dw)
D × CT					
D_{High} × CT1	3445 ± 240	5.10 ± 0.16	43.73 ± 0.84 a	9.88 ± 0.49	2.78 ± 0.11
D_{Low} × CT1	4125 ± 207	5.13 ± 0.16	46.87 ± 1.84 a	10.27 ± 0.32	2.78 ± 0.09
D_{High} × CT2	2300 ± 110	2.35 ± 0.11	35.94 ± 1.26 b	8.11 ± 0.26	2.53 ± 0.08
D_{Low} × CT2	2584 ± 352	2.61 ± 0.13	31.13 ± 1.24 c	8.56 ± 0.32	2.30 ± 0.13
	ns	ns	***	ns	ns
CV × CT					
Eleonora × CT1	4279 ± 174	4.58 ± 0.10	48.31 ± 1.95	8.83 ± 0.43 bc	2.65 ± 0.12
Aroma 2 × CT1	2953 ± 224	5.42 ± 0.13	45.81 ± 1.36	10.65 ± 0.39 a	2.94 ± 0.11
Italiano Classico × CT1	4123 ± 142	5.35 ± 0.13	41.79 ± 1.16	10.74 ± 0.23 a	2.74 ± 0.11
Eleonora × CT2	2902 ± 328	2.20 ± 0.12	35.02 ± 1.28	7.84 ± 0.30 d	2.37 ± 0.05
Aroma 2 × CT2	1710 ± 211	2.68 ± 0.06	32.82 ± 2.47	9.18 ± 0.30 b	2.71 ± 0.05
Italiano Classico × CT2	2714 ± 146	2.57 ± 0.18	32.77 ± 1.60	8.00 ± 0.20 cd	2.16 ± 0.16
	ns	ns	ns	*	ns

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.5. Quantification of Phenolic Acids

Total phenolic acids were affected by the factors under investigation and their interactions (**Table 5**). Rosmarinic acid was the most prevalent compound, followed by chicoric, caffeic, and ferulic acids. 'Italiano Classico' showed the highest content of rosmarinic (144.0 μg g^{-1} dw) and chicoric acids (74.49 μg g^{-1} dw) with an overall higher accumulation of 44.2% (on average) in total phenolic acids, compared to the other two cultivars. The density influenced the content of the most abundant phenolic acids (rosmarinic and chicoric acids), as well as the total phenolic acids content. Except for rosmarinic acid, the cut impacted all the phenolic profile. In addition, the interaction between cultivar and density affected rosmarinic, chicoric, and caffeic acids including total phenolic acids. Moreover, for all cultivars, D_{Low} density led to an increase of rosmarinic, chicoric, and total phenolic acids by 58.3, 84.2 and 55.2%, respectively. In addition, the concentration of all phenolic acids and their sum (total phenolic acids) was affected by the density × cut interaction with D_{Low} × CT2 combination resulting in their highest accumulation. Lastly, the phenolic profile was strongly affected by CV × CT, increasing from the first to the second cut for all the studied cultivars.

Table 5. Phenolic acids and total polyphenols of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	Caffeic acid (μg g^{-1} dw)	Chicoric acid (μg g^{-1} dw)	Rosmarinic acid (μg g^{-1} dw)	Ferulic acid (μg g^{-1} dw)	Total phenolic acids (μg g^{-1} dw)
Cultivar (CV)					
Eleonora	40.94 ± 3.55 b	56.59 ± 8.21 c	46.75 ± 3.49 c	4.63 ± 0.72 a	145.50 ± 11.90 c
Aroma 2	55.69 ± 8.77 a	67.35 ± 12.00 b	111.90 ± 16.60 b	4.88 ± 0.64 a	237.90 ± 35.50 b
Italiano Classico	55.51 ± 1.70 a	74.49 ± 19.50 a	144.00 ± 12.60 a	3.24 ± 0.38 b	276.40 ± 32.30 a
	***	***	***	***	***
Density (D)					
D_{High}	46.71 ± 3.21	46.55 ± 6.09	78.15 ± 9.62	3.83 ± 0.44	172.40 ± 12.80
D_{Low}	54.72 ± 5.77	85.74 ± 13.40	123.70 ± 15.30	4.53 ± 0.52	267.50 ± 31.30
t-test	ns	*	*	ns	**
Cut (CT)					
CT1	39.76 ± 2.77	35.28 ± 2.45	85.10 ± 10.40	2.67 ± 0.13	159.80 ± 12.90
CT2	61.66 ± 4.87	97.01 ± 12.00	116.70 ± 15.70	5.50 ± 0.44	279.80 ± 28.70
t-test	***	***	ns	***	***
CV × D					
Eleonora × D_{High}	48.68 ± 5.16 bc	47.27 ± 2.21 b	38.89 ± 4.09 b	3.91 ± 0.40	135.30 ± 10.70 c
Aroma 2 × D_{High}	36.21 ± 6.20 c	59.49 ± 17.30 b	72.24 ± 5.70 b	5.09 ± 0.80	173.00 ± 29.80 c
Italiano Classico × D_{High}	55.23 ± 1.56 b	32.91 ± 1.91 b	123.30 ± 13.10 a	2.54 ± 0.13	212.10 ± 10.70 bc
Eleonora × D_{Low}	33.21 ± 2.20 c	65.92 ± 16.00 ab	54.61 ± 3.47 b	4.98 ± 1.06	158.70 ± 21.70 c
Aroma 2 × D_{Low}	75.17 ± 12.20 a	75.22 ± 17.80 ab	151.60 ± 23.50 a	4.67 ± 1.06	306.70 ± 53.50 ab
Italiano Classico × D_{Low}	55.78 ± 3.19 b	116.10 ± 31.40 a	164.70 ± 19.00 a	3.95 ± 0.65	340.50 ± 53.50 a
	***	***	***	ns	***
D × CT					
D_{High} × CT1	37.46 ± 4.40 c	33.17 ± 4.24 c	79.64 ± 18.20 b	2.91 ± 0.25 c	152.20 ± 20.80 b
D_{Low} × CT1	42.07 ± 3.43 bc	37.40 ± 2.51 bc	90.56 ± 11.20 b	2.51 ± 0.11 c	171.30 ± 16.60 b
D_{High} × CT2	55.95 ± 1.71 ab	59.94 ± 9.73 b	76.67 ± 7.87 b	4.44 ± 0.64 b	195.80 ± 12.00 b
D_{Low} × CT2	67.37 ± 9.47 a	134.10 ± 13.30 a	156.8 ± 24.30 a	6.55 ± 0.36 a	363.80 ± 40.30 a
	**	***	***	***	***
CV × CT					
Eleonora × CT1	33.05 ± 2.53 c	39.56 ± 4.34 b	39.87 ± 4.89 b	2.69 ± 0.21 b	113.80 ± 4.46 c
Aroma 2 × CT1	35.28 ± 5.74 c	28.44 ± 3.49 b	79.83 ± 8.88 b	2.88 ± 0.26 b	144.10 ± 18.50 bc
Italiano Classico × CT1	50.96 ± 0.70 bc	37.85 ± 3.96 b	135.60 ± 9.12 a	2.46 ± 0.14 b	225.40 ± 7.82 b
Eleonora × CT2	48.84 ± 4.90 bc	73.63 ± 12.70 ab	53.63 ± 3.27 b	5.59 ± 0.81 a	180.20 ± 11.20 bc
Aroma 2 × CT2	76.10 ± 11.80 a	106.30 ± 4.59 a	144.0 ± 26.90 a	6.88 ± 0.35 a	331.80 ± 43.10 a
Italiano Classico × CT2	60.05 ± 1.98 ab	111.10 ± 33.50 a	152.5 ± 24.30 a	4.03 ± 0.61 b	327.70 ± 59.00 a
	***	***	***	***	***

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.6. Volatile Profile Estimation

The percentage of the major volatile compounds are shown in **Table 6**. Linalool was the most prevalent compound, followed by eucalyptol, eugenol, α-bergamotene, 1-octen-3-ol, and β-cis-ocimene. Except for eucalyptol, all volatile compounds detected were affected significantly by the cultivar. 'Eleonora' recorded the highest concentration of 1-octen-3-ol and α-bergamotene but the lowest linalool concentration; instead, 'Italiano Classico' showed the lowest β-cis-ocimene value while 'Aroma 2' the lowest eugenol percentage. The density only influenced the β-cis-ocimene content, with the highest value recorded in D_{Low}. Conversely, all volatile compounds, except β-cis-ocimene, were affected by the cut. In contrast to linalool, eugenol, and α-bergamotene, the highest percentage values of eucalyptol and 1-octen-3-ol were obtained at the second cut. 1-octen-3-ol, β-cis-ocimene, and linalool buildup were influenced exclusively by the interaction between cultivar and density, with the latter exhibiting the lowest value in 'Eleonora' × D_{Low} (36.1%). The interaction between the density and cut showed significant variations for eucalyptol, linalool, and α-bergamotene. Specifically, eucalyptol content was higher in D_{High} × CT2 (31.1 %). Interaction between cultivar and cut resulted in differences exclusively for eucalyptol and α-bergamotene content, with the latter showing the maximum value in 'Eleonora' × CT1.

Table 6. Most abundant volatile compounds of Genovese basil cultivars Eleonora, Aroma 2, and Italiano Classico in light of density and cut treatments.

Source of variance	1-Octen-3-ol (%)	Eucalyptol (%)	β-cis-Ocimene (%)	Linalool (%)	Eugenol (%)	α-Bergamotene (%)
Cultivar (CV)						
Eleonora	4.03 ± 0.05 a	25.72 ± 1.45	3.09 ± 0.15 a	38.49 ± 1.01 b	4.59 ± 0.22 a	5.17 ± 0.56 a
Aroma 2	2.86 ± 0.11 c	25.71 ± 1.83	2.97 ± 0.30 a	44.56 ± 0.87 a	3.92 ± 0.25 b	3.13 ± 0.35 b
Italiano Classico	3.30 ± 0.13 b	25.58 ± 0.63	2.36 ± 0.29 b	44.84 ± 0.94 a	4.51 ± 0.29 a	2.97 ± 0.16 b
	***	ns	***	***	**	***
Density (D)						
D_{High}	3.47 ± 0.13	26.90 ± 1.31	2.19 ± 0.17	43.32 ± 0.74	4.17 ± 0.23	3.51 ± 0.47
D_{Low}	3.32 ± 0.15	24.44 ± 0.80	3.42 ± 0.15	41.94 ± 1.24	4.51 ± 0.20	4.01 ± 0.29
t-test	ns	ns	***	ns	ns	ns
Cut (CT)						
CT1	3.17 ± 0.15	22.73 ± 0.75	2.84 ± 0.17	44.14 ± 1.03	5.03 ± 0.13	4.55 ± 0.43
CT2	3.62 ± 0.11	28.61 ± 0.97	2.77 ± 0.26	41.13 ± 0.91	3.65 ± 0.14	2.97 ± 0.25
t-test	*	***	ns	*	***	**
CV × D						
Eleonora × D_{High}	3.99 ± 0.09 a	26.40 ± 2.92	2.81 ± 0.15 bc	40.88 ± 1.15 b	4.55 ± 0.34	5.22 ± 1.06
Aroma 2 × D_{High}	2.91 ± 0.17 c	27.67 ± 2.84	2.19 ± 0.34 cd	45.98 ± 1.10 a	3.57 ± 0.35	2.66 ± 0.47
Italiano Classico × D_{High}	3.51 ± 0.13 b	26.63 ± 0.81	1.57 ± 0.10 d	43.11 ± 0.70 ab	4.40 ± 0.41	2.63 ± 0.18
Eleonora × D_{Low}	4.07 ± 0.05 a	25.05 ± 0.73	3.37 ± 0.21 ab	36.11 ± 0.94 c	4.63 ± 0.31	5.11 ± 0.51
Aroma 2 × D_{Low}	2.81 ± 0.16 c	23.75 ± 2.26	3.75 ± 0.21 a	43.14 ± 1.15 ab	4.27 ± 0.32	3.60 ± 0.47
Italiano Classico × D_{Low}	3.09 ± 0.20 c	24.52 ± 0.81	3.14 ± 0.33 ab	46.58 ± 1.48 a	4.62 ± 0.44	3.31 ± 0.18
	*	ns	***	***	ns	ns

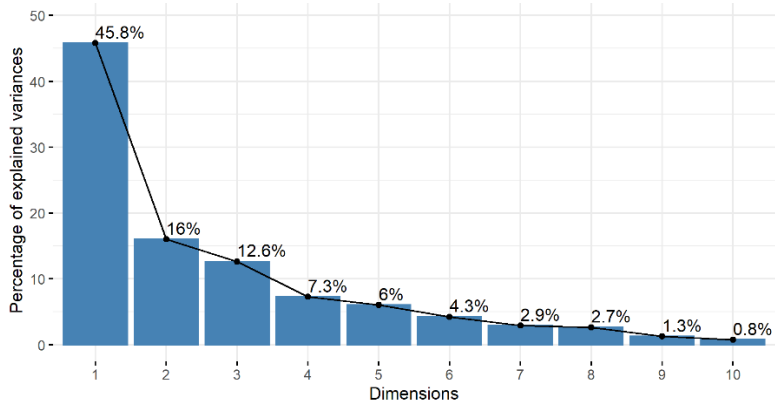
Cont. **Table 6**

Source of variance	1-Octen-3-ol (%)	Eucalyptol (%)	β-cis-Ocimene (%)	Linalool (%)	Eugenol (%)	α-Bergamotene (%)
D × CT						
D_{High} × CT1	3.28 ± 0.19	22.70 ± 1.09 c	2.46 ± 0.19 b	44.59 ± 1.06	4.88 ± 0.18	4.63 ± 0.76 a
D_{Low} × CT1	3.05 ± 0.24	22.76 ± 1.10 c	3.22 ± 0.23 a	43.69 ± 1.83	5.18 ± 0.20	4.47 ± 0.45 a
D_{High} × CT2	3.66 ± 0.16	31.10 ± 1.30 a	1.92 ± 0.26 b	42.06 ± 0.92	3.46 ± 0.24	2.39 ± 0.24 b
D_{Low} × CT2	3.59 ± 0.16	26.11 ± 0.88 b	3.62 ± 0.18 a	40.20 ± 1.58	3.83 ± 0.14	3.55 ± 0.34 ab
	ns	***	***	ns	ns	**
CV × CT						
Eleonora × CT1	3.92 ± 0.06	21.92 ± 0.89 cd	3.25 ± 0.26	40.17 ± 1.34	5.20 ± 0.07	6.67 ± 0.49 a
Aroma 2 × CT1	2.52 ± 0.04	20.17 ± 0.82 d	3.17 ± 0.18	46.23 ± 1.29	4.63 ± 0.17	4.05 ± 0.36 b
Italiano Classico × CT1	3.06 ± 0.17	26.11 ± 0.77 b	2.10 ± 0.20	46.01 ± 1.62	5.27 ± 0.32	2.92 ± 0.13 bc
Eleonora × CT2	4.14 ± 0.06	29.52 ± 1.64 a	2.93 ± 0.13	36.82 ± 1.26	3.98 ± 0.24	3.67 ± 0.50 b
Aroma 2 × CT2	3.20 ± 0.09	31.25 ± 1.33 a	2.77 ± 0.60	42.90 ± 0.76	3.21 ± 0.21	2.21 ± 0.24 c
Italiano Classico × CT2	3.53 ± 0.15	25.04 ± 1.03 bc	2.61 ± 0.55	43.67 ± 0.84	3.75 ± 0.19	3.02 ± 0.31 bc
	ns	***	ns	ns	ns	***

ns, *, **, ***, non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). Density and Cut factors are compared according to Student's t-test. All data are expressed as mean ± standard error, $n = 3$.

3.7. Principal Component Analysis (PCA)

A principal component analysis was conducted for all the agronomical and physico-chemical composition parameters assessed in this study, which were shaped by the investigated factors and their significant interactions. The first two components accounted for 61.8% of the total variance (**Supplementary Figure 2**). The two-dimensional component plot uncovered an internal structure of the data consistent with the experimental factors (**Figure 2**). Samples were separated coherently along the PC1 based on the density, with all D_{Low} samples (respectively, D_{High}) in the positive (resp, negative) PC1 plot area. Considering the prominent contribution of the first component (45.8% of total variance), the density factor associated with the largest linearly projected variance in the measured basil traits. Moreover, samples were much more distributed at the lower planting density, indicating that the total common variance of the basil traits is restrained when plants grow tighter. Considering the cut, there was good separation along the PC2 for nearly all samples. The clustering of the samples according to the cultivar indicated that the genotype-dependent effect on the measured traits does not vary strongly depending on the conditions, and it is inferior to the other pre-harvest factors, as the three varieties consistently clustered according to the level of the other two factors (cut and density). It should be added that PCA orthogonally transforms data, and the grouping of the cultivars may be also interpreted considering a possible non-linear genotypic-dependent response to the cut and density of the different varieties. Overall, the multivariate analysis indicated that most of the variance can be explained considering the two growing conditions, and that, at higher density, the variability of the measured traits due to the genotype and cut factors is less extensive.



Supplementary Figure 2. Scree plot of the eigenvalues of the principal components.

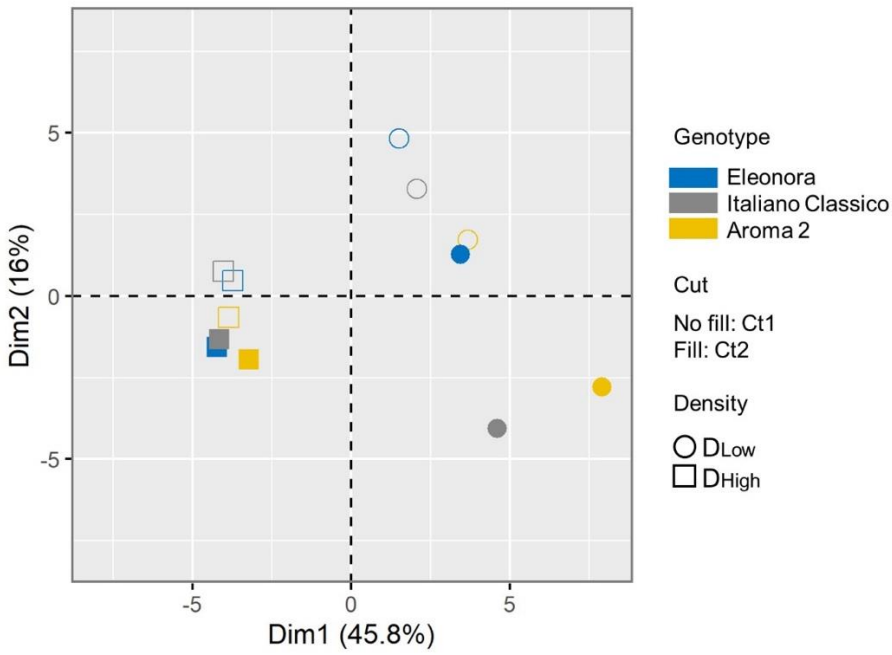


Figure 2. Principal Component Analysis of the basil response. Symbol shape indicates the growing density (DLow: circle; DHigh: square). Each condition is colored according to the variety ('Eleonora': blue; 'Italiano Classico': grey; 'Aroma 2': gold). For each cut, the plot displays the symbol empty (CT1) or filled (CT2). The color and symbol legends are reported on the right-side.

4. Discussion

The FRS is a valuable tool to deseasonalize, anticipate, and improve basil plants' productivity, useful also to understand plant response to the combined action of different pre-harvest factors on various classes of basil traits. The biometric parameters were the most affected, followed by polyphenols, considering the relative presence of three-way interactions. From an applied perspective, it is noteworthy that the fresh biomass per area was affected by each factor and all their interactions. Among the yield components, the number of leaves was the highly sensitive parameter to the various factors and interaction. Also, total polyphenols were highly affected by all the factors and this is reasonable considering their inducible accumulation and, as indicated by our data, that distinct major polyphenols of sweet basil vary differently according to the pre-harvest factors.

Our data showed an improved production performance of the tested cultivars, both in fresh yield and in advance production, achieving yields about two-fold higher than those obtained by Nicoletto et al.[32] in the open field. Regardless of plant density and

cuts, 'Aroma 2' exhibited a better adaptability to the floating raft system, ensuring higher fresh yield and dry biomass per square meter, which can be ascribed to a better photosynthetic performance and a higher number of leaves and nodes per plant. On the contrary, a recent comparative study illustrated for the same cultivars grown in the autumn-winter season, a diametrically opposite production response, indicating a high impact of the environmental factors[40].

Apart from plant material, both the cut and density affected yield and yield-related parameters. Similarly, to Zheljazkov et al.[41] and Puccinelli et al.[42], a linear increase in fresh yield, dry biomass, number of leaves, and nodes per plant were marked from the first to the second cut. As suggested by Zheljazkov et al.[41], the increase in production could be due to a well-formed root system at the second cut that facilitates a faster regrowth of the epigeal part. Moreover, the suppression of apical dominance would have stimulated lateral buds' emission, which led to an increase in the number of nodes and leaves per plant, and consequently to a decrease in the leaf to stem ratio[43]. Other studies on herbaceous crop suggested that the cut may increase cytokinin concentration, hence stimulating cell division and regulating the leaf primordia emission[44,45].

Prior to the second harvest, gas exchange measurements showed a decrease of plants main physiological parameters, such as transpiration rate, net CO_2 fixation, and increased stomatal resistance compared to CT1. These results could be attributed to the combined effects of cut and tissues lignification of plant nearing the end of their life cycle. Scientific evidence demonstrated a direct relationship between the end of life cycle and the reduction in the photosynthetic activity, attributed to a degradation of RuBisCO activity and the alteration of redox processes involving the electron transport chain[46]. The minimal reduction in net CO_2 assimilation rate, transpiration, and F_v/F_m ratio would confirm the onset of leaf senescence processes in the plants at the second cut. The observed phenomenon was also confirmed by the increase in dry matter, due to the progressive lignification of plant tissues[47]. Noteworthy, for the industrial processing of pesto, the dry matter content is a crucial technological parameter. An excessive fibrousness would extend the processing duration, thus causing oxidation with a decrease in the quality of the final product (pesto blackening)[32]. Another crucial industrial requirement is basil leaves' color, which drives consumer choice[48]. Colorimetric parameters were not affected by genotype, like the results obtained in a recent open field trial wherein the same cultivars were compared for production and quality[38]. However, the cut resulted in a reduction in perceived color intensity (Chroma), attributable to both a* and b* variations, probably due to the lower nitrate content in basil leaves[49].

On the other hand, density choice did not affect food processing key parameters such as dry matter and leaf to stem ratio, in contrast to the observations of Miceli et al. (2003)[25], which reported an increase in dry matter with density growth. This result can

be attributed to the different plant material and the different densities that were almost double (226 and 593 plants m^{-2}) compared to those tested (159 and 317 plants m^{-2}) in the current study. However, the double density (D_{High}) in our experiment led to an increased fresh yield and dry shoot biomass for all assayed cultivars, as supported by the results reported in the reviewed literature[22,25,50]. Nonetheless, the increased fresh yield and dry biomass at the higher density is due to the higher number of plants per unit area (Maboko and Du Plooy, 2013)[22], as highlighted by the lower number of leaves and nodes per plant. It should be added that in hydroponics, neighboring plants little compete for below-ground resources (water and nutrients). The reduction in the number of nodes is probably caused by the lower light capture of the canopy because the resources competition increases with the distance decrease (Postma et al., 2020)[51]. An interesting study by Ballaré and Pierik (2017)[52] revealed that plants grown at high densities, due to a reduced ratio between red and far-red light (R:FR) in the canopy, reduce the diameter of the stem, corroborating our findings.

Our results showed a significant cultivar-dependent response for mineral accumulation, in agreement with the findings of Licina et al. (2014)[53], who compared the mineral composition of different basil genotypes. The positive lower nitrate accumulation recorded in 'Aroma 2' emphasizes the genotype's key role in accumulating this potentially risky dietary compound for human health[29]. This may be connected to a different expression of genes involved in nitrate transport, as shown in lettuce[54] and/or a higher nitrate reductase activity[55]. Magnesium is a central cation of the chlorophyll molecule and involved in RuBisCO activation, promoting CO_2 assimilation[56]. The higher magnesium content in 'Aroma 2' is reflected in the higher SPAD and net CO_2 assimilation values, which resulted in higher fresh yield. In contrast to the density effect, successive cuts resulted in a decrease in all analyzed minerals. However, the overall mineral profile reduction was associated with a significant increase in dry matter (about twice as much) from the first to the second cut. This would explain the decrease in minerals as an effect of dilution and not directly attributable to cut-induced distress[57].

Besides synthesizing primary compounds for growth and development, plants produce a wide range of specialized metabolites, such as phenolics, which act as passive defense barriers[58]. Their biosynthesis is strongly affected by genotype and environmental stressors[59]. As outlined in our investigation, phenolic acids were strongly influenced by genotype. 'Aroma 2' and 'Italiano Classico' phenolic profiles had a higher concentration of rosmarinic acid (a compound found to be the more predominant in basil), in contrast to 'Eleonora' that accumulated more chicoric acid. A recent study performed in an FRS provided comparable results, highlighting a significant cultivar-dependent response to chicoric and rosmarinic acid accumulation using the same cultivars of Genovese basil[40]. Rosmarinic acid accumulation was higher than the one

obtained by Sgherri et al. (2010)[12] in a soilless experiment, but well−below the values of Javanmardi et al. (2002)[60] in the open field. These discrepancies can be ascribed to the different growing conditions, extraction and determination methods, and various plant material adopted by each author[2]. A study carried out by Kwee and Niemeyer (2011)[61] revealed in the spice basil (*Ocimum basilicum* × *O. americanum*) a lower content of chicoric acid compared to our findings. In contrast, Thai basil (*Ocimum basilicum* var. *thyrsiflorum*) had a higher chicoric acid content, underlining the impact of genotype on biosynthesis and accumulation of phenolic acids. Concerning the total phenolic acid content, this study showed values about four-fold lower than those obtained by the same cultivars in an open field experiment[38]. The higher values obtained in the open field may be imputable to pedoclimatic conditions, less favorable than those in the soilless system, leading to an oxidative stress that fostered phenolic acids accumulation as a defense mechanism[12]. Furthermore, continuous exposure of field-grown plants to UV radiation can prompt higher phenylalanine ammonia-lyase (PAL) activity resulting in increased phenolic acid accumulation[62,63]. Additionally, specialized metabolite biosynthesis is also influenced by perceived solar radiation, varying with seasonality and planting density. Therefore, the rise of total phenolic acids with the lowest density (D_{Low}) could be due to a lower shading of the plants. Apart from having a positive effect on primary metabolism, light is a critical parameter for producing carbon compounds in plants such as phenolic acids[64]. Similarly, the accumulation of phenolic acids is stimulated by stress factors that cause the evolution of "Reactive Oxygen Species (ROS)" in plant tissues[65-69]. Like other biotic and abiotic stresses, the cut led to a linear increase in the total phenolic acid content in sweet basil, as confirmed by Nicoletto et al. (2013)[32] and Ciriello et al. (2021)[38]. The increase in total phenolic acids in response to cut suggests that this agronomic practice might promote PAL activity; in addition, better production performance at the second harvest might have led to an increased allocation of photosynthates to the shikimic acid pathway[70,71].

Basil is also endowed with aromatic molecules belonging to different chemical groups (i.e., monoterpenes, sesquiterpenes, and phenylpropanoids), whose composition confers the characteristic aroma and taste of the plant[7]. The tested cultivars showed either the absence of undesirable aromatic compounds (e.g., estragole, thymol, and carvacrol) or a predominance (more than 60%) of oxygenated monoterpenes such as linalool and eucalyptol, typical volatiles of Genovese cultivars used for pesto sauce production[7]. Variations in volatiles composition among cultivars were attributable to the different percentage content of minor aromatic compounds, mainly related to different genotypes' intrinsic characteristics[72]. The higher concentration of 1-octen-3-ol and α-bergamotene in 'Eleonora' and the lower of β-cis-Ocimene in 'Italiano Classico' are traits fixed by the genotype. Recent experiments carried out under different conditions and growth systems with the same cultivars showed an increased accumulation of the

above-mentioned minor compounds, which contribute to enrich and diversify the aromatic bouquet of basil[38,40]. In dill (*Anethum graveolens* L.) plants grown in the open field, the employment of high densities resulted in significantly increased amounts of major aroma compounds due to the root competition for water and nutrients[73]. However, in our experiment, independently from the cultivar, the density choice did not induce significant variations in eucalyptol and linalool values. On the other hand, the aroma profile of basil changed in response to successive cuts. In agreement with Ciriello et al. (2021)[38], the cut significantly impacted the expression of the major volatiles (eucalyptol and linalool), thus confirming the strict link between the volatiles' biosynthesis and stressors. However, concerning the results of several open field trials, the second cut reduced the linalool content[38,41,74]. This difference could be attributed either to using different growing systems (open field vs. floating raft system) or the different climatic conditions that characterized the experiments[17]. In contrast to linalool content, eucalyptol increased significantly at the second cut; probably, the cut induced a better expression of the enzyme 1,8-cineole synthase, which converts geranyl pyrophosphate (GPP) to eucalyptol, at the expense of the enzyme linalool synthase (LIS), which catalyzes the GPP-Linalool reaction[75]. Apart from the factors under investigation, the cut caused a decrease in eugenol as observed in an open field study on basil[74]. Similarly, research on sorrel (*Rumex acetosa* L.), showed a significant reduction of sesquiterpenes concentration, evidenced by the reduced α-bergamotene at the second cut[76].

5. Conclusions

The increased demand of the food industry for fresh basil with standardized technological and aromatic attributes has fostered the diffusion of hydroponics. Among the tested cultivars, 'Aroma 2' ensured the best production performance, the lowest nitrate content, and the highest dry matter percentage. The latter, as well as the aromatic profile, were not affected by the density, whereas the yield was increased with the highest density. Successive cuts, ordinarily performed for basil production, also increased the yield per area and favored the accumulation of phenolic acids (+75.1%), without modifying linalool content, though triggering Eucalyptol (+25.9%) and 1-octen-3-ol (+15.1%) accumulation. Our work provides useful information on the productive and qualitative response of the main basil cultivars used for the food industry. The observed wide-ranging responsiveness also suggests that an assessment under different climatic conditions (e.g., autumn cycle) will be a useful complement to manage the year-round production of Genovese leaves for the food industry. Finally, future research may also explore the here described impact of the cut on the phenolic acids' accumulation as a possible fortification means to extend the pesto sauce shelf-life, reducing the need of added antioxidants and thermal processing.

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Chapter 7
Sweet Basil Functional Quality as Shaped by Genotype and Macronutrient Concentration Reciprocal Action

Abstract: Basil (*Ocimum basilicum* L.) is among the most widespread aromatic plants due to its versatility of use and its beneficial health properties. This aromatic plant thrives in hydroponics, which is a valid tool to improve the production and functional quality of crops, but nevertheless, it offers the possibility to de-seasonalize production. A floating raft system was adopted to test the production and quality potential during autumn season of three different genotypes of Genovese basil (Aroma 2, Eleonora, and Italiano Classico) grown in three nutrient solutions with crescent electrical conductivity (EC: 1, 2, and 3 dS m^{-1}). The aromatic and phenolic profiles were determined by GC/MS and HPLC analysis, respectively. The combination Aroma 2 and the EC 2 dS m^{-1} resulted in the highest production, both in terms of fresh weight and dry biomass. The 2 dS m^{-1} treatment determined the major phenolic content, 44%, compared to the other two EC. Italiano Classico showed a higher total polyphenolic content in addition to a different aromatic profile compared to the other cultivars, characterized by a higher percentage of Eucalyptol (+37%) and Eugenol (+107%) and a lower percentage of linalool (−44%). Correct management of the nutritional solution combined with adequate genetic material managed an improvement in the production and the obtainment of the desired aromatic and phenolic profiles.

Keywords: *Ocimum basilicum* L.; Nutrient solution management; Gas chromatography; Volatile compounds; Caffeic acid; Chicoric acid; Rosmarinic acid

1. Introduction

Basil (*Ocimum basilicum* L.) is undoubtedly a transcendent aromatic plant of the Lamiaceae family[1]. Despite its tropical origin, basil is widely used in Mediterranean cuisine as an indispensable ingredient of traditional dishes due to its fresh and aromatic leaves[2]. Apart from its gastronomic value, the pharmaceutical and cosmetic industries have made basil a "versatile herb", sought for its distinctive chemical composition, which includes volatile compounds and phytochemicals beneficial for human health[3-5]. Several studies highlighted that bioactive compounds such as rosmarinic acid, caffeic acid and chicoric acid[4,6-9] confer to basil treasured antioxidant, antiviral, antibacterial, antimutagenic and anti-allergic properties[10-13]. The affluent and intense aromatic profile of basil leaves represents a distinctive quality attribute over other aromatic herbs[14]. This aromatic profile is mainly characterized by the presence of phenylpropanoids (e.g., estragole, eugenol and methyl-eugenol) and monoterpenes (e.g., linalool and eucalyptol) produced by the leaves and secreted through dedicated structures known as peeled glandular trichomes[15].

Chadha and Rajendra[16] identified 160 species of *Ocimum*, characterized by a great genetic variability that influences the morphological characteristics, composition and concentration of odorous molecules and the accumulation of secondary metabolites such as phenols[17,18]. However, in addition to the genetic aspect, the accumulation and biosynthesis of these biomolecules is influenced by physiological and environmental factors such as climate, cultivation technique, plant nutrition, phenological phase, environmental stress, plant ontogenesis and their mutual interactions[3,17-19]. For example, studies conducted by Di Cesare et al.[20,21] showed an evident influence of the environment on the aromatic profile of Genovese basil. For instance, basil plants cultivated in Liguria (Italy) showed a prevalence of linalool, eucalyptol, eugenol and methyl-eugenol that is not present in basil leaves grown in other regions of Italy. A further study conducted by Hussain et al.[22] showed that the chemical composition of essential oils varies according to the harvest season, with a prevalence of sesquiterpenes in summer and oxygenated monoterpenes in winter.

Basil is a versatile leafy vegetable for which demand is constantly increasing[23]. Notwithstanding, ensuring its off-season production with high quality is not simple with traditional cultivation methods. Consumers' interest in fresh leafy vegetables united with their eco-sustainable and high-quality features has prompted producers to develop innovative production systems. Therefore, the floating raft system is a valid alternative for short cycle cultivation that occurs for basil. Control of the growing parameters through careful management of the nutrients, absence of soil and telluric organisms and recycling of the nutrient solution ensure high yields and high-quality attributes due to the accumulation of secondary metabolites with antioxidant activity and flavour enhancers[3,24]. Indeed, among the preharvest factors, mineral nutrition

management is one of the most effective methods to improve the yield and functional quality of horticultural products[25]. In this regard, hydroponics represents a key tool, as it provides accurate control of the nutritional status of the plant[26,27]. Moreover, the shortage of agricultural land makes soilless cultures an interesting alternative to traditional open field cultivation, as it is well suited to constantly developing urban areas[28]. Additionally, Saha et al.[28] observed that soilless cultivation of basil guarantees a higher marketable yield compared to the open field. Furthermore, Sgherri et al.[10] detected a higher antioxidant activity of sweet basil grown hydroponically than those grown in soil. In soilless cultures, nutrient solution management is a critical preharvest factor that plays an imperative role in plant growth. The concentration and composition of the nutrient solution can modulate the organoleptic, functional and productive attributes of vegetable. Herein, positive nutritional stress can lead to increased vegetable quality due to physiological adjustments and accumulation of secondary metabolites as an adaptive response to suboptimal nutritional levels[27].

The reviewed scientific literature showed a positive correlation between modulation of the nutrient solution and biosynthesis of secondary metabolites which provide a qualitative boost[3,27,29-31]. Nevertheless, the lack of specific information about the optimal macronutrient concentration of the nutrient solution as well as the outcomes on production and quality attributes of sweet basil requires further investigation. Our research aimed to assess the impact of three nutrient solutions with different macronutrient concentrations (1 dS m^{-1}, 2 dS m^{-1}, and 3 dS m^{-1}) on the production, aromatic and phenolic profiles of three different Genovese basil cultivars grown in a floating raft system, thus identifying the best combination of nutrient solution and Genovese basil cultivar to guarantee in the autumn season a correct balance between production and quality and, concomitantly, ensuring a low environmental impact and paving the way for future work.

2. Materials and Methods

2.1. Basil Cultivars, Nutrient Solution Concentrations, Growing Conditions and Experimental Design

This research aimed to evaluate the end results of three nutrient solutions with different macronutrient concentrations on the quantitative and qualitative attributes of three Genovese basil cultivars in a floating raft system. The experiment was carried out in an unheated greenhouse located at the University of Naples Federico II, Department of Agriculture (DIA) in Portici (Naples, Italy; 40°48′ N, 14°20′ E, 29 m.s.l. Three cultivars of basil (*Ocimum basilicum* L.), Eleonora (Enza Zaden, Enkhuizen, Noord-Holland, The Netherlands), Aroma 2 (Fenix, Belpasso, Catania, Italy) and Italiano Classico (La Semiorto, Sarno, Salerno, Italy), were transplanted on 1st November 2019 in 54-hole

polystyrene trays (52 × 33 cm) at a density of 317 plants m^{-2}. Each experimental unit consisted of a tray containing 54 plants.

The experimental project was organized in a factorial design with three replicates, consisting of three cultivars (C) (i.e., Eleonora, Aroma 2 and Italiano Classico) and three nutrient solution (NS) strengths (i.e., 1 dS m^{-1}, 2 dS m^{-1} and 3 dS m^{-1}). The cultivation system consisted of twenty-four water tanks with a maximum capacity of 35 L, each filled with 30 L of NS. The macronutrient concentrations of the standard NS (i.e., 2 dS m^{-1}) were 14.0 mM nitrate, 1.5 mM phosphorous, 3.0 mM potassium, 1.75 mM sulfur, 4.5 mM calcium, 1.5 mM magnesium and 1.0 mM ammonium, while the micronutrient concentrations were 15 μM iron, 9 μM manganese, 0.3 μM copper, 1.6 μM zinc, 20 μM boron and 0.3 μM molybdenum. The 1 dS m^{-1} and 3 dS m^{-1} NS concentrations were obtained by halving or increasing (×1.5) the macronutrients concentrations, respectively. During the experiment, the pH of the different NS was monitored every other day and kept around 5.8 ± 0.2 using a portable pH-meter (HI 991301, Hanna Instruments, Milan, Italy). To avoid anoxia in the aqueous medium, an immersion pump was used in each water tank. During the growing period, the greenhouse mean day/night air temperature was 23/13 °C.

2.2. Harvesting, Biometric Analysis and Sampling

Basil plants were harvested at 34 DAT, where part of the harvest was used to determine the main biometric indices: total fresh weight per square meter, leaf and node number per plant, leaf/stem ratio (by weight), percentage of dry matter, and total dry weight per square meter. The latter parameter was obtained by placing the plant material in a ventilated stove at a temperature of 70 °C for a total of 72 h. The remaining harvest was immediately placed in a freezer at −80 °C to preserve the quality of the final product.

2.3. Colorimetric Measurement, SPAD Index and Maximum Quantum Efficiency Determination

Prior to harvest, the colorimetric indices (L*, a* and b*) were evaluated with the Minolta Chroma meter CM-2600d (Minolta Camera Co. Ltd., Osaka, Japan). The instrument, composed of a portable spectrophotometer, was properly calibrated with the Minolta standard; then, the measurements were made on the top leaf blade of 15 fully expanded leaves per experimental unit. The measurements of the green index (SPAD) were made with a handheld Minolta Chlorophyll Meter SPAD-502 (Minolta Camera Co. Ltd., Osaka, Japan), taking the third fully expanded leaf from the top as reference. The observations involved 10 plants per experimental unit. Leaves from the same phenological stage were used for fluorescence measurements through a portable fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA). For each experimental unit, 4 measurements were made in the 11:00–13:00 timeslot. The maximum quantum

efficiency of PSII photochemistry expressed as Fv/Fm was calculated as (Fm-Fo)/Fm, where Fo and Fm represented, respectively, the initial fluorescence and the maximum fluorescence of a sample adapted to darkness for 10 min. The fluorometer was calibrated using the "autoset" option on a leaf similar to the leaves to be measured. The instrument uses an algorithm that allows automatic optimal setting to the modulated light intensity and gain.

2.4. Activities Extraction and Determination of Basil Aromatic Profile

Determination of the aromatic profile of basil was carried out by gas chromatography combined with the mass spectrometer (GC/MC) (Agilent, Santa Clara, CA, USA) after having extracted and concentrated the volatile molecules (VOC's) using the solid phase microextraction (SPME) technique. A GC 6890N equipped with a 5973 mass spectrometer was used, while the spectrometer was set to 70 eV. The 10 mL vial containing 500 mg of fresh frozen basil was stirred continuously with a magnetic stir bar for 10 min (min) at 30 °C. Subsequently, 50/30 µm thick DVB/CAR/PDMS fibre (Supelco® Bellofonte, PA, USA), coated with stationary phase, was introduced into the vial. After 10 min of contact, the SPME fibre was injected into the GC/MS injector where it underwent a thermal desorption of the analytes for 10 min at 250 °C. Split-less injection was used for the samples. The volatiles molecules were separated in a DB-5 capillary column (5% Phenyl, 95% dimethylpolysiloxane, 30 m × 0.250 mm, 0.25 µm; Agilent, Santa Clara, CA, USA). For the first 2 min, the furnace temperature was maintained at 50 °C and then increased to 10 °C min^{-1} from 50 °C to 150 °C and later from 150 °C to 280 °C to 15 °C min^{-1}. The ion and injection source temperatures were 230 °C and 250 °C, respectively. Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The compounds were identified after verification with retention indexes using NIST Atomic Spectra Database version 1.6 (U.S. Department of Commerce, Gaithersburg, MD, USA). Three replicates were performed for each sample with results expressed in percentage (%).

2.5. Extraction, Determination and Quantification of Phenolic Acids

All reagents, standards and solvents were purchased from Sigma Aldrich (Milan, Italy) and were HPLC grade. Preparation of the phenolic extracts followed the method of Ferracane et al.[32] with minor modifications. 2 mL of 70% (*v*/*v*) methanol–water mixture was added to 100 mg of freeze-dried basil sample. This mixture was vortexed, sonicated and agitated for 1, 20 and 10 min, respectively. It was then centrifuged for 10 min at 6800 rpm and finally filtered with a 45-µm membrane filter (Phenomenex, Torrance, CA, USA). The supernatant was pipetted into a sterile falcon from amber glass and analysed to quantify the following compounds: caffeic acid, rosmarinic acid, chicoric acid and ferulic acid. The chromatographic separation of phenolic compounds using a 20-µL sample injection loop was performed using an Agilent 1100 Series HPLC system (Santa

Clara, CA, USA) equipped with a degasser (G4225A), quaternary pump (G13111A) and diode matrix detector (G1315B). The used column was a reverse phase C18 (150 × 4.6 mm d.i.; particle size 5 μm; Kinetex® 100 Å column C18; Phenomenex, Torrance, CA, USA), while the eluents were 0.1% (v/v) trichloroacetic acid in H_2O (A) and acetonitrile (B). The gradient program set was 0–50% for 50 min at a constant flow of 1 mL min^{-1}. Detection of individual phenolic compounds was performed at 280 nm. Identification was then performed by comparing the retention times with the standards. Calibration curves were built for each standard using seven concentration levels (0.15, 0.5, 1, 10, 20, 50 and 100 mg L^{-1}). For each sample, three replicates were performed. Results were expressed as μg g^{-1} dry matter.

2.6. Statistical Analysis

All data were subjected to variance analysis (ANOVA) using the IBM SPSS 20 package (www.ibm.com/software/analytics/spss). For each significant identified variable ($p < 0.05$), a multiple Duncan interval test (DMRT) was performed.

3. Results

3.1. Biometric Measurements, Dry Biomass and Dry Matter Percentage

As illustrated in **Table 1**, only the mean effect of the cultivars determined the difference in leaf number per plant, where Aroma 2 registered the highest value followed by Eleonora and then Italiano Classico. However, the interaction between the two examined factors (cultivar and nutrient solution) was dominant for the rest of the morphometric parameters. The combination Aroma 2 × NS 2 dS m^{-1} guaranteed the highest production of fresh and dry biomass (3.36 and 0.25 kg m^{-2}, respectively). The same cultivar grown in nutrient solution at 1 dS m^{-1} presented the highest dry matter value (7.97%), while the lowest percentage value was displayed by Italiano Classico grown in nutrient solution, 1 dS m^{-1} (5.91%), whereas Eleonora maintained a steady dry matter between all of the different nutrient solutions. Eleonora and Italiano Classico, both cultivated in medium concentration NS (2 dS m^{-1}), recorded the highest number of nodes per plant and the highest leaf/stem ratio, respectively. Regardless of the used nutrient solution, Aroma 2 showed the lowest values for the latter parameter.

Table1. Sweet basil biometric parameters in light of the cultivar and the nutrient solution electrical conductivity.

Source of Variance	Leaf Number (No. Plant^{-1})	Node Number (No. Plant^{-1})	Fresh Yield (kg m^{-2})	Dry Biomass (kg m^{-2})	Leave/Stem Ratio	Dry Matter (%)
Cultivar (C)						
Eleonora	13.07 ± 0.32 b	4.83 ± 0.08 a	3.06 ± 0.05 a	0.20 ± 0.00 b	2.50 ± 0.05 b	6.44 ± 0.08 b
Aroma 2	16.94 ± 0.88 a	4.22 ± 0.05 b	2.87 ± 0.14 b	0.22 ± 0.01 a	1.94 ± 0.07 c	7.59 ± 0.13 a
Italiano Classico	10.65 ± 0.25 c	4.01 ± 0.01 c	3.04 ± 0.06 a	0.20 ± 0.01 b	2.72 ± 0.10 a	6.46 ± 0.14 b
Nutrient solution concentration (NS)						
1 dS m^{-1}	12.99 ± 0.80	4.25 ± 0.10 b	2.93 ± 0.11	0.19 ± 0.00 b	2.30 ± 0.12	6.71 ± 0.33
2 dS m^{-1}	14.08 ± 1.35	4.40 ± 0.17 a	3.06 ± 0.08	0.21 ± 0.01 a	2.44 ± 0.17	6.87 ± 0.12
3 dS m^{-1}	13.60 ± 0.96	4.41 ± 0.12 a	2.98 ± 0.08	0.21 ± 0.00 a	2.42 ± 0.12	6.90 ± 0.15
C × NS						
Eleonora × 1 dS m^{-1}	12.67 ± 0.61	4.63 ± 0.00 c	3.23 ± 0.03 ab	0.20 ± 0.01 c	2.63 ± 0.11 bc	6.23 ± 0.13 de
Eleonora × 2 dS m^{-1}	13.42 ± 0.40	5.04 ± 0.11 a	2.97 ± 0.08 c	0.20 ± 0.00 cd	2.45 ± 0.10 c	6.57 ± 0.10 cd
Eleonora × 3 dS m^{-1}	13.13 ± 0.75	4.83 ± 0.11 b	2.99 ± 0.02 c	0.19 ± 0.00 cd	2.42 ± 0.04 c	6.51 ± 0.08 cd
Aroma 2 × 1 dS m^{-1}	15.58 ± 1.06	4.12 ± 0.01 e	2.54 ± 0.12 e	0.20 ± 0.01 c	1.88 ± 0.03 d	7.97 ± 0.23 a
Aroma 2 × 2 dS m^{-1}	18.42 ± 2.21	4.17 ± 0.04 e	3.36 ± 0.09 a	0.25 ± 0.01 a	1.86 ± 0.05 d	7.32 ± 0.10 b
Aroma 2 × 3 dS m^{-1}	16.83 ± 1.18	4.36 ± 0.08 d	2.72 ± 0.11 de	0.20 ± 0.01 c	2.08 ± 0.21 d	7.47 ± 0.12 b
Italiano Classico × 1 dS m^{-1}	10.71 ± 0.48	4.01 ± 0.01 e	3.02 ± 0.01 bc	0.18 ± 0.00 d	2.39 ± 0.11 c	5.91 ± 0.06 e
Italiano Classico × 2 dS m^{-1}	10.42 ± 0.67	4.00 ± 0.00 e	2.87 ± 0.04 cd	0.19 ± 0.00 cd	3.00 ± 0.07 a	6.73 ± 0.09 c
Italiano Classico × 3 dS m^{-1}	10.83 ± 0.15	4.03 ± 0.03 e	3.24 ± 0.07 ab	0.22 ± 0.01 b	2.78 ± 0.02 ab	6.73 ± 0.04 c
Significance						
Cultivar (C)	***	***	*	***	***	***
Nutrient solution (NS)	ns	*	ns	**	ns	ns
C × NS	ns	*	***	***	**	***

ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). All data are expressed as mean ± se (n = 3)

3.2. Colorimetric Indices, SPAD Index and Fluorescence

According to **Table 2**, the statistical analysis listed significant variations among basil cultivars for all colorimetric components. As far as the L* (brightness) parameter is concerned, only the cultivar mean effect was significant, the highest values were obtained in both Eleonora and Italiano Classico. The latter cultivar had the highest b* value and an absolute negative value for a*. The macronutrients concentration in the nutrient solution significantly influenced the parameters a* and b*. The increase in electrical conductivity of the nutrient solution resulted in a 17% increase in a* values. On the other hand, the switch from the most concentrated to the most diluted nutrient solution produced a 29% increase for b* values, meaning an increase towards the yellow axe, which is inversely correlated with the SPAD index that decreased with reduction of the macroelements. The Fv/Fm ratio, which defines the fluorescence index, was significantly influenced by both the cultivar and the nutrient solution but not by their interaction. Particularly, Italiano Classico and a concentration of 1 dS m^{-1} defined the lowest values. Concerning the SPAD index, the interaction of the two considered factors was significant: the combinations Aroma 2 × NS 2 and 3 dS m^{-1}, and Eleonora × NS 3 dS m^{-1} showed the highest SPAD values (avg. 33.0).

Table 2. Colorimetric indices, SPAD index and fluorescence in light of the cultivar and the nutrient solution electrical conductivity.

Source of Variance	L*	a*	b*	SPAD Index	Fluorescence Fv/Fm
Cultivar (C)					
Eleonora	46.46 ± 0.22 a	−9.81 ± 0.31 b	25.98 ± 0.99 b	30.81 ± 0.46 b	0.81 ± 0.00 a
Aroma 2	44.69 ± 0.18 b	−6.76 ± 0.41 a	14.94 ± 1.28 c	32.54 ± 0.49 a	0.82 ± 0.00 a
Italiano Classico	46.12 ± 0.46 a	−11.27 ± 0.18 c	29.90 ± 0.87 a	30.63 ± 0.41 b	0.80 ± 0.00 b
Nutrient solution concentration (NS)					
1 dS m^{-1}	45.67 ± 0.46	−10.06 ± 0.53 c	26.66 ± 1.93 a	30.08 ± 0.35 c	0.80 ± 0.00 b
2 dS m^{-1}	45.83 ± 0.44	−9.21 ± 0.79 b	23.51 ± 2.63 b	31.54 ± 0.46 b	0.81 ± 0.00 a
3 dS m^{-1}	45.77 ± 0.33	−8.57 ± 0.76 a	20.66 ± 2.35 c	32.37 ± 0.46 a	0.82 ± 0.00 a
C × NS					
Eleonora × 1 dS m^{-1}	46.20 ± 0.64	−10.36 ± 0.63	28.53 ± 1.13	29.65 ± 0.47 c	0.81 ± 0.01
Eleonora × 2 dS m^{-1}	46.72 ± 0.28	−9.99 ± 0.51	26.46 ± 0.78	30.46 ± 0.35 c	0.81 ± 0.00
Eleonora × 3 dS m^{-1}	46.47 ± 0.23	−9.07 ± 0.22	22.95 ± 1.40	32.34 ± 0.50 ab	0.82 ± 0.00
Aroma 2 × 1 dS m^{-1}	44.89 ± 0.28	−8.27 ± 0.14	19.58 ± 0.62	30.94 ± 0.71 bc	0.81 ± 0.01
Aroma 2 × 2 dS m^{-1}	44.61 ± 0.51	−6.28 ± 0.46	13.50 ± 1.58	32.94 ± 0.45 a	0.82 ± 0.00
Aroma 2 × 3 dS m^{-1}	44.58 ± 0.13	−5.74 ± 0.08	11.75 ± 0.08	33.76 ± 0.19 a	0.82 ± 0.00
Italiano Classico × 1 dS m^{-1}	45.93 ± 1.25	−11.53 ± 0.42	31.87 ± 1.57	29.65 ± 0.47 c	0.79 ± 0.01
Italiano Classico × 2 dS m^{-1}	46.16 ± 0.89	−11.37 ± 0.29	30.55 ± 0.94	31.22 ± 0.80 bc	0.81 ± 0.00
Italiano Classico × 3 dS m^{-1}	46.27 ± 0.37	−10.90 ± 0.07	27.28 ± 0.32	31.02 ± 0.63 bc	0.80 ± 0.00
Significance					
Cultivar (C)	**	***	***	***	*
Nutrient solution (NS)	ns	***	***	***	*
C × NS	ns	ns	ns	*	ns

ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). All data are expressed as mean ± se (n = 3).

3.3. Sweet Basil Aromatic Profile

The solid phase microextraction (SPME)-GC/MS analysis of volatile components enabled determination of the aromatic profile of basil cultivars subjected to three different concentrations of macronutrients. In **Table 3**, the percentage of the seven main compounds is reported. Among these, eucalyptol, linalool and eugenol constituted, on average, about 70% of the whole aromatic profile, followed by 1-Octen-3-ol, α-bergamotene, trans-2-hexenal and β-cis-ocimene. The genotype significantly influenced biosynthesis of all reported odorous molecules. Italiano Classico was characterized by a higher amount of eucalyptol (+37%) and eugenol (+107%) but by a lower percentage of linalool (−44%), compared to the other two cultivars. On the other hand, the interaction between the two considered factors determined relevant differences between the following compounds: trans-2-hexenal, eugenol and α-bergamotene. In general, Italiano Classico reached the highest levels of trans-2-hexenal and eugenol. Specifically, the transition from the concentrated nutrient solution to the diluted one determined an increase of about 118% for eugenol. Instead, trans-2-hexenal showed an opposite trend, with the highest percentage obtained from the combination Italiano Classico × 3 dS m^{-1}. Use of the most concentrated solution determined the highest percentage of α-bergamotene for Eleonora, whereas this same NS gave similar percentages to the 2 dS m^{-1} solution for the other two cultivars.

Table 3. Sweet basil aromatic profile in light of the cultivar and the nutrient solution electrical conductivity.

Source of Variance	Trans-2-Hexenal (%)	1-Octen-3-ol (%)	Eucalyptol (%)	β-cis-Ocimene (%)	Linalool (%)	Eugenol (%)	α-Bergamotene (%)
Cultivar (C)							
Eleonora	1.79 ± 0.18 b	4.44 ± 0.18 a	33.56 ± 1.42 b	1.99 ± 0.18 b	35.57 ± 1.73 a	5.78 ± 0.65 b	3.93 ± 0.48 a
Aroma 2	2.68 ± 0.16 a	3.08 ± 0.14 b	32.09 ± 1.26 b	2.45 ± 0.13 a	37.99 ± 1.54 a	3.59 ± 0.28 c	2.21 ± 0.22 b
Italiano Classico	2.82 ± 0.17 a	3.49 ± 0.10 b	45.13 ± 1.24 a	1.50 ± 0.14 c	20.74 ± 2.45 b	9.70 ± 1.33 a	3.61 ± 0.16 a
Nutrient solution concentration (NS)							
1 dS m^{-1}	2.52 ± 0.12	3.65 ± 0.19	36.21 ± 2.07	1.80 ± 0.16 b	32.58 ± 3.51	7.42 ± 1.85 a	2.81 ± 0.36 b
2 dS m^{-1}	2.39 ± 0.21	3.61 ± 0.26	38.34 ± 2.76	1.77 ± 0.21 b	30.05 ± 3.48	6.28 ± 0.76 ab	3.06 ± 0.34 b
3 dS m^{-1}	2.38 ± 0.32	3.75 ± 0.28	36.24 ± 2.37	2.36 ± 0.17 a	31.67 ± 2.89	5.37 ± 0.62 b	3.89 ± 0.43 a
C × NS							
Eleonora × 1 dS m^{-1}	2.34 ± 0.18 bc	4.34 ± 0.20	32.66 ± 1.70	1.76 ± 0.26	37.08 ± 2.01	4.49 ± 0.63 cde	2.86 ± 0.13 bc
Eleonora × 2 dS m^{-1}	1.64 ± 0.23 cd	4.41 ± 0.39	33.50 ± 3.00	1.79 ± 0.37	35.05 ± 4.47	7.02 ± 1.07 bc	3.41 ± 0.79 b
Eleonora × 3 dS m^{-1}	1.37 ± 0.24 d	4.56 ± 0.43	34.54 ± 3.40	2.41 ± 0.24	34.56 ± 3.18	5.84 ± 1.37 bcde	5.52 ± 0.35 a
Aroma 2 × 1 dS m^{-1}	2.77 ± 0.26 ab	3.30 ± 0.07	32.68 ± 1.93	2.26 ± 0.20	40.04 ± 1.06	3.19 ± 0.53 e	1.59 ± 0.28 d
Aroma 2 × 2 dS m^{-1}	2.86 ± 0.11 ab	2.85 ± 0.16	33.33 ± 2.49	2.35 ± 0.07	35.60 ± 2.49	3.96 ± 0.60 de	2.16 ± 0.11 cd
Aroma 2 × 3 dS m^{-1}	2.41 ± 0.41 b	3.10 ± 0.40	30.27 ± 2.54	2.73 ± 0.30	38.34 ± 4.04	3.62 ± 0.36 e	2.88 ± 0.28 bc
Italiano Classico × 1 dS m^{-1}	2.46 ± 0.17 b	3.29 ± 0.12	43.28 ± 2.69	1.39 ± 0.06	20.62 ± 5.73	14.58 ± 1.26 a	3.97 ± 0.15 b
Italiano Classico × 2 dS m^{-1}	2.65 ± 0.23 ab	3.57 ± 0.12	48.19 ± 1.78	1.17 ± 0.16	19.48 ± 5.95	7.86 ± 1.12 b	3.60 ± 0.41 b
Italiano Classico × 3 dS m^{-1}	3.35 ± 0.21 a	3.60 ± 0.25	43.91 ± 0.96	1.93 ± 0.21	22.12 ± 1.41	6.65 ± 0.43 bcd	3.26 ± 0.08 bc
Significance							
Cultivar (C)	***	***	***	***	***	***	***
Nutrient solution (NS)	ns	ns	ns	**	ns	*	**
C × NS	*	ns	ns	ns	ns	***	**

ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). All data are expressed as mean ± se (n=3).

3.4. Antioxidant activities

The phenolic profile of the three Genovese basil cultivars grown with different concentrations of macronutrients (**Table 4**) revealed significant differences. The main abundant phenolic acids in basil were rosmarinic acid followed by chicoric acid and caffeic acid, with Eleonora accumulating the least polyphenols in general and Italiano Classico accumulating the most. A significant interaction between the cultivars and the nutrient solutions was noted for all phenolic acids. Eleonora showed no significant improvement in its total polyphenols content in any of the NS treatments. Moreover, the highest accumulation of caffeic acid and chicoric acid was noted in the combination Italiano Classico × 1 dS m^{-1}, whereas a combination of the same cultivar with 2 dS m^{-1} NS resulted in the highest value of rosmarinic acid (512.2 μg g^{-1} dw). However, the higher content of the less represented ferulic acid was obtained from the combination Aroma 2 × 2 dS m^{-1}. Overall, the accumulation of the polyphenols was triggered for only Aroma 2 and Italiano Classico, with different combinations, where the best combination for a higher content was revealed with Italiano Classico × 2 dS m^{-1} (804.1 μg g^{-1} dw).

Table 4. Sweet basil phenolic acids profile in light of the cultivar and the nutrient solution electrical conductivity.

Source of Variance	Caffeic Acid (μg g^{-1} dw)	Chicoric Acid (μg g^{-1} dw)	Rosmarinic Acid (μg g^{-1} dw)	Ferulic Acid (μg g^{-1} dw)	Total Polyphenols (μg g^{-1} dw)
Cultivar (C)					
Eleonora	16.10 ± 1.04 c	32.74 ± 0.64 c	14.71 ± 1.61 c	2.96 ± 0.20 c	66.50 ± 1.65 c
Aroma 2	23.57 ± 1.58 b	50.54 ± 6.99 b	151.62 ± 18.01 b	12.07 ± 2.03 a	237.76 ± 15.41 b
Italiano Classico	50.67 ± 1.43 a	205.20 ± 31.70 a	326.03 ± 54.21 a	5.67 ± 0.55 b	587.49 ± 79.71 a
Nutrient solution concentration (NS)					
1 dS m^{-1}	32.32 ± 6.14 a	121.30 ± 43.50 a	148.92 ± 45.81 b	7.67 ± 1.44 a	310.19 ± 93.99 b
2 dS m^{-1}	29.68 ± 5.04 b	115.90 ± 31.01 a	218.21 ± 75.41 a	9.09 ± 2.42 a	372.81 ± 110.81 a
3 dS m^{-1}	28.34 ± 4.92 b	51.30 ± 8.18 b	125.21 ± 31.21 b	3.94 ± 0.47 b	208.76 ± 37.33 c
C × NS					
Eleonora x 1 dS m^{-1}	12.49 ± 0.24 f	33.35 ± 0.98 d	20.09 ± 0.83 e	3.40 ± 0.18 de	69.33 ± 0.03 e
Eleonora × 2 dS m^{-1}	19.28 ± 1.07 de	32.88 ± 1.84 d	14.60 ± 1.36 e	3.10 ± 0.36 de	69.86 ± 1.85 e
Eleonora × 3 dS m^{-1}	16.52 ± 0.43 e	31.98 ± 0.22 d	9.44 ± 0.35 e	2.37 ± 0.22 e	60.31 ± 0.63 e
Aroma 2 × 1 dS m^{-1}	29.72 ± 0.65 c	35.25 ± 0.70 d	105.8 ± 2.77 d	12.68 ± 1.39 b	183.46 ± 5.33 d
Aroma 2 × 2 dS m^{-1}	20.29 ± 0.83 de	78.17 ± 3.23 c	127.72 ± 10.21 d	18.29 ± 2.02 a	244.49 ± 12.67 c
Aroma 2 × 3 dS m^{-1}	20.68 ± 0.46 d	38.19 ± 0.75 d	221.23 ± 5.43 c	5.23 ± 0.73 cde	285.34 ± 5.71 c
Italiano Classico × 1 dS m^{-1}	54.74 ± 1.08 a	295.2 ± 7.31 a	320.93 ± 34.02 b	6.92 ± 1.02 c	677.78 ± 37.34 b
Italiano Classico × 2 dS m^{-1}	49.48 ± 3.01 b	236.5 ± 7.82 b	512.23 ± 11.12 a	5.88 ± 0.87 cd	804.08 ± 8.68 a
Italiano Classico × 3 dS m^{-1}	47.80 ± 1.02 b	83.74 ± 1.62 c	144.84 ± 12.83 d	4.23 ± 0.06 cde	280.62 ± 12.34 c
Significance					
Cultivar (C)	***	***	***	***	***
Nutrient solution (NS)	**	***	***	***	***
C × NS	***	***	***	***	***

, * Significant at $p \leq 0.01$ and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). All data are expressed as mean ± se (n=3).

4. Discussion

The best production and quality performance combined with the efficient use of water and nutrients that characterize closed-loop soilless cultivation[33] have captured the attention of the entire agricultural sector. Among the various pre-harvesting factors, the genetic starting material and the nutrition management portray crucial factors in defining quality parameters[25]. Whilst the choice of genotype is dictated by the final destination of the product, hydroponics represents a valid tool, as it guarantees precise control of plant nutrition, thus offering many advantages over traditional cultivation in agricultural soil[24]. In the present experiment, the sweet basil cultivars grown in a floating system determined on average a yield about 7 times higher than the production of 38 basil cultivars grown in soil[28]. This result is mainly justified by the adopted high density (317 m^{-2}), which features cultivation of basil on floating panels[34,35]. Although numerous studies underlined how the composition and concentration of the nutrient solution affects growth and yield[36–38], in this case, the average nutrient solution effect did not lead to differences in fresh production, leaf number and dry matter percentage. It can be partly attributed to the moderate salinity tolerance of basil[39,40] but, above all, to the short growing cycle (34 DAT), which minimizes the influence of macronutrients concentrations in the solution on growth parameters[26]. These results are also confirmed by the Fv to Fm ratio that showed a similar efficiency of the PSII system for all the applied treatments[41] without any evidence of stress or deficiency. This confirms the light significant differences in yield and growth parameters between the treatments. On the contrary, the cultivar mean effect clearly influenced all the measured morphometric parameters, underlining again the key role played by genetics. In particular, Aroma 2 was characterized by a higher percentage of dry matter and a lower leaf/stem ratio (in weight) resulting from a higher production of leaves per plant regardless of the nutrient solution. The latter two aspects represent technological characteristics greatly sought after by the agro-industry, as the excessive presence of the stem could increase the processing time due to its greater fibrousness, thus determining an early blackening of "pesto."[2] Identically to the observation of Raimondi et al.[42], an interaction between the genetic background of the different cultivars and the different concentrations of the nutrient solution was obvious, specifically in the case of fresh and dry biomass. Each cultivar achieved the best production with a specific nutrient solution, highlighting how tolerance to the concentration of salts in the nutrient solution is highly dependent on the genotype[39,43]. The use of nutrient solution 2 dS m^{-1} determined on average an increase of Aroma 2 fresh biomass of about 30% compared to those recorded with the other two nutrient solutions, confirming how the yield is negatively affected by nutrient solutions that are too concentrated and/or too diluted[44]. On the contrary, the Eleonora and Italiano Classico cultivars reached the highest production of fresh and dry biomass when grown under sub- and supraoptimal nutrient conditions, at 1 and 3 dS m^{-1}, respectively.

On the other hand, colour is an important quality characteristic for leafy vegetables as it influences consumers' acceptability and preferences[45,46]. The evaluation of colorimetric parameters as well as the SPAD index revealed a strong influence by the cultivar. The leaves of Aroma 2 were characterized by a lower brightness (L*) and intensity of yellow (b*) but higher SPAD values. Instead, Italiano Classico had a higher intensity of green (a*) in absolute. The same parameters were also influenced by the concentration of macronutrients in nutrient solution as also observed by Raimondi et al.[42] and by Walters and Currey[38]. It is noteworthy that the increase of the nutrient solution concentration, independent from the cultivar effect, determined a simultaneous increase of SPAD values and a decrease of the yellow intensity (b*). These results confirm what was observed by Fallovo et al.[44], where low concentrations of nutrients reduce the content of chlorophyll with subsequent yellowing (b*) due to lower levels of N that, in this case, characterized the most diluted nutrient solution (1 dS m^{-1}). The interaction between the two considered factors defined significant differences only for SPAD values. SPAD is a nondestructive tool widely used to indirectly monitor the chlorophyll content of leaves[47,48]. The higher SPAD values obtained from the combination Aroma 2 × 2 dS m^{-1} could explain the higher production resulting from this combination.

On the other hand, a relevant quality aspect in an aromatic plant is certainly the composition of its aromatic profile, which indiscriminately defines its taste. The flavour of basil is mainly owed to the presence of odorous molecules produced and stored in peeled glandular trichomes located in the aerial parts of the plant[15]. The aromatic compounds characteristic of sweet basil belongs to two distinct groups: terpenes and phenylpropenes that are synthesized by two different metabolic pathways[49]. As observed by several authors[15,18,50], the cultivars Eleonora and Aroma 2, used for the industrial production of "Pesto Genovese", were characterized by a higher presence of linalool, oxygenated monoterpene that contributes to the good quality of the essential oil and therefore of the famous Italian sauce[5]. Nevertheless, Italiano Classico was characterized by a dominant presence of eucalyptol, a metabolite extremely interesting for its biological activity thanks to its wide use in the pharmaceutical field[18,51]. However, it was never indicated as a predominant aromatic compound of the Genovese type. However, considering that the two molecules linalool and eucalyptol have the same precursor (geranyl pyrophosphate (GPP)), the conversion to linalool and eucalyptol is mediated by specific enzymes (linalool synthase (LIS) and 1,8-cineol synthase), as suggested by Chang et al.[52], but could also be influenced in a genotype × environment interaction, since the different enzymatic activities are highly sensitive to environmental conditions[53]. In addition to the genetic and environmental component, plant nutrition is to be considered a further liable factor to quanti-qualitatively modify the aromatic profile[54]. The different concentrations of macronutrients in nutrient solution

significantly influenced the content of trans-2-hexenal, α-bergamotene and eugenol. The latter aromatic compound is associated with sensory quality[5] and has a recognized antioxidant power[55]. As observed by Nurzyńska-Wierdak and Borowski[56], the content of this relevant aromatic molecule especially in Italiano Classico was negatively affected by the increased presence of the macrocations, whereas for Eleonora, the use of more concentrated nutrient solutions determined a linear increase of α-bergamotene. The greater biosynthesis of this sesquiterpene typical of sweet basil[57] could be related to the greater availability of potassium, which as always observed by Nurzyńska-Wierdak and Borowski[56], and Singh et al.[58] determined in *Ocimum basilicum* L. a greater production of aromatic molecules belonging to this chemical class.

The growing interest in this aromatic plant beyond the culinary field is mainly attributed to the high presence of a multitude of phenolic compounds considered as powerful antioxidants[9,59–61]. Phenolic compounds are secondary metabolites produced by plants through the shikimate pathway[62] and facilitate adaptation of the plant to the surrounding environment[58]. The content of phenolic composition, as shown by our results, is strongly influenced by the genetic aspect[59,63,64]. Regardless of the nutrient solution effect, Italiano Classico compared to Aroma 2 and Eleonora was characterized by higher total polyphenol concentrations of 147% and 783%, respectively. The phenolic profile of Italiano and Aroma 2 is in line with that reported in the literature[3,60,65], where a high content of rosmarinic acid is registered. This latter acid is an ester of caffeic acid, synthesized from the amino acids L-tyrosine and L-phenylalanine[62,66]. Rosmarinic acid is a characteristic secondary metabolite of sweet basil to which is attributed a high antioxidant capacity[67]. In general, the average concentration of rosmarinic acid of the two basil cultivars (Aroma 2 and Italiano Classico) grown in floating was lower than that obtained by Kiferle et al.[23] and Javanmardi et al.[60] but higher than that recorded by Sgherri et al.[10]. These results, besides underlining the influence of the genotype, may have been due to the different extraction methods and solvents used for the determination of such a phenolic acid[67] but, most importantly, to the different growth conditions. In this specific case, the lower production of phenolic compounds compared to basil grown in the open field[60] could be related to the different methods of cultivation. In fact, the hydroponic cultivation optimizing the growing conditions reduces the possibility of oxidative stress[10] and, therefore, the biosynthesis and the accumulation of phenolic compounds that are actively involved in neutralization of the free radicals formed just under conditions of oxidative stress[30]. Although rosmarinic acid is constantly defined as the most represented phenolic acid in basil[3,4,60,67], the results showed that the phenolic profile of Eleonora was characterized by a dominant presence of chicoric acid. The influence of the genotype on the major biosynthesis of this phenolic acid is confirmed by the work of Kwee et al.[68], where nine varieties of basil were identified with an absolute higher content of chicoric acid. The use of nutrient solutions

with different concentrations of macronutrients determined significant differences for all the evaluated phenolic compounds, as nutritional eustress induces physiological responses and molecular mechanisms that cause accumulation or decrease of bioactive compounds needed by the plant to adapt to suboptimal conditions[69]. In line with previous work on both basil[3,70,71] and other vegetables of interest[25,69], the nutrient limitation, regardless of the cultivar effect, led to higher production of caffeic acid and chicoric acid. These results are not surprising since adverse conditions such as low nutrient levels, particularly N, intensify the activity of phenylalanine ammonia-lyase (PAL) and other enzymes regulating the biosynthesis of phenolic compounds[72,73]. In addition, these suboptimal nutrient conditions limit growth without blocking photosynthetic activity, resulting in excessive production of carbohydrates that could be partly converted into C-based secondary metabolites such as phenols[3]. Nevertheless, it should be noted that the use of the more diluted solution (1 dS m^{-1}) did not increase the production of rosmarinic acid and ferulic acid, since as observed by Heimler et al.[73], the increase of secondary metabolites in conditions of nutritional deficit does not involve in general all phenolic compounds. Additionally, as observed by Jakovljević et al.[70], the decrease in the concentration of macroelements in the nutrient solution has not resulted in a greater accumulation of phenolic compounds for all cultivars, confirming how the availability of nutrients modulates the phenolic content in a genotype-dependent way[69,74]. That said, it is interesting to note that, for Italiano classic, the use of 2 dS m^{-1} NS has simultaneously reduced biomass production but increased the total polyphenols content, particularly affecting the concentration of rosmarinic acid. This result partially confirms how the accumulation of secondary metabolites could lead to a slower growth rate[62,75].

5. Conclusions

The challenge imposed on the agricultural sector to provide nourishment to a growing population has led to alternative production techniques such as hydroponics. However, the urgent need to reduce chemical inputs in alternative cropping systems has paved the way for biostimulants, which currently represent an environmentally sustainable strategy for horticultural production. Under the experimental conditions of our study, the varietal comparison showed that 'Eleonora' provided the highest fresh yield (6576.81 g m^{-2}). At the same time, 'Italiano Classico' had the highest total phenol concentration (1590.04 μg g^{-1} dw). The use of NS with different concentrations did not result in significant differences in fresh yield, regardless of the cultivar, but positively impacted the aroma and phenolic profile. Specifically, the HS increased total phenols by 32.5%, compared to the FS that ensured the highest content of eucalyptol (22.0%) and linalool (53.4%). The application of biostimulants in the NS increased all biometric parameters (such as the number of leaves, fresh and dry yield) and the linalool content proportionally to the dose used, while the highest total phenol concentration was

obtained from the lowest dose ($B_{0.15}$). Based on the excellent results achieved, the application of biostimulants in NS turned out to be a valid strategy to reduce chemical input. For this reason, it should also be investigated on other leafy crops to define a new production technique that can improve both yield and quality.

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Chapter 8

Biostimulatory Action of Vegetal Protein Hydrolysates Compensates for Reduced Strength Nutrient Supply in a Floating Raft System by Enhancing Performance and Qualitative Features of 'Genovese' Basil

Abstract: The floating raft constitutes a valuable system for growing herbs as it effectuates high yield and prime functional quality. However, the pressing need for advancing sustainability in food production dictates the reduction of chemical fertilizer inputs in such intensive production schemes through innovative cultivation practices. In this perspective, our work appraised the productive and qualitative responses of two 'Genovese' basil genotypes (Eleonora and Italiano Classico) grown in a floating raft system with nutrient solutions of varied electrical conductivity (EC; 2 and 1 dS m^{-1}) combined with root application of protein hydrolysate biostimulant at two dosages (0.15 and 0.3 0 ml L^{-1} of Trainer®). The phenolic composition, aromatic profile and antioxidant activities (ABTS, DPPH, and FRAP) of basil were determined by UHPLC/HRMS, GC/MS, and spectrophotometry, respectively. 'Eleonora' demonstrated higher number of leaves (37.04 leaves per plant), higher fresh yield (6576.81 g m^{-2}) but lower polyphenol concentration (1440.81 μg g^{-1} dry weight) compared to 'Italiano Classico'. The lower EC solution (1 dS m^{-1}) increased total phenols (+32.5%), ABTS, DPPH, and FRAP antioxidant activities by 33.2, 17.1 and 15.8%, respectively, and decreased linalool relative abundance by 5.5%. Biostimulant application improved crop performance and increased total phenolic concentration in both genotypes, with the highest phenolic concentration (1767.96 μg g^{-1} dry weight) registered at the lowest dose. Significant response in terms of aromatic profile was detected only in 'Eleonora'. Our results demonstrate that the application of protein hydrolysate may compensate for reduced strength nutrient solution by enhancing yield and functional quality attributes of 'Genovese' basil for pesto.

Keywords: *Ocimum basilicum* L.; Biostimulants; hydroponic; Nutrient solution concentration; Volatiles; Phenolics; Antioxidant activities; UHPLC/HRMS

1. Introduction

The ongoing quest for a healthy lifestyle and modern-day awareness exemplified in "*we are what we eat*" usher consumers to dietary schemes characterized by regular consumption of fruits and vegetables. The association between high consumption of healthy foods and low incidence of chronic disorders is attributed to the beneficial effects of the phytochemical antioxidants typical of plants[1]. Phytochemicals are classified into three groups according to their metabolic pathway: phenylpropanoids, alkaloids, and terpenoids[2]. Better known as secondary metabolites, these biomolecules, crucial in defense and functional environment-plant interaction[3], have always been a natural and indispensable resource for cosmetic, pharmaceutical, and agri-food industries[4,5]. Minor plant species, such as herbs, due to a heterogeneous and not fully explored reservoir of secondary metabolites, have rekindled the interest of both consumers and academics[6,7]. Basil (*Ocimum basilicum* L., *Lamiaceae*) is an irreplaceable ingredient for traditional Italian dishes ('pesto' and pizza 'Margherita') and the pharma-cosmetic sector[4,8,9] due to the biosynthesis of low molecular weight organic compounds (i.e., monoterpenes and phenylpropanoids), responsible for its distinctive aroma[10]. On the other hand, the outstanding nutraceutical value of basil is mainly attributable to a heterogeneous phenolic profile (rosmarinic acid, chicoric acid, caffeic acid, p-coumaric acid) that, like aroma, is strongly affected by the interaction between genotype and environment[4,11]. To date, phenolic compounds have become among the most investigated natural molecules[10]. In addition to having a considerable impact on quality attributes (flavor and color), they possess antioxidant, antifungal, and antimicrobial properties, such as being considered multitarget drugs with potential applications in the agri-food sector as surrogates for artificial preservatives[12]. Increasingly extreme environmental conditions combined with the demand for high-quality agricultural production have led the growers to alternative cropping systems[1,6,9]. In this context, the soilless growing systems is a viable strategy for the conversion and redevelopment of abandoned urban and peri-urban areas to full-scale green farms[13]. Among hydroponic systems, the floating system is undoubtedly the one that best lends itself to the production of aromatic herbs such as basil[9,14]. This growing system, in addition to being more economically sustainable (lower production and set-up costs), would guarantee, in line with today's market demands, a higher production all year round with standardized characteristics[15–18]. In addition, the potential to control and manipulate the composition of the nutrient solution (NS) would positively change secondary metabolism by enhancing the phytochemical properties of grown horticultural products[16,18–21]. The unclear effects of dilute NS (nutrient stresses) on leafy vegetable yield parameters[2,14,22,23] have prompted growers to use concentrated NS that exceed crop needs[24]. Consistent with the guidelines of the European Commission, the need to reduce chemical input while improving yield and quality in intensive production environments has prompted the agricultural sector to become

increasingly interested in biostimulants[1,25,26]. The combination of hydroponic and biostimulants appears to be a promising ecological strategy for controlled environment production of high-quality vegetables. Colla et al.[27] reported that plant protein hydrolysates (PH's) are innovative strategies to address the above challenges. Recent work by Rouphael et al.[28] pointed out that the use of PH's by foliar application improved the production performance of 'Genovese' basil in protected cultures. The effectiveness of these natural products (derived from agricultural by-products) is also confirmed in the work of Caruso et al.[29] and Cristofano et al.[30] on arugula (*Eruca sativa* Mill.) and lettuce (*Lactuca sativa* L.), respectively. These results are attributable to bioactive molecules (amino acids, signaling peptides) that exert a plethora of physiological and growth effects on plants while inducing up-regulation of increasingly sought-after secondary metabolites[29,31,32]. The possibility of sustainably increasing resource use efficiency[28] by partially ameliorating environmental drawbacks associated with overfertilization makes the application of PH's in floating raft systems (FRS) even more attractive. The complete absence of interactions between the roots and the agricultural soil makes the FRS suitable for studying in vivo the real plant responses to biostimulant integration[33]. To date, there is a lack of information in the literature on the application mode recommended for this cropping system. However, considering that PH's improve the uptake, assimilation, and translocation of nutrients through modifications of the root system, the possibility of applying the biostimulant directly in contact with the root system (in NS) could further enhance its potential. The benefits of biostimulus on production and quality in a soilless superintensive system could be an effective tool to reduce chemical fertilizer inputs, and to improve economic and environmental sustainability. Our work aimed to evaluate the use of a PH in a NS at two different doses to assess the effects on the production performance and quality of two Genovese basil genotypes (Eleonora and Italiano Classico) grown in a FRS with two different nutrient concentrations (1 and 2 dS m^{-1}).

2. Materials and Methods

2.1. Plant Material, Experimental Design, and Growth Conditions

The experimental trial was carried out in a passive ventilation greenhouse at the experimental site of the Federico II University of Naples - Department of Agriculture (DIA) located in Portici (Naples, Italy; lat. 40°51'N, long. 14°34'E; 60 m above sea level), during the summer growing season in 2020. The experimental design was trifactorial in which two 'Genovese' basil (*Ocimum basilicum* L.) genotypes [(1) Eleonora, Enza Zaden, Enkhuizen, Noord-Holland, The Netherlands: erect stem, large green, slightly serrated leaves; suitable for open field cultivation; intermediate resistance to *Peronospora belbahrii* and (2) Italiano Classico, La Semiorto Sementi, Sarno, SA, Italy; erect stem, medium height with bright green, slightly blistered "spoon" leaves] were grown in a FRS with

two different concentrations of NS (1 dS m^{-1}-Half Strength and 2 dS m^{-1}-Full Strength) and two doses of biostimulants (0.15 and 0.30 ml L^{-1}) plus an untreated control (hereafter $B_{0.15}$, $B_{0.30}$, and Control, respectively). The treatments were performed in triplicate and arranged in a completely randomized block design. On 9 June (18 days after sowing), at the phenological stage of 2-3 true leaves, basil seedlings were transplanted into 54-hole polystyrene trays (52 × 32 × 6 cm; upper hole diameter: 4.5 cm; bottom hole diameter 3 cm; volume: 0.06 L) at a density of 317 plants m^{-2}. The experimental design comprised 36 experimental units, each consisting of a 54-hole tray floating in a 40-liter tank filled with 35 L of NS. The oxygenation of the NS was provided by a submersible pump (Aquaball 60, Eheim, Stuttgart, Germany).

2.2. Nutrient Solutions Management, Biostimulant Application, and Harvest

The NS (half strength and full strength) were prepared from osmosis water. The half strength NS was obtained by halving the macronutrient concentration of the full-strength stock NS (14.0 mM nitrate, 1.5 mM phosphorus, 3.0 mM potassium, 1.75 mM sulfur, 4.5 mM calcium, 1.5 mM magnesium, and 1.0 mM ammonium). Micronutrient concentrations were for both solutions 15 μM iron, 9 μM manganese, 0.3 μM copper, 1.6 μM zinc, 20 μM boron, and 0.3 μM molybdenum. During the trial, the pH was continuously monitored and maintained at values of 5.8 ± 0.2. At transplanting, a legume PH (Trainer®) was applied to the NS at two different doses (0.15 ml L^{-1} and 0.30 ml L^{-1}). The biostimulant used, which was free of plant hormones[34,35], contained soluble peptides and amino acids such as Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, which comprised 5% of the total nitrogen content along with soluble sugars and phenols. At the end of the experiment, 25 plants per experimental unit were sampled to determine biometric parameters such as the number of leaves per plant and fresh yield. The harvested plant material was then placed in a ventilated oven at 60 °C until a constant weight was reached to determine the dry yield and the percentage of dry matter (DM = 100 × dry weight/fresh weight). Instead, a homogeneous pool of 20 plants per experimental unit was sampled and placed immediately at −80 °C for future qualitative analysis. A plant material sample was freeze-dried (Alpha 1-4, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and finely ground with a KM13 rotating blade grinder (Bosch, Gerlingen, Germany).

2.3. CIELab Color Space Determination

At harvest, color coordinates were recorded on the adaxial surface of ten healthy and fully expanded leaves per experimental unit using a Minolta Chromameter CR-400 portable colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). As described by the International Commission on Illumination (CIE), the color was expressed by L, a*, and b* coordinates by which the Chroma and Hue angle were determined as follows:

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{0.5}$$
$$\text{Hue angle} = \tan^{-1} b^*/a^*$$

2.4. Determination of ABTS, DPPH, and FRAP Antioxidant Activities

The antioxidants activities were determined following the protocols described by Graziani et al.[36]. For the determination of ABTS antioxidant activity a stock solution was prepared by mixing 44 ml of potassium persulfate (2.45 mM) with 2.50 ml of aqueous solution (7 mM) of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate radical ($ABTS^+$) and placed at 20 °C (room temperature) for 12 h. The ABTS solution was diluted (1:88) with ethanol until it reached an absorbance of 0.700 ± 0.005 at 734 nm. After that, a 1 ml aliquot of ABTS solution was added to 100 ml of the filtered sample and incubated at room temperature for 2.5 min. For the antioxidant 2,2-diphenyl-1-picryl-hydrazyl (DPPH) activity, 1 ml of methanolic solution of DPPH 100 µM (absorbance of 0.90 ± 0.02 at 517 nm) was added to 0.2 ml of diluted leaf extract and incubated at room temperature for 10 min. For ferric reduction/antioxidant power (FRAP) antioxidant activity determination a FRAP reagent was prepared by mixing 1.25 ml of 2,4,6-triridyl-striazine (TPTZ; 10 mM) in 40 mM Hydrochloric acid, 1.25 ml of 20 mmol ferric chloride in water, and 12.5 ml of 0.3 M sodium acetate (pH 3.6). An aliquot of 2.850 ml of FRAP reagent was added to 0.015 ml of leaf extract and incubated at room temperature for 4 min. The absorbances of the ABTS, DPPH, and FRAP assays were measured with a UV-VIS spectrophotometer (Shimadzu, Japan) at 734, 517, and 593 nm, respectively. Results were expressed as mmol Trolox equivalents kg^{-1} dry weight (dw) of the sample. All determinations were made in triplicate.

2.5. Determination of the Polyphenol Profile by Ultra-High Performance Liquid Chromatography (UHPLC) and Orbitrap High-Resolution Mass Spectrometry (HRMS) Analysis

2.5.1. Extraction of Polyphenolic Compounds

Polyphenolic compounds were extracted as described by Corrado et al.[37]. Briefly, 0.1 g of finely ground and freeze-dried leaves were extracted in 5 ml of an aqueous methanol solution (60:40, *v*/*v*). Then, the obtained solution was sonicated and centrifuged at 4,000 rpm for 15 min, and 0.05 ml of supernatant was collected, filtered, and analyzed.

2.5.2. Quantification of Phenolic Compounds

Quantification and separation of phenolic compounds were performed by UltraHigh-Pressure Liquid Chromatography (Dionex UltiMate 3000 UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) coupled to the Q Exactive Orbitrap LC-MS/MS Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) as described by El-

Nakhel et al.[38]. The polyphenols were separated by using a Luna Omega PS (1.6 m, 50 × 2.1 mm, Phenomenex, Torrance, CA, USA) at 25 ° C. The mobile phase was a two-phase solution containing water (phase A) and acetonitrile (phase B). Both mobile phases contained 0.1% formic acid (*v*/*v*). Polyphenolic compounds were eluted using the following gradient schedule: 0-1.3 min 5% B, 1.3-9.3 min 5-100% B, 9.3-11.3 min 100% B, 11.3-13.3 min 100-5% B, 13.3-20 min 5% B. The flow rate was 0.2 ml min^{-1}. For all compounds of interest, an ESI source (Thermo Fisher Scientific, Waltham, MA, USA) was used in negative ion mode, with Full ion (MS) and All ion fragmentation (AIF) scanning events. Data acquisition and processing were performed with Quan/Qual Browser Xcalibur software, v. 3.1.66.10 (Xcalibur, Thermo Fisher Scientific, (Thermo Fisher Scientific, Waltham, MA, USA). Polyphenols were expressed as μg g^{-1} dw.

2.5.3. Determination of Volatile Compounds

The extraction and quantification of volatile compounds (VOCs) was performed by gas chromatography combined with the mass spectrometer technique (GC/MS) after solid phase microextraction (SPME), as described in detail by Ciriello et al.[9].

2.5.4. Extraction of Volatile Compounds by the SPME Technique

An aliquot of 0.5 g of frozen sample was placed in glass vials with a screw cap and placed on a heated stirrer (30 °C for 10 min) to facilitate the migration of volatile compounds into the headspace. The adsorption of VOCs was performed by introducing a divinylbenzene/carboxane/polydimethylsiloxane fiber (1 cm long and 50/30 μm thick; Supelco® (Bellefonte, PA, USA) into the headspace for 10 min.

2.5.5. Extraction of Volatile Compounds by the SPME Technique

SPME fiber containing the adsorbed analytes was introduced into the split-splitless injector of the gas chromatograph (GC 6890N; Agilent, Santa Clara, CA, USA) coupled to the mass spectrometer (MS 5973N; Agilent, Santa Clara, California, USA). The thermal desorption of the analytes occurred at 250 ° C for 10 min. The oven temperature was maintained at 50 °C for 2 min and increased from 50 °C to 150 °C at 10 °C/min and from 150 °C to 280 °C at 15 °C/min. The injection and ion source temperatures were 250 °C and 230 °C, respectively, and helium (99.999%) was used as a carrier gas with a flow rate of 1 ml min^{-1}. The gas chromatograph was equipped with a capillary column (30 m × 0.250 mm) coated with a 0.25 μm 5% diphenyl/95% dimethylpolysiloxane film (Supelco®, Bellefonte, PA, USA). The mass spectrometer was set at 70 eV. Identification of VOCs identification was performed using the National Institute of Standards and Technology (NIST) Atomic Spectra Database version 1.6 (U.S. Department of Commerce, Gaithersburg, Maryland, United States).

2.6. Statistics

The experiment consisted of a randomized block design with three factors: Cultivar-CV, Biostimulant-B, and Nutrient Solution Concentration-NSC. Analysis of variance (ANOVA) was conducted for the main effects and their interactions. In the absence of significant interactions, significant main effects for factors applied at only two levels (CV and NSC) also denote significant differences between the two means. In the case of significant two-way interactions (CV × B, B × NSC, and CV × NSC), interaction means were compared using the Tukey-Kramer HSD test with statistical significance determined at the $p < 0.05$ level. All data are presented as mean ± standard error. Statistical analysis was performed using IBM SPSS 20 (Armonk, NY, USA) package for Microsoft Windows 10. Statistical processing was performed using IBM SPSS 20 (Armonk, NY, USA) package for Microsoft Windows 10.

3. Results

3.1. Yield and Yield Parameters

Regarding the main yield parameters, the cultivar factor significantly influenced all the parameters reported in **Table 1**, except the dry yield. Although 'Eleonora' had the highest number of leaves and the highest fresh yield, 'Italiano Classico' was characterized by a higher percentage of dry matter. Biostimulant treatment significantly influenced all yield parameters compared to the Nutrient Solution Concentration that affected dry yield and dry matter (**Table 1**).

Table 1. Analysis of variance and mean comparisons for leaf number, fresh yield, dry yield, and dry matter of Eleonora and Italiano Classico genotypes grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®].

Treatment	Leaf number No. plant^{-1}	Fresh Yield (g m^{-2})	Dry Yield (g m^{-2})	Dry matter (%)
Cultivar (CV)				
Eleonora	37.04±0.35	6576.81±126.42	486.40±10.47	7.40±0.06
Italiano Classico	31.34±0.64	6232.70±124.58	481.99±11.89	7.76±0.05
Biostimulant (B)				
Control	31.86±1.15c	5863.99±69.31c	434.65±5.43c	7.40±0.06c
$B_{0.15}$	34.49±0.88b	6365.64±99.00b	490.34±6.12b	7.78±0.05a
$B_{0.30}$	36.21±0.71a	6984.64±95.94a	527.60±10.88a	7.57±0.10b
Nutrient Solution Concentration (NSC)				
Half Strength (HS)	34.33±0.82	6406.93±97.33	479.18±7.47	7.48±0.05
Full Strength (FS)	34.05±0.91	6402.59±159.72	489.21±13.88	7.68±0.08
CV × B				
Eleonora × Control	35.46±0.44bc	6016.81±102.01	435.60±9.8d	7.22±0.03d
Eleonora × $B_{0.15}$	37.23±0.28ab	6601.58±120.67	503.25±6.46bc	7.66±0.08bc
Eleonora × $B_{0.30}$	38.43±0.32a	7112.04±133.79	520.34±13.32ab	7.32±0.06d
Italiano Classico × Control	28.27±0.68f	5711.17±37.28	433.70±5.77d	7.58±0.05c
Italiano Classico × $B_{0.15}$	31.74±0.55d	6129.71±79.32	477.42±7.49c	7.89±0.04a
Italiano Classico × $B_{0.30}$	34.00±0.35c	6857.24±126.91	534.86±17.95a	7.81±0.11ab
B ×NSC				
Control × HS	32.40±1.82	5979.71±119.42cd	442.53±7.57c	7.38±0.06c
$B_{0.15}$ × HS	34.58±0.99	6525.28±136.88b	500.34±8.59b	7.67±0.07b
$B_{0.30}$ × HS	36.01±1.11	6715.79±72.40b	494.67±5.69b	7.40±0.09c
Control × FS	31.33±1.55	5748.27±38.99d	426.76±6.88c	7.42±0.12c
$B_{0.15}$ × FS	34.39±1.56	6206.01±119.12c	480.33±7.13b	7.88±0.06a
$B_{0.30}$ × FS	36.42±0.96	7253.49±79.66a	560.53±7.39a	7.74±0.14ab

Cont. **Table 1**

Treatment	Leaf number No. plant^{-1}	Fresh Yield (g m^{-2})	Dry Yield (g m^{-2})	Dry matter (%)
CV × NSC				
Eleonora × HS	37.15±0.36	6624.76±102.91	487.73±9.05ab	7.34±0.05d
Eleonora × FS	36.92±0.63	6528.87±238.23	485.06±19.59ab	7.46±0.10c
Italiano Classico × HS	31.50±0.85	6189.10±133.44	470.63±11.72b	7.63±0.05b
Italiano Classico × FS	31.17±1.01	6276.31±218.37	493.35±20.76a	7.89±0.06a
Significance				
CV	***	***	**ns**	***
B	***	***	***	***
NSC	**ns**	**ns**	*	***
CV× B	**	**ns**	**	**
B × NSC	**ns**	***	***	**
CV × NSC	**ns**	**ns**	*	*

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

Biostimulant treatment showed a linear increase in leaf number, fresh yield, and dry yield as a function of the dose used (Control > $B_{0.15}$ > $B_{0.30}$), in contrast to dry matter, which showed the highest value at $B_{0.15}$. Regarding the CV × B interaction for both 'Eleonora' and 'Italiano Classico', the $B_{0.30}$ dose determined, compared to the Control, an average increase of 13.65 and 21.38% in the number of leaves and dry yield, respectively. The dry matter did not show the same trend since, in 'Eleonora', the highest value was obtained at $B_{0.15}$, while in 'Italiano Classico', the highest values were obtained at $B_{0.15}$ and $B_{0.30}$. The CV × B interaction did not influence fresh yield that was significantly influenced by the B × NSC interaction (**Figure 1**).

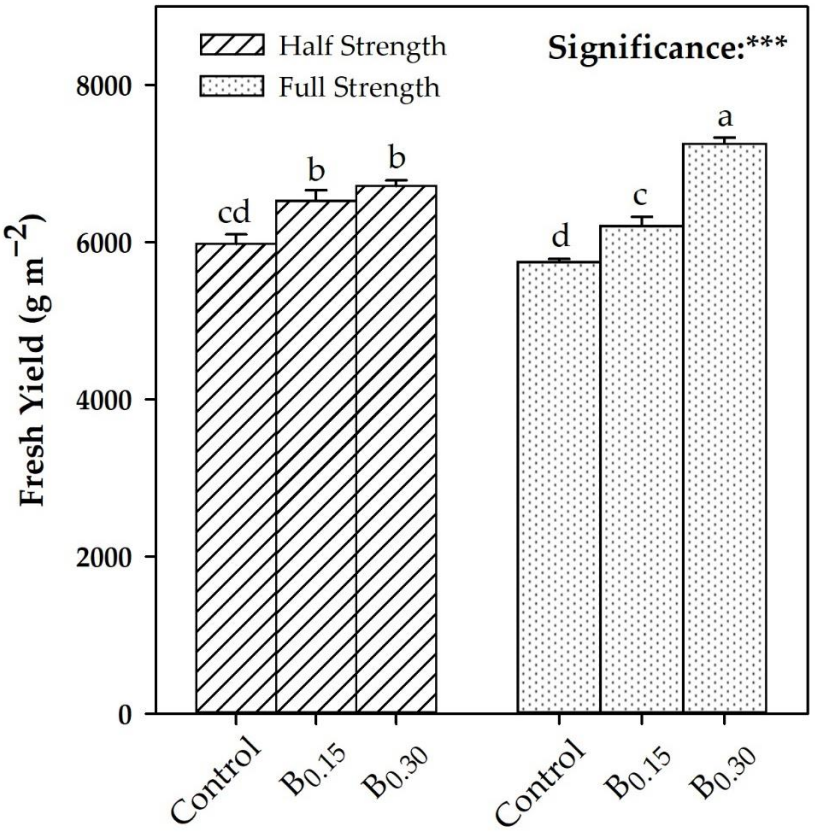

Figure 1. Effects of Biostimulant × Nutrient Solution Concentration interaction for fresh yield [Nutrient solution concentration treatments: Half strength and Full Strength; biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®]. Different letters denote significant differences ($p < 0.05$) according to Tukey–Kramer HSD test. ***Significant effect at the 0.001 level.

Regardless of the NSC, the use of the biostimulant increase fresh yield (**Figure 1**) and dry yield (**Table 1**). However, for the full-strength solution (FS; 2 dS m^{-1}), a linear increase of the above two parameters was observed as the concentration of the biostimulant increased. The highest dry matter values were obtained at $B_{0.15}$ for both half strength [HS;1 dS m^{-1}; (7.67%)] and FS (7.88%) nutrient solutions. On the contrary, the highest values were already observed at $B_{0.15}$ for the HS nutrient solution, which did not show significant differences compared to the $B_{0.30}$ dose. The CV × NSC interaction showed significant differences only for dry yield and dry matter. In 'Italiano Classico', the use of the FS increase by 4.82% and 3.40% dry yield and dry matter, respectively. The same trend was observed in 'Eleonora' only for dry matter (+1.60%).

3.2. CIELab colorimetric parameters

Except for the Hue angle, significant differences were observed between genotypes for leaf colorimetric characteristics (**Table 2**). 'Italiano Classico' showed the highest values of L, a*, b*, and Chroma. Greenness (a*) was the only parameter influenced by the biostimulant, with the highest value obtained at $B_{0.30}$. The different NSC influenced the colorimetric parameters L, a*, and Hue angle, as opposed to b* and Chroma. The latter were significantly affected by the CV × B and CV × NSC interactions (**Table 2**). The CV × NSC interaction also influenced the a* and Hue angle parameters. The biostimulants in the HS solutions did not affect a* and Hue angle, compared to the FS nutrient solution, where the $B_{0.15}$ and $B_{0.30}$ doses increased these parameters, compared to the Control. The CV × NSC interaction showed significant differences only for the parameters L and b*. In both 'Eleonora' and 'Italiano Classico', the use of HS increased L; the opposite trend was observed for the b* in Eleonora, while no significant differences were registered in 'Italiano Classico'.

Table 2. Analysis of variance and mean comparisons for CIELab colorimetric parameters of Eleonora and Italiano Classico genotypes grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®].

Treatment	L*	a*	b*	Chroma	Hue angle
Cultivar (CV)					
Eleonora	44.99±0.15	–6.92±0.14	15.97±0.17	17.04±0.16	112.69±0.11
Italiano Classico	45.36±0.10	–7.17±0.12	16.54±0.12	18.09±0.18	112.52±0.18
Biostimulant (B)					
Control	45.15±0.17	–6.82±0.06b	16.38±0.19	17.83±0.26	112.51±0.14
$B_{0.15}$	45.32±0.14	–7.04±0.21ab	16.35±0.23	17.40±0.17	112.61±0.21
$B_{0.30}$	45.06±0.18	–7.28±0.15a	16.04±0.16	17.47±0.33	112.70±0.19
Nutrient Solution Concentration (NSC)					
Half Strength (HS)	45.57±0.06	–6.73±0.08	16.17±0.16	17.45±0.18	112.41±0.15
Full Strength (FS)	44.78±0.11	–7.36±0.13	16.34±0.16	17.68±0.24	112.80±0.13
CV × B					
Eleonora × Control	44.93±0.28	–6.72±0.08	15.90±0.16bc	17.39±0.18c	112.53±0.14
Eleonora × $B_{0.15}$	45.18±0.26	–6.92±0.33	16.33±0.45ab	17.21±0.31cd	112.57±0.24
Eleonora × $B_{0.30}$	44.86±0.25	–7.13±0.25	15.67±0.14c	16.51±0.24d	112.96±0.11
Italiano Classico × Control	45.37±0.16	–6.91±0.07	16.85±0.22a	18.27±0.44ab	112.49±0.26
Italiano Classico × $B_{0.15}$	45.46±0.09	–7.16±0.29	16.37±0.18ab	17.58±0.10bc	112.65±0.36
Italiano Classico × $B_{0.30}$	45.27±0.24	–7.43±0.17	16.41±0.19ab	18.43±0.22a	112.43±0.35

Cont. **Table 2**

Treatment	L*	a*	b*	Chroma	Hue angle
B × NSC					
Control × HS	45.56±0.14	–6.88±0.08b	16.61±0.23ab	17.61±0.19ab	112.84±0.14ab
$B_{0.15}$ × HS	45.59±0.09	–6.41±0.08b	15.74±0.18c	16.99±0.18b	112.05±0.19c
$B_{0.30}$ × HS	45.57±0.12	–6.91±0.15b	16.17±0.31bc	17.76±0.46ab	112.32±0.31bc
Control × FS	44.73±0.19	–6.75±0.08b	16.14±0.30bc	18.04±0.50a	112.18±0.16c
$B_{0.15}$ × FS	45.06±0.21	–7.67±0.18a	16.96±0.23a	17.81±0.14ab	113.16±0.16a
$B_{0.30}$ × FS	44.56±0.15	–7.65±0.15a	15.91±0.10c	17.18±0.48ab	113.07±0.09a
CV × NSC					
Eleonora × HS	45.53±0.10a	–6.54±0.09	15.71±0.15b	17.06±0.21	112.61±0.17
Eleonora × FS	44.46±0.10c	–7.30±0.19	16.23±0.28a	17.01±0.27	112.76±0.13
Italiano Classico × HS	45.62±0.08a	–6.92±0.11	16.64±0.17a	17.85±0.25	112.21±0.23
Italiano Classico × FS	45.11±0.13b	–7.41±0.19	16.45±0.17a	18.34±0.25	112.84±0.24
Significance					
CV	***	*	***	***	ns
B	ns	**	ns	ns	ns
NSC	***	***	ns	ns	**
CV× B	ns	ns	*	***	ns
B × NSC	ns	***	***	**	***
CV × NSC	**	ns	**	ns	ns

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD.

3.2. Phenolic Acids

The total phenols were influenced by the factors under investigation and their mutual interactions (**Table 3**). Chicoric acid was the predominant compound, followed by feruloyl tartaric acid, salvianolic acid K, rosmarinic acid, caftaric acid, salvianolic acid L, and chlorogenic acid. 'Italiano Classico' showed the highest content of chicoric acid, salvianolic acid K, rosmarinic acid, salvianolic acid L, and chlorogenic acid, while 'Eleonora' showed the highest concentration of feruloyl tartaric acid. B and NSC treatments significantly affected the entire phenolic profile (**Table 3**). Specifically, the biostimulant at $B_{0.15}$ dose increased the total phenols by 35.63% compared to the Control. Similarly, the HS increased the total phenol by 32.50%, compared to the HS one. CV × B interaction affected all the parameters reported in **Table 3**. The $B_{0.15}$ dose increased caftaric acid, feruloyl tartaric acid, salvianolic acid K, salvianolic acid L, and total phenols for both genotypes, compared to the Control. On the other hand, the highest chicoric acid values were obtained from the Italiano Classico × $B_{0.15}$ combination (1290.32 μg g^{-1} dw) and Italiano Classico × $B_{0.30}$ (1309.98 μg g^{-1} dw). In comparison, the lowest value was obtained from the combination Eleonora × $B_{0.30}$ (11.68 μg g^{-1} dw). Except for the most and least representative phenolic acids (chicoric and chlorogenic acids, respectively), all phenolic acids were affected by the B × NSC interaction. The $B_{0.15}$ × HS combination provided the highest total phenol concentration (2045.94 μg g^{-1} dw) and feruloyl tartaric acid, salvianolic acid L, salvianolic acid K, and caftaric acid, while the highest concentration of rosmarinic acid was obtained from the Control × HS combination. Compared to the CV × NSC interaction, for both 'Eleonora' and 'Italiano Classico', the HS, compared to the FS, increased total phenols by 53.99 and 15.95%, respectively. Except for caftaric acid, the highest concentration of all phenolic acids was recorded for both genotypes in the HS

Table 3. Analysis of variance and mean comparisons for phenolic acids in Eleonora and Italiano Classico genotypes grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®]. All data are expressed as μg g^{-1} dw.

Treatment	Caftaric acid	Chlorogenic acid	Feruloyl tartaric acid	Salvianolic acid K	Salvianolic acid L	Rosmarinic acid	Cichoric acid	Total Phenols
Cultivar (CV)								
Eleonora	48.41±4.78	40.29±1.36	243.49±9.56	42.27±8.03	39.52±9.65	36.28±6.21	990.57±59.63	1440.81±92.40
Italiano Classico	49.30±5.66	42.84±2.01	166.68±13.6	71.62±5.66	50.96±7.56	61.45±3.08	1147.18±59.55	1590.04±86.65
Biostimulant (B)								
Control	32.74±6.48c	35.38±1.39c	166.50±18.61c	45.01±8.31c	28.99±4.75b	57.78±5.52a	937.10±49.81c	1303.50±80.99c
$B_{0.15}$	63.45±5.05a	46.04±1.85a	250.84±13.19a	74.86±10.64a	77.73±13.62a	49.12±5.80b	1205.92±71.14	1767.96±111.20a
$B_{0.30}$	50.38±4.10b	43.27±1.77b	197.92±13.86b	50.97±7.43b	29.00±2.32b	39.69±8.69c	1063.60±85.47	1474.83±98.81b
Nutrient Solution Concentration (NSC)								
Half Strength (HS)	58.34±4.32	45.45±1.33	232.89±15.13	78.49±6.65	65.08±9.21	60.38±5.75	1186.69±39.37	1727.32±65.05
Full Strength (FS)	39.37±5.07	37.68±1.59	177.28±11.40	35.40±4.82	25.40±4.81	37.35±4.24	951.05±68.12	1303.54±84.79
CV × B								
Eleonora × Control	47.08±9.54c	38.36±1.61d	223.51±12.48c	28.72±4.59d	27.56±9.44c	62.87±10.17b	1032.96±38.36	1461.07±83.74d
Eleonora × $B_{0.15}$	56.88±9.52b	42.28±3.02c	269.12±20.79a	66.02±20.85bc	65.8±25.4b	34.28±4.61d	1121.53±133.9	1655.90±216.47c
Eleonora × $B_{0.30}$	41.27±5.16d	40.22±2.38cd	237.85±11.21b	32.06±5.65d	25.19±2.90c	11.68±2.24e	817.21±84.00c	1205.47±113.17e
Italiano Classico × Control	18.40±3.35e	32.41±1.55e	109.49±8.22e	61.30±13.28c	30.41±3.09c	52.68±4.47c	841.24±75.96c	1145.93±109.18e
Italiano Classico × $B_{0.15}$	70.02±2.06a	49.80±0.57a	232.56±14.12bc	83.69±5.65a	89.67±10.67a	63.96±6.24ab	1290.32±38.46	1880.01±50.23a
Italiano Classico × $B_{0.30}$	59.49±3.76b	46.33±2.1b	158.00±9.03d	69.88±8.24b	32.81±3.09c	67.70±3.67a	1309.98±28.34	1744.19±33.61b
B ×NSC								
Control × HS	47.03±9.56c	38.72±1.44	189.49±27.69c	64.88±11.67b	42.57±3.27b	73.92±5.34a	1061.13±26.38	1517.73±58.58c
$B_{0.15}$ × HS	76.00±1.56a	49.57±0.61	289.62±11.66a	104.13±4.63a	117.13±5.68a	61.03±7.53b	1348.48±38.73	2045.94±47.29a
$B_{0.30}$ × HS	51.98±0.47b	48.07±1.35	219.56±19.39b	66.46±9.76b	35.54±1.92bc	46.19±13.22c	1150.48±70.42	1618.28±77.63b
Control × FS	18.45±3.38d	32.04±1.42	143.50±23.35e	25.15±3.07e	15.40±3.86c	41.64±1.09cd	813.07±63.78	1089.26±84.11e
$B_{0.15}$ × FS	50.89±6.83bc	42.52±3.13	212.06±5.27b	45.59±11.57c	38.34±12.76bc	37.21±5.89de	1063.37±112.4	1489.97±145.81c
$B_{0.30}$ × FS	48.77±8.53bc	38.48±1.67	176.29±16.78d	35.47±7.19d	22.46±1.73bc	33.20±11.86e	976.72±155.44	1331.38±169.42d

Cont. **Table 3**

Treatment	Caftaric acid	Chlorogenic acid	Feruloyl tartaric acid	Salvianolic acid K	Salvianolic acid L	Rosmarinic acid	Cichoric acid	Total Phenols
CV × NSC								
Eleonora × HS	66.37±3.78a	45.35±1.07a	276.41±10.05a	65.26±11.9b	67.16±14.2a	48.77±10.03b	1177.81±63.18a	1747.12±102.13
Eleonora × FS	30.45±1.51c	35.22±0.58c	210.57±4.01b	19.27±0.61d	11.88±1.86c	23.79±4.92c	803.33±48.52c	1134.50±49.01c
Italiano Classico ×	50.30±6.99b	45.55±2.53a	189.37±19.95c	91.71±1.51a	63.00±12.55a	71.99±2.49a	1195.58±50.74a	1707.51±86.35a
Italiano Classico ×	48.3±9.34b	40.14±2.98b	143.99±16.09d	51.54±5.76c	38.92±7.01b	50.91±2.53b	1098.78±109.14	1472.58±144.94
Significance								
CV	ns	***	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
NSC	***	***	***	***	***	***	***	***
CV× B	***	***	***	***	**	***	***	***
B × NSC	***	ns	***	***	***	***	ns	***
CV × NSC	***	***	***	*	***	*	***	***

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD.

3.3. Antioxidant activities

The results of the ABTS, DPPH, and FRAP assay are presented in **Table 4** and are expressed as Trolox equivalents mmol kg^{-1} dw. The CV factor did not result in any significant differences for all antioxidant activities, in contrast to what was observed for the B and NSC factors. Specifically, application of Biostimulant at $B_{0.15}$ dose increased ABTS, DPPH, and FRAP by 32.37, 31.37, and 19.80%, respectively, compared to $B_{0.30}$ dose. Relative to the effect of nutrient solution concentrations, HS resulted in a significant increase in all antioxidant activities compared to FS. The CV × B and B × NSC interactions did not result in significant differences for all parameters reported in **Table 4**, compared to the CV × NSC interaction where differences were observed only for DPPH antioxidant activity. In 'Italiano Classico', the different nutrient solution concentration did not lead to significant differences for this parameter (DPPH). In contrast, in 'Eleonora', the FS reduced DPPH by 27.32%, compared to the HS.

Table 4. Analysis of variance and mean comparisons for ABTS, DPPH, and FRAP antioxidant activities of Eleonora and Italiano Classico genotypes grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®].

Treatment	ABTS (mmol Trolox eq. kg^{-1} dw)	DPPH (mmol Trolox eq. kg^{-1} dw)	FRAP (mmol Trolox eq. kg^{-1} dw)
Cultivar (CV)			
Eleonora	39.84±2.87	28.63±1.79	49.59±2.24
Italiano Classico	39.02±2.23	31.15±1.62	51.86±2.11
Biostimulant (B)			
Control	40.49±2.69ab	30.30±1.58ab	50.60±2.34ab
$B_{0.15}$	44.32±3.50a	33.71±2.47a	55.36±3.04a
$B_{0.30}$	33.48±2.41b	25.66±1.56b	46.21±1.95b
Nutrient Solution Concentration (NSC)			
Half Strength (HS)	45.05±2.42	32.25±1.47	54.44±1.95
Full Strength (FS)	33.81±1.93	27.53±1.80	47.01±2.05
CV × B			
Eleonora × Control	42.18±3.95	30.42±1.61	52.34±3.79
Eleonora × $B_{0.15}$	44.65±6.03	31.81±3.98	51.16±4.34
Eleonora × $B_{0.30}$	32.71±4.06	23.66±2.59	45.28±3.50
Italiano Classico × Control	38.81±3.88	30.18±2.89	48.87±2.92
Italiano Classico × $B_{0.15}$	44.00±4.18	35.61±3.09	59.57±3.83
Italiano Classico × $B_{0.30}$	34.24±2.98	27.66±1.54	47.14±2.05

Cont. **Table 4**

Treatment	ABTS (mmol Trolox eq. kg^{-1} dw)	DPPH (mmol Trolox eq. kg^{-1} dw)	FRAP (mmol Trolox eq. kg^{-1} dw)
B × NSC			
Control × HS	46.84±1.75	31.87±1.37	55.34±2.68
$B_{0.15}$ × HS	52.19±4.23	36.88±2.99	59.04±3.57
$B_{0.30}$ × HS	36.13±3.45	28.01±1.78	48.93±2.91
Control × FS	34.14±3.54	28.72±2.84	45.86±2.81
$B_{0.15}$ × FS	36.46±3.36	30.54±3.73	51.68±4.74
$B_{0.30}$ × FS	30.83±3.29	23.31±2.30	43.49±2.31
CV × NSC			
Eleonora × HS	47.85±3.69	33.16±2.34a	55.36±3.22
Eleonora × FS	31.84±2.33	24.10±1.75b	43.82±1.62
Italiano Classico × HS	42.25±3.04	31.34±1.86ab	53.51±2.37
Italiano Classico × FS	35.78±3.06	30.95±2.78ab	50.20±3.55
Significance			
CV	ns	ns	ns
B	**	**	*
NSC	***	*	**
CV× B	ns	ns	ns
B × NSC	ns	ns	ns
CV × NSC	ns	*	ns

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

3.4. Volatile Compounds

The percentages of the main volatile compounds are shown in **Table 5**. Linalool was the predominant compound, followed by eucalyptol, α-Bergamotene, eugenol, 1-Octen-3-ol, and β-cis-Ocimene. Except for eugenol, all volatile compounds detected were significantly affected by CV. 'Eleonora' recorded the highest content of eucalyptol, α-Bergamotene, 1-Octen-3-ol, and β-cis-Ocimene, while 'Italiano Classico' showed the highest value of linalool (**Table 5**). The biostimulant influenced the whole aroma profile with the highest content of linalool and eucalyptol obtained at $B_{0.30}$ and $B_{0.15}$ doses, respectively. The same compounds increased with increasing NSC (HF > HS) while the highest values of α-Bergamotene, eugenol, and β-cis-Ocimene were obtained using the HS solution. The CV × B interaction affected the entire profile of volatile compounds (**Table 5**). For 'Eleonora', the $B_{0.30}$ dose increase linalool by 27.33%, compared to the control, in contrast to 'Italiano Classico', where the application of the biostimulant did

not result in significant differences. Furthermore, for 'Eleonora', the $B_{0.15}$ dose increased eucalyptol and 1-Octen-3-ol. The highest values of α-Bergamotene, eugenol, and β-cis-Ocimene were obtained from the Eleonora × Control combination. Relative to the B × NSC interaction, at both nutrient solution concentrations, the $B_{0.30}$ dose increase linalool (+11.81%, on avg.) compared with Control. Regardless of dose, the biostimulant in the HS reduced eugenol and α-Bergamotene. The highest values of 1-Octen-3-ol (2.95%) were obtained from the combination of $B_{0.15}$ × HS combination. Except for eucalyptol, all volatile compounds were affected by the CV × NSC interaction (**Table 5**). For 'Eleonora', the FS increased linalool and 1-Octen-3-ol, compared to the HS. The opposite trend was observed for α-Bergamotene, eugenol, and β-cis-Ocimene. For 'Italiano Classico', only linalool was affected by the different nutrient concentrations, with the highest values recorded by the Italiano Classico × FS combination.

Table 5. Analysis of variance and mean comparisons for volatile compounds in Eleonora and Italiano Classico genotypes grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; $B_{0.15}$ = 0.15 ml L^{-1} of Trainer®; $B_{0.30}$ = ml L^{-1} of Trainer®]. All data are expressed as percentage relative abundance (%).

Treatment	1-Octen-3-ol	Eucaliptol	β-cis-Ocimene	Linalool	Eugenol	α-Bergamotene
Cultivar (CV)						
Eleonora	2.73±0.07	25.42±0.63	2.24±0.14	43.08±1.23	3.86±0.27	7.72±0.55
Italiano Classico	2.38±0.05	17.44±0.30	1.67±0.03	60.77±0.30	3.72±0.20	5.48±0.15
Biostimulant (B)						
Control	2.48±0.06b	20.78±0.90	2.11±0.18a	49.33±3.40	4.35±0.35a	7.39±0.97a
$B_{0.15}$	2.81±0.10a	22.64±1.83	1.9±0.16ab	51.3±2.86b	3.28±0.23b	6.27±0.25b
$B_{0.30}$	2.37±0.05c	20.86±1.06	1.85±0.10b	55.16±1.93	3.74±0.17b	6.14±0.15b
Nutrient Solution Concentration (NSC)						
Half Strength (HS)	2.53±0.08	20.83±1.06	2.13±0.13	50.46±2.49	4.09±0.27	7.24±0.63
Full Strength (FS)	2.57±0.07	22.03±1.09	1.78±0.10	53.40±2.09	3.49±0.17	5.95±0.18
CV × B						
Eleonora × Control	2.57±0.1b	23.3±0.74b	2.63±0.16a	38.38±1.6d	5.06±0.46a	9.81±1.31a
Eleonora × $B_{0.15}$	3.10±0.04a	28.59±0.61	2.06±0.32b	42.00±1.09	3.010±0.2c	6.91±0.21b
Eleonora × $B_{0.30}$	2.50±0.02b	24.37±0.20	2.03±0.18b	48.87±0.62	3.50±0.14b	6.43±0.21b
Italiano Classico × Control	2.38±0.03c	18.26±0.68	1.60±0.06c	60.27±0.63	3.64±0.36b	4.97±0.22d
Italiano Classico × $B_{0.15}$	2.52±0.11b	16.69±0.40	1.74±0.05bc	60.61±0.47	3.56±0.41b	5.62±0.24c
Italiano Classico × $B_{0.30}$	2.23±0.07d	17.36±0.18	1.67±0.05bc	61.45±0.36	3.97±0.29b	5.84±0.16c

Cont. **Table 5**

Treatment	1-Octen-3-ol	Eucaliptol	β-cis-Ocimene	Linalool	Eugenol	α-Bergamotene
B ×NSC						
Control × HS	2.33±0.02c	19.74±0.98	2.18±0.28	47.14±5.43	5.21±0.40a	8.91±1.71a
$B_{0.15}$ × HS	2.95±0.09a	22.14±2.74	2.19±0.26	49.82±4.44	3.04±0.19c	6.30±0.49b
$B_{0.30}$ × HS	2.33±0.06c	20.61±1.58	2.01±0.17	54.40±2.95	4.03±0.28b	6.51±0.17b
Control × FS	2.62±0.08b	21.83±1.46	2.04±0.24	51.51±4.41	3.5±0.30bc	5.87±0.50b
$B_{0.15}$ × FS	2.68±0.18b	23.14±2.66	1.61±0.11	52.78±3.93	3.53±0.43b	6.23±0.18b
$B_{0.30}$ × FS	2.41±0.09c	21.12±1.57	1.68±0.07	55.91±2.73	3.44±0.13b	5.76±0.15b
CV × NSC						
Eleonora × HS	2.65±0.12b	24.66±1.00	2.62±0.11a	40.94±1.90	4.34±0.44a	8.95±0.95a
Eleonora × FS	2.81±0.08a	26.18±0.74	1.86±0.19b	45.23±1.30	3.38±0.23b	6.48±0.15b
Italiano Classico × HS	2.42±0.09c	17.00±0.35	1.64±0.04b	59.98±0.33	3.85±0.32a	5.53±0.22c
Italiano Classico × FS	2.34±0.05c	17.88±0.45	1.70±0.05b	61.57±0.32	3.60±0.25b	5.42±0.21c
Significance						
CV	***	***	***	***	ns	***
B	***	***	*	***	***	***
NSC	ns	**	***	***	***	***
CV× B	***	***	**	***	***	***
B × NSC	***	ns	ns	*	***	***
CV × NSC	***	ns	***	**	*	***

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HS

3.5. Principal Component Analysis

A principal Component analysis (PCA) for yield, visual and quality attributes was conducted to further explore differences between the two 'Genovese' basil genotypes (Eleonora and Italiano Classico), grown in a FRS with two different concentrations of NS (1 dS m^{-1}-Half Strength [HS] and 2 dS m^{-1}-Full Strength [FS]) and two doses of biostimulants (0.15 and 0.30 ml L^{-1}, compared to an untreated control). The first two principal components (PCs) explained 60.7% of the cumulative variance, with PC1 and PC2 accounting for 36.1% and 24.6%, respectively (**Figure 2**). PC1 was positively correlated with all target polyphenols, volatile compounds as well as the antioxidant assays. Also, PC1 correlated negatively with the visual attributes (L, a*, b*). Furthermore, PC2 correlated positively with the three antioxidant activities and target polyphenols (**Figure 2**). Based on the angle between vectors of the examined variables, cichoric acid, chlorogenic acid, total phenols, DPPH and FRAP were found to be positively and significantly correlated among them (angle < 90°) and negatively correlated with eucalyptol (angle > 90°) (**Figure 2**). The PC1 and PC2 score plot discriminated tested treatments into different cluster groups. On the positive side of PC1, 'Italiano Classico' fertigated with HS and treated with 0.15 ml L^{-1} of PH delivered basil leaves of premium quality with high concentration of target polyphenols and antioxidant activities. At the lower right quadrant, 'Eleonora' supplied with HS solution, showed the highest aroma profile, while the 'Eleonora' cultivar fertigated with FS (irrespective of the biostimulant treatment) was positioned in the lower left quadrant distinguished by the poorest nutritional value (**Figure 2**).

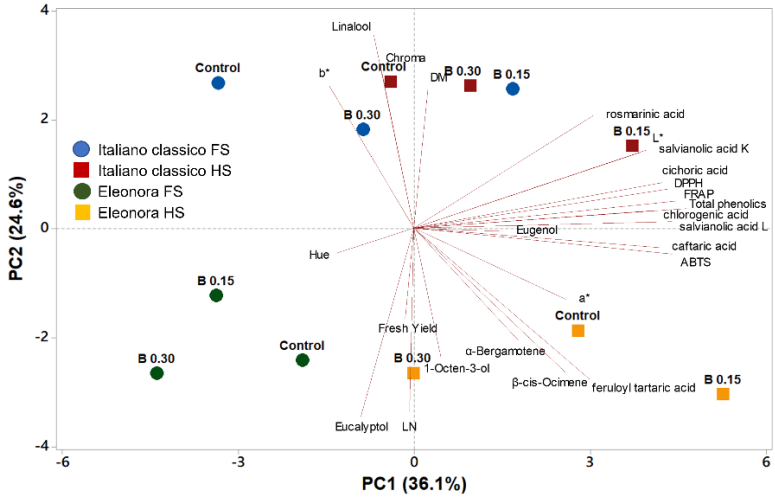

Figure 2. Loading plot and scores of principal component analysis (PCA) for yield, yield components, leaf colorimetric parameters, phenolic acids profile, antioxidant activities, and volatile compounds in two Genovese basil genotypes (Eleonora and Italiano Classico) grown hydroponically under two nutrient solution and dose of biostimulant. [Nutrient solution concentration treatments: HS = half strength; FS = full strength; Biostimulant treatments: Control; B0.15 = 0.15 ml L^{-1} of Trainer®; B0.30 = ml L^{-1} of Trainer®].

4. Discussion

4.1. The PH's in Nutrient Solution Boosted Basil Yield

Soilless systems are increasingly used to maximize the yields of premium quality vegetables. Among these, the FRS is characterized by ease of use, low management costs and high functionality, allowing early production with standard characteristics even on a large scale. The surprising yield obtained in the present study confirms the high efficiency of the FRS for basil cultivation compared to soil cultivation. Compared to the results obtained by Zheljazkov et al.[39] on 38 basil cultivars grown in the open field, we recorded average yields approximately 15 times higher. This result is attributable to a better allocation of water and nutritional resources and the high density adopted (317 m^{-2})[14]. Regardless of the growing system, genetic material plays a crucial role in the productive response of basil[17]. It should be noted that 'Eleonora' showed better adaptability to the selected cropping system, producing more leaves per plant, and thus providing a higher fresh yield than 'Italiano Classico', which, in contrast, had a higher dry matter percentage (**Table 1**). In hydroponic systems, yield is primarily determined by the formulation of the NS, and to this end, numerous studies have focused on seeking optimal mineral levels to achieve ad hoc crop-specific 'recipes'[23]. For example, some studies have shown reduced yields in spinach (*Spinacia oleracea* L.)[40] and lettuce[23] when

grown in nutrient solutions with suboptimal mineral concentrations. Moreover, in our study, we did not observe any significant change in fresh yield in basil grown in HS (1 dS m^{-1}) and FS (2 dS m^{-1}) nutrient solutions. Our result is in line with the observations of Hosseini et al.[23], who reported reductions in fresh yield in basil and lettuce grown on nutrient solutions with lower EC of 0.9 dS m^{-1} and corroborated the studies of Walters and Currey[41] on 'Sweet', 'Lemon', and 'Holy' basil, which did not observe yield increase with EC between 1 and 4 dS m^{-1}. This shows that excess nutrients in the solution provide no benefit in terms of basil yield and negatively affect resource efficiency, economic viability, and environmental sustainability of hydroponic systems. The pressing need to ensure high yields of high-quality vegetables by adopting efficient and environmentally friendly cultivation methods makes the application of biostimulants in NS a promising ecological strategy. In our study, the application of PH's (Trainer®) in the NS increased the fresh yield, the dry yield, and the number of leaves proportional to the dose used (**Table 1**). These results highlight that applying the biostimulant directly to the NS is a beneficial strategy to increase yield in hydroponic systems, as also shown by Cristofano et al.[30] in lettuce. The beneficial effects of PH's on yield parameters, also obtained in arugula[29,42], celery (*Apium graveolens* L.)[43] and basil[28], can be attributed to the peptides and bioactive amino acids characteristic of commercial formulations[28]. Peptides, involved in cell differentiation and division, due to recognized hormone-like activity, modify root architecture and growth, improving uptake and crop yield[31,44,45]. The above effects are also attributable to amino acids (easily absorbed by roots) that are involved in essential signaling processes in addition to performing physiological functions[33]. These molecules found in Trainer® could have promoted nitrogen uptake in the rhizosphere and regulated key transcription factors and photosynthesis[46]. In contrast to what was observed for the yield, the higher accumulation of dry matter in plants treated with the biostimulant (**Table 1**) is not widely supported in the literature. As an example, Caruso et al.[29] recorded results comparable to ours in arugula, while Consentino et al.[43] obtained opposite results in celery. In spinach, Rouphael et al.[47] observed no significant difference for this parameter. The contrasting results highlight how the effects of biostimulants depend on factors such as time and mode of application, growth conditions, and genotype[1]. In line with the above, although the biostimulant, on average, increased the fresh and dry yield, integration of the PH's into the FS and HS solutions showed dose-dependent responses. While, for the FS, increasing the dose led to linear increases in fresh and dry yields, there was no apparent dose-dependent effect for the HS. Under these operating conditions, the above points out that the impact of the biostimulant could also be influenced by the mutual interaction between the application dose and the concentration of the NS.

4.2. Different Genotypes Impacted Visual Attributes of Basil Leaves

Color is a characteristic of light measurable in terms of wavelength and intensity, related to the observer's perception and to the light conditions under which it is observed, able of influencing consumer choice about food quality[48]. In basil, the bright green color of the leaves and the attraction of consumer interest is a critical industrial requirement for the preparation of a 'pesto' sauce, as it reduces the use of artificial colorants[4]. Although the basil genotypes tested all belonged to the 'Genovese' cultivar, CIELab colorimetric parameters (L, a*, b*) showed cultivar-dependent variations, with 'Italiano Classico' recording the highest values of all above parameters, confirming the results of Ciriello et al.[14] on the same basil genotypes grown in FRS. The higher values of L (brightness) and a* (greenness) in 'Italiano Classico' are consistent with Chroma values, indicating a higher color intensity perceived by the consumer. The latter parameters (L and a*) were also influenced by the NSC. In particular, the FS increased leaf brightness (higher L) and greenness (higher a*) compared to the HS, although it did not show any productive differences (**Table 2**). As argued by Fallovo et al.[22], the more intense green leaf color could be attributed to a higher chlorophyll content (data not shown) related to the higher nitrogen levels of the FS (2 dS m^{-1}). Our color results showed that applying the biostimulant at the highest dose ($B_{0.30}$) in the NS increased only the parameter a *, compared to the Control, in agreement with Consentino et al.[43]. This result could be related again to the increase induced by biostimulants in chlorophyll content, as observed by Vernieri et al.[49] and Aktsoglou et al.[16] in arugula and peppermint (*Mentha* × *piperita*), respectively. However, Caruso et al.[29] and Giordano et al.[42], despite observing an increase in SPAD (an indirect index of chlorophyll content), did not record a change in color in arugula after the application of PH's. These results confirm once again how the effects of biostimulants differ primarily by species, but also by dose, mode of application, and different growth and development conditions.

4.3. Impact of Interactions Between Investigated Factors on Basil Quality Attributes

The inability to 'escape' from possible environmental threats has 'bound' plants to passive defense mechanisms based on the production of specialized metabolites that have allowed their survival over time[50]. In medicinal plants, specialized metabolites are characterized by significant structural and chemical diversity that uniquely confers the desired technological and nutritional attributes[10,12]. Although we had used 'Genovese' genotypes characterized by a similar phenolic profile in our study, the concentration of total phenolic acids differed considerably (**Table 3**). The higher total phenolic concentration in 'Italiano Classico' (**Table 3**), also obtained in other works conducted under different growth conditions, again demonstrates how the accumulation of these compounds is strongly influenced by genetics[17]. Despite this, the antioxidant activities reported in **Table 4** were not affected by the effect of the cultivar. The explanation for

this could lie in the fact that between the two genotypes tested there was only a 9.4% difference in the concentration of total phenols, but it could also be due to the synergistic effects between polyphenols and other chemical constituents, such as ascorbic acid and carotenoids that contribute to overall antioxidant activity[36]. The data in **Table 3** clearly show the influence of genetics on the diversity of the phenolic profile of the basil genotypes. Although rosmarinic acid is referred to as the most represented phenolic acid in basil[12,14,51], in our study, both 'Eleonora' and 'Italiano Classico' were characterized by a predominant concentration of chicoric acid. The influence of genotype on the predominant biosynthesis of chicoric acid was also confirmed by Kwee and Niemeyer[52] in basil. The authors showed that 9 basil varieties out of 15 tested had the highest absolute concentration of chicoric acid. Furthermore, it is important to note that the discrepancy with the results reported in the literature is attributable not only to the genetic material but also to the different extraction methods and solvents used to determine the phenolic acids and the different growth conditions adopted[12]. Regardless of the cultivar, the present work confirms that basil leaves contain, in addition to high levels of chicoric acid, significant amounts of salviolanic acids K and L. The important and recognized pharmacological properties of salviolanic acid could further increase the nutraceutical value of basil[18]. The change in the entire phenolic profile in response to changing concentrations of NS (**Table 3**) confirms that nutritional stress can affect the biosynthesis and accumulation of specialized metabolites[5]. The use of a HS increased the levels of the entire phenolic profile in both genotypes compared to what was observed in the FS, similar to what was observed in basil[11], lettuce[2], artichoke (*Cynara cardunculus* subsp. *scolymus* L.), and cardoon (*Cynara cardunculus* L.)[53]. The increase in the phenolic profile showed the same trend as the ABTS, DPPH, and FRAP assays (**Table 4**), indicating how the limitation of nutrition induced an improvement in antioxidant activity. This result and the increase in the phenolic profile are probably related to the halving of nitrate in the HS, which as observed by Chishaki and Horiguchi[54] has a more significant influence on the accumulation of phenolic acids than potassium and phosphorus deficiency. Low nitrogen levels would stimulate phenylpropanoid metabolism, inducing the accumulation of phenylalanine ammonia-lyase (PAL) and other critical enzymes involved in the biosynthesis of phenolic compounds[5,55,56]. This would suggest that low nitrogen levels, by decreasing growth requirements, would promote the accumulation of specialized metabolites[18]. However, in our study, we did not observe a reduction in fresh yield at HS (**Table 1**), which justifies the high phenolic concentration as a result of ex novo synthesis rather than a deceleration of primary metabolism by increased activity of PAL or its substrate (phenylalanine)[57]. Similarly to the yield parameters (**Table 1**), the application of the biostimulant in the NS significantly increase the phenolic concentration in basil. A probable reason for elucidating this interesting result could be related to the increase in production due to a better

photosynthetic activity mediated by the biostimulant, which would have promoted secondary metabolism[31]. However, the bioactive signal molecules characteristic of PH's, in addition to providing the plethora of physiological effects mentioned above, may have triggered the induction of the production of specialized metabolites. Based on a recent work[58], in which a positive influence was observed on basil secondary metabolism after applying amino acids, our results could be traced to the composition of Trainer®, which is characterized by the presence of these organic molecules. One of the crucial functions of amino acids and molecules derived from them is their ability to serve as precursors for specialized plant metabolites, acting both as substrates and as activators of key enzymes such as chorismate mutase, creating points of interconnection in the biosynthesis of phenolic compounds[58,59]. Interestingly, regardless of the cultivar and concentration of the nutrient solution, among the biostimulant doses tested ($B_{0.15}$ and $B_{0.30}$), the highest accumulation of total phenolics was obtained after application in the nutrient solution of the lowest dose ($B_{0.15}$) of the biostimulant, confirming that this result was not dose dependent. The justification behind the above could stem from the fact that at dose $B_{0.30}$, the biostimulant prioritized production over secondary metabolism. The variability in the composition of essential oils among basil types gives this aromatic herb a multitude of uses. The non-unique aroma of basil is determined by the various compounds that constitute its essential oils, mainly terpenoids (synthesized through the mevalonate pathway and the 2-methylitritol 4-phosphate pathway) and phenylpropanoids (synthesized through the shikimate pathway)[5,60]. The distinctive aroma of 'Genovese' basil and its derivative products (such as pesto sauce), is attributable to the dominant presence of critical aromatic molecules such as linalool and the complete absence of mint (menthol) and anise (estragole)[4]. Not surprisingly, in the basil genotypes tested, linalool was the predominant, a compound that, in addition to uniquely characterizing the flavor of the 'Genovese' genotypes, also has documented therapeutic properties[61]. However, the differences found in 'Eleonora' (higher content of 1-Octen-3-ol, eucalyptol, β-cis-Ocimene, and α-Bergamotene) and 'Italiano Classico' (higher content of linalool) in the full aroma profile reported in **Table 5** underscore the significant impact of genotype. These differences could be due to the different leaf morphology, the density of oil glands, vegetative growth, and biosynthesis of volatile odorous compounds[62]. Compared to the latter, the higher content of linalool but lower contents of eucalyptol and β-cis-ocimene contents, recorded in 'Italiano Classico', compared to 'Eleonora', highlights a clear genotypic effect on gene expression that regulates the conversion of its sole precursor (geranyl pyrophosphate) from the enzymes linalool synthase, 1,8-cineole synthase and β-cis-ocimene synthase[63]. The basil genotypes tested showed a different response to the biostimulant (**Table 5**). As seen in peppermint and spearmint (*Mentha romana* L.)[16], biostimulant in the NS did not result in any significant difference in the composition of the aroma profile of Italiano Classico. On the

one hand, this result could indicate a low sensitivity of the cultivar to the biostimulant and, on the other hand, it could result from the use of insufficient doses to induce alterations in the overall composition of volatile oils. On the contrary, in 'Eleonora', there was a significant effect on the whole aromatic profile caused by the application of the biostimulant. We observed a direct correlation between increasing the dose of biostimulant and the linalool content, contrary to what was observed for eugenol, β-cis-Ocimene and α-Bergamotene, which instead decreased regardless of the dose used. Since plant nutrition is known to influence the content of volatile oils[16], it is not surprising that the use of NS at different concentrations resulted in significant differences in basil flavor profile (**Table 5**). As with the biostimulant, different responses were observed for the NSC between the two basil genotypes used in the present study. In 'Italiano Classico', the different NSC changed only the content of the most represented compound (linalool), while in 'Eleonora', all compounds, except eucalyptol, were significantly affected by the different availability of nutrients in the nutrient solution. In any case, in both genotypes, the more concentrated nutrient solution (FS) increased the linalool content. As also seen on *Salvia sclarea* L.[64], the higher availability of nutrients, especially nitrogen, led to an increase in the linalool content, as nitrogen, involved in the biosynthesis of primary and secondary metabolites, could positively interact in its metabolic pathway, confirming our results[62].

5. Conclusions

The challenge imposed on the agricultural sector to provide nourishment to a growing population has led to alternative production techniques such as hydroponics. However, the urgent need to reduce chemical inputs in alternative cropping systems has paved the way for biostimulants, which currently represent an environmentally sustainable strategy for horticultural production. Under the experimental conditions of our study, the varietal comparison showed that 'Eleonora' provided the highest fresh yield (6576.81 g m^{-2}). At the same time, 'Italiano Classico' had the highest total phenol concentration (1590.04 μg g^{-1} dw). The use of NS with different concentrations did not result in significant differences in fresh yield, regardless of the cultivar, but positively impacted the aroma and phenolic profile. Specifically, the HS increased total phenols by 32.5%, compared to the FS that ensured the highest content of eucalyptol (22.0%) and linalool (53.4%). The application of biostimulants in the NS increased all biometric parameters (such as the number of leaves, fresh and dry yield) and the linalool content proportionally to the dose used, while the highest total phenol concentration was obtained from the lowest dose ($B_{0.15}$). Based on the excellent results achieved, the application of biostimulants in NS turned out to be a valid strategy to reduce chemical input. For this reason, it should also be investigated on other leafy crops to define a new production technique that can improve both yield and quality.

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Chapter 9
Biostimulatory Action of a Plant-Derived Protein Hydrolysate on Morphological Traits, Photosynthetic Parameters, and Mineral Composition of Two Basil Cultivars Grown Hydroponically under Variable Electrical Conductivity

Abstract: In Hydroponics is a viable alternative to open field cultivation for year-round vegetable production in urban areas. However, the total dependence on external chemical inputs (fertilizers) makes these systems often less environmentally sustainable. In this perspective, the use of biostimulants could represent a valuable and eco-friendly tool to limit the excessive use of fertilizers without a negative impact on the yield. To this end, our work aimed to evaluate the productive and physio-logical response of two cultivars of 'Genovese' basil (Eleonora and Italiano Classico) for the industrial production of "pesto" grown for 22 days in two nutrient solutions with different electrical conductivity (1 and 2 dS m^{-1}) and the application of two doses of protein hydrolysates (0.15- and 0.30-mL L^{-1} of Trainer® in the nutrient solution). The mineral profile was evaluated by ion chromatography coupled with a conductivity detector, while pigments were evaluated by UV-Vis spectrophotometry. Generally, the nutrient solution concentration did not significantly affect the fresh yield of the two cultivars tested. On the contrary, the use of the maximum dose of biostimulant (BT_2 = 0.30 mL L^{-1} of nutrient solution) increased fresh yield, leaf area, and ACO_2 by 20.7, 27.5, and 17.6%, respectively, compared with the control. Using the lowest dose of biostimulant (BT_1 = 0.15 mL L^{-1} of the nutrient solution) reduced nitrate by 6.6% compared with the control. The results obtained showed that basil cultivation in a floating raft system combined with biostimulant in the nutrient solution could be an excellent solution to improve productivity, reduce nitrate, and cut fertilizer costs.

Keywords: *Ocimum basilicum* L.; Biostimulants; Floating raft system; Nutrient solution concentration; Ion chromatography; Nitrate

1. Introduction

Climate change and rapid unplanned urbanization aggravate the erosion of agricultural land, a valuable nonrenewable resource. A situation imperiled by the steady growth of the world's population (which will reach 10 billion by 2050) challenges the agricultural sector to adopt extensive cropping systems and techniques to ensure food security[1,2]. In this scenario, hydroponics is an effective and practical solution to meet the rapidly changing needs of agriculture. Soilless crops provide better space optimization, including abandoned urban areas that are not suitable for traditional agriculture, where environmental conditions do not interfere, leading to higher yields because of a high-er-density setup[3]. Not least, the sudden change in the lifestyle of consumers in the most industrialized countries, who are increasingly conscious of their "waistlines" and are pressured to eat fast meals to keep up with their hectic lives, has increased the consumption of fresh-cut herbs (such as *Lactuca sativa* L., *Spinacia Oleracea* L., *Beta vulgaris* L., *Eruca sativa* Mill.), which are increasingly grown in soilless systems[4]. However, leaning completely on nutrient solutions that exceed plants' nutrient exigencies undermines the sustainability of hydroponic systems[5,6].

As widely observed in the field, even in superintensive agricultural sectors such as soilless systems, biostimulants increase nutrient use efficiency, partially reducing the use of traditional chemical input while improving crop yield and quality[7-11]. However, an in vivo understanding of the physiological and molecular influence of biostimulants is still under investigation to clarify and improve their efficiency[12]. Therefore, the beneficial effects depend on the mode and timing of application, dose, and composition[7]. Among the different categories of nonmicrobial biostimulants are plant-derived protein hydrolysates (PH) that differ from the rest in the distinctive functions they perform. PHs are produced from organic waste biomass, recycling by-products deriving from anthropogenic activities with positive repercussions from both economic and ecological points of view[13-15]. These hydrolysates are a heterogeneous mixture of oligopeptides, polypeptides, and amino acids (e.g., aspartic acid, glutamic acid, and essential amino acids), produced primarily by enzymatic processes[16]. The latter, as observed by Noroozlo et al.[17] and Souri and Hatamian[18], play different crucial roles in plant metabolism.

As reported in the literature, PHs represent a successful ecological strategy to reduce chemical input by promoting the availability, uptake, and metabolic use of macro and micronutrients and improving crop production and quality performance, especially under suboptimal growth conditions[16,19]. The abovementioned beneficial PHs effects are attributable to signal peptides with a hormone-like activity that can stimulate shoot growth, modulate root architecture, and improve nutrient uptake[20,21]. The biostimulation activity of PHs triggers molecular and physiological processes involving increased hormonal activities, enzymatic antioxidants (catalase, ascorbate peroxidase,

superoxide dismutase, peroxidase, and glutathione reductase), nonenzymatic secondary metabolites and pigments, and the activation of processes and key enzymes involved in C metabolism and the nitrogen cycle (GOCAT, GS, NiR, and NR)[2,22]. As pointed out by several authors[23-25], the use of PHs is a potential eco-friendly and effective solution to overcome the en-vironmental problems resulting from excessive use of fertilizers, often produced from nonrenewable resources[26].

However, an investigation of PHs effects should be carried out on a wider range of staple foods and medicinal plants. Among the latter, basil (*Ocimum basilicum* L.) is undoubtedly one of the most cultivated in Italy, with a total annual production of approximately 8000 tons[27] for the gastronomic sector, as young leaves are the main ingredient in typical regional dishes (pesto sauce and pizza Margherita)[28]. In addition, the great morphological and phytochemical variability of the Ocimum genus[29] has also allowed this medicinal plant to find wide use in the pharma-cosmetic sector[30]. The necessity to meet the growing demands of the food processing industry, which requires a deseasonalized and well-standardized production, has pushed the whole production sector towards hydroponic cultivation of Genovese basil[30]. As well as guaranteeing higher yields, these systems can improve functional and organoleptic quality while reducing the incidence of pests and pathogens.

Thus far, the scientific community has focused its research mainly on the evaluation of the effects induced by microbial biostimulants on basil, and only recently, Rouphael, et al.[21] investigated the effects of biostimulants based on plant and animal origin protein hydrolyses (Trainer® and Siapton®) on sweet basil cultivar Gecom grown in agricultural soil. To the authors' knowledge, this work is the first to test the effect of PHs applied directly and constantly in contact with the root zone of basil grown in a floating system. The integration of biostimulants into traditional cropping systems could be a beneficial resource for reducing chemical inputs. In the light of this, our study analyzes in detail if and how the use of biostimulants of plant origin can reduce the use of chemical fertilizers for hydroponic basil production. The present study constitutes a continuation of our previous work, where the nutritive, aroma profile, and phytochemical aspects of two basil cultivars indicated that, in certain conditions, the application of PH can improve the functional quality attributes (i.e., total phenolic concentration) of Genovese basil for pesto. Taking into account the importance of soilless basil cultivation, we evaluated the productive, mineral composition, and physiological response to root integration of a PH (Trainer®) at two doses on two cultivars of Genovese basil (Eleonora and Italiano Classico) grown at two levels of nutrient solution concentration.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

The A floating raft system (FRS) experiment was carried out at the Department of Agriculture, University of Naples "Federico II", Portici, Italy (40°48′ N, 14°20′ E, 29 m.s.l.) from June 9 to June 30, 2020, in a passively ventilated greenhouse. A trifactorial randomized complete block experimental design was used, in which two different nutrient solution concentrations (NSC) (1 dS m^{-1} and 2 dS m^{-1}, hereafter NSC1 and NSC_2, respectively), two basil cultivars (*Ocimum basilicum* L.) (Eleonora, Enza Zaden, Enkhuizen, NH, The Netherlands and Italiano Classico, La Semiorto Sementi, Sarno, Italy) and two doses of biostimulants (0.15 and 0.30 mL L^{-1}, hereafter BT_1 and BT_2, respectively) plus an untreated control were considered as factors. Each experimental treatment was replicated three times (n = 3) for a total of 36 experimental units, each consisting of a polystyrene tray containing 54 plants floating in a tank filled with 35 L of nutrient solution. Both nutrient solutions (NSC_1 and NSC_2) were prepared from osmosis water and contained the same concentrations of micronutrients (15 μM iron, 9 μM manganese, 0.3 μM copper, 1.6 μM zinc, 20 μM boron, and 0.3 μM molybdenum). NSC_1 was obtained by halving the macronutrient concentration of NSC_2 characterized by: 14.0 mM nitrate, 4.5 mM calcium, 5.0 mM potassium, 1.75 mM sulfur, 1.5 mM phosphorus, 1.5 mM magnesium, and 1.0 mM ammonium. For each tank, the nutrient solution oxygenation was provided by a submersible pump (Aquaball 60, Eheim, STU, Deizisau, Germany), and pH was continuously monitored and maintained at values of 5.8 ± 0.2. At transplanting (9 June), the commercial biostimulant Trainer® (plant PH obtained by enzymatic hydrolysis of legume biomass; **Supplementary Figure S1**) was applied directly to the nutrient solution at two different doses (0.15 mL L^{-1} and 0.30 mL L^{-1}). To prevent large fluctuations in EC, pH, and ionic concentrations, the nutrient solutions were completely renewed from all tanks weekly.

Density	1.21 kg L^{-1}
Dry matter	46%
pH	4.0
Free amino acids and soluble peptides	310 g kg^{-1}
FRAP antioxidant activity	41.9 mmol Fe^{2+} g^{-1} f.w.
Total phenolics	8.93 mg of gallic acid eq. per g of f.w. product
Total flavonoids	0.95 mg of quercetin eq. per g of f.w. product
Soluble sugars	90 g kg^{-1} f.w.
N–NO_3	3.13 µg g^{-1} f.w.
N–NH_4	6.00 µg g^{-1} f.w.
Phytohormones	ND
Mineral composition (g kg–1 f.w.)	
N	50.0
P	0.9
K	41.1
Ca	10.9
Mg	0.5
Fe	0.024
Zn	0.010
Mn	0.001
B	0.005
Cu	0.001
Aminogram (g kg–1 f.w.)	
Ala	12
Arg	19
Asp	33
Cys	4
Glu	54
Gly	13
His	8
Ile	12
Leu	24
Lys	19
Met	4
Phe	16
Pro	15
Ser	17
Thr	11
Trp	4
Tyr	13
Val	16

Supplementary Figure S1. Trainer® composition

2.2. Harvest and Soil Plant Analysis Development Index (SPAD), Leaf Gas Exchange, and Chlorophyll Fluorescence Determination

At the end of the experiment (30 June, 22 days after transplanting (DAT)), twenty-five plants from each experimental unit were collected to perform biometric measurements. The selected plants were separated into leaves and stems to determine fresh weights (g $plant^{-1}$), stem diameter (cm), node number, leaf-to-stem ratio, and leaf

area (cm^2) using ImageJ software version 1.50 (US National Institutes of Health, Bethesda, MD, USA). The sampled material was placed in a ventilated oven at 65 °C for approximately 72 h and then stored for mineral analysis.

At harvest, measurements of the SPAD index were made on the adaxial side of twenty fully expanded young leaves per experimental unit using a portable SPAD-502 m (Konica Minolta Co. Ltd., Osaka, Japan). At 22 DAT, on the same leaves used for the determination of the SPAD index, between 10:30 and 12:30, via a portable fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA), measurements of the maximum quantum efficiency of PSII (expressed as Fv/Fm) were made. Chlorophyll fluorescence was performed after adaptation of leaves to darkness (for at least 10 min) using specific leaf clips. The maximum quantum efficiency of photosystem II (PSII) Fv/Fm was calculated as (Fm − F0)/Fm, where F0 and Fm were the ground fluorescence signal and maximum fluorescence intensities in the dark-adapted state, respectively. The determination of net CO_2 assimilation rate (A_{CO2}; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}), and transpiration (E; mmol H_2O m^{-2} s^{-1}) was performed using an LI-6400 (LI-COR Biosciences, Lincoln, NE, USA). The CO_2 of the gas exchange analyzer chamber was set at ambient values (approximately 400 ppm) and photosynthetically active radiation at 1000 μmol m^{-2} s^{-1}. Instantaneous water use efficiency (WUEi) was calculated as A_{CO2}/E.

2.3. Determination of Minerals

For the determination of minerals, 0.25 g of finely ground dried sample (MF10.1 cutting head mill, IKA®, Staufen im Breisgau, Germany), sieved (MF0.5, 0.5-mm hole; IKA®, Staufen im Breisgau, Germany), and extracted in ultrapure water (Arium® Advance EDI (Sartorius, Goettingen, Germany) by stirred water bath (80 °C for 10 min; SW22, Julabo, Seelbach, Germany), were analyzed by ion chromatography (ICS 3000, Thermo Fisher Scientific™ Dionex™, Sunnyvale, CA, USA) according to the method described by Formisano et al.[31] An analytical column IonPac CS12A, an IonPac CG12A precolumn, and a self-healing electrolyte suppressor CERS5000 (Thermo ScientificTM Dionex™, Sunnyvale, CA, USA) while an IONPAC® ATC-HC 9 × 75 mm trap, an IONPAC® AG11-HC 4 × 50 mm guard column and an IONPAC® AG11-HC 4 × 50 mm column were used for anions and cations, respectively. Each treatment was analyzed in triplicates, and the results, except for nitrate (expressed as mg kg^{-1} fresh weight-fw), were expressed as g kg^{-1} dw. All columns were purchased from Thermo Fisher Scientific™ Dionex™ (Sunnyvale, CA, USA).

2.4. Determination of Chlorophylls and Carotenoids

The determination of total chlorophylls (chlorophyll a + chlorophyll b) and total carotenoids was performed according to the methods described by El-Nakhel, et al.[32] with some modifications. Briefly, 0.50 g of fresh frozen leaves were extracted in the dark

(15 min) in ammonia acetone (90% *v*/*v*; Carlo Erba Reagents Srl, Milan, Italy). Subsequently, the extracts were centrifuged (3000 rpm for 5 min; R-10M (Remi Elektrotechnik Ltd., Mumbai, India), and the pigment concentration was determined by UV-Vis spectro-photometry (DR 4000, Hach Co, Loveland, CO, USA), by reading the absorbances at 647, 664, and 470 nm (for chlorophyll a, b, and carotenoids, respectively). Total chlorophylls were calculated as the sum of chlorophyll a and b. All the pigments were expressed as mg g^{-1} fw.

2.6. Statistics

A two-way analysis of variance (ANOVA) was implemented to assess the significance of the effects and interactions between the factor pairs: Cultivar × Biostimulant Treatment (CV × BT), Biostimulant Treatment × Nutrient Solution Concentration (BT × NSC), and Cultivar × Nutrient Solution Concentration (CV × NSC). One-way ANOVA was used to compare the mean effect of Biostimulant Treatment (BT), whereas Cultivar (CV) and Nutrient Solution Concentration (NSC) were compared according to Student's t-test. The statistical significance was determined at the $p < 0.05$ level using the Tukey–Kramer HSD test for CV × BT, BT × NSC, and CV × NSC interactions and for the BT factor. All data were presented as mean ± standard error. All statistical analyses were performed using IBM SPSS 20 (Armonk, NY, USA) package for Microsoft Windows 11.

3. Results

3.1. Yield and Yield Parameters

BT factor had a highly significant main effect ($p \leq 0.001$) on all biometric variables reported in **Table 1**. Contrary to the effect of CV, the NSC factor did not significantly influence the total fresh weight. Supplementation with biostimulants in the nutrient solution, regardless of doses (BT_1 and BT_2), increased all measured biometric variables compared with the control.

The CV × BT interaction resulted in significant differences for all parameters, except the leaf-to-stem ratio and stem diameter. Particularly, for Eleonora and Italiano Classico, compared with the control, the BT_2 dose of biostimulant increased the total fresh weight by 18.99 and 22.63%, respectively. Unlike the other parameters, in Eleonora, the biostimulant did not significantly affect the node number, which increased by 11.78% (on average) in Italiano Classico compared with the control.

The BT × NSC resulted in significant differences for all parameters (**Table 1**). When the biostimulant dose BT_2 was used, the total fresh weight increased by 14.46% in the NSC_1 and 27.34% in the NSC_2 compared with controls. For leaf stem-to-stem ratio and node number parameters, the use of the biostimulant did not determine significant

differences in plants grown in NSC_1. In contrast, in NSC_2, the biostimulant, regardless of the dose, increased (on average) leaf-to-stem ratio and node number by 11.41 and 11.15 compared with the control. The highest stem diameter value (6.10 mm) was obtained from the $BT_1 \times NSC_1$ combination.

Regarding the CV × NSC, in Eleonora, different concentrations of the nutrient solution did not lead to significant differences in leaf-to-stem ratio. On the contrary, increasing the concentration of the nutrient solution increased the number of nodes (+5.4%) but decreased the diameter of the stem (–3.1%). On the other hand, in Italiano Classico, the increase in the concentration of the nutrient solution did not determine significant differences in node number. In contrast, it increased the leaf-to-stem ratio (+6.8%) and de-creased the diameter (–7.2%).

Table 1. Analysis of variance and mean comparisons for total fresh weight, leaf-to-stem ratio, node number, and stem diameter of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Total Fresh Weight	Leaf-to-Stem Ratio	Node Number	Stem Diameter
	g Plant^{-1}		n° Plant^{-1}	mm
Cultivar (CV)				
Eleonora	21.02 ± 0.41 a	1.17 ± 0.01 b	4.18 ± 0.05 a	6.03 ± 0.05 a
Italiano Classico	19.91 ± 0.42 b	1.51 ± 0.02 a	3.93 ± 0.06 b	5.52 ± 0.07 b
Biostimulant Treatment (BT)				
Control	18.52 ± 0.24 c	1.31 ± 0.05 c	3.86 ± 0.07 b	5.64 ± 0.11 b
BT_1	20.50 ± 0.25 b	1.37 ± 0.06 a	4.12 ± 0.06 a	5.84 ± 0.12 a
BT_2	22.36 ± 0.28 a	1.35 ± 0.05 b	4.19 ± 0.06 a	5.84 ± 0.08 a
Nutrient Solution Concentration (NSC)				
NSC_1	20.39 ± 0.33	1.32 ± 0.04 b	4.02 ± 0.05 b	5.92 ± 0.06 a
NSC_2	20.53 ± 0.51	1.37 ± 0.05 a	4.09 ± 0.07 a	5.62 ± 0.09 b
CV × BT				
Eleonora × Control	19.06 ± 0.36 c	1.14 ± 0.01	4.08 ± 0.03 ab	5.91 ± 0.12
Eleonora × BT_1	21.31 ± 0.11 b	1.21 ± 0.03	4.26 ± 0.08 a	6.09 ± 0.07
Eleonora × BT_2	22.68 ± 0.45 a	1.18 ± 0.01	4.21 ± 0.12 a	6.08 ± 0.07
Italiano Classico × Control	17.98 ± 0.06 d	1.47 ± 0.02	3.65 ± 0.05 c	5.36 ± 0.07
Italiano Classico × BT_1	19.69 ± 0.06 c	1.54 ± 0.05	3.98 ± 0.05 b	5.58 ± 0.17
Italiano Classico × BT_2	22.05 ± 0.29 ab	1.52 ± 0.03	4.18 ± 0.02 ab	5.61 ± 0.06
BT × NSC				
Control × NSC_1	18.94 ± 0.41 d	1.34 ± 0.08 bc	3.91 ± 0.12 cd	5.84 ± 0.15 b
BT_1 × NSC_1	20.56 ± 0.42 c	1.28 ± 0.06 cd	4.09 ± 0.02 bc	6.10 ± 0.07 a
BT_2 × NSC_1	21.68 ± 0.20 b	1.32 ± 0.06 bcd	4.07 ± 0.06 bc	5.82 ± 0.07 b
Control × NSC_2	18.10 ± 0.11 e	1.27 ± 0.07 d	3.81 ± 0.09 d	5.43 ± 0.10 c
BT_1 × NSC_1	20.44 ± 0.32 c	1.46 ± 0.09 a	4.15 ± 0.12 ab	5.57 ± 0.17 c
BT_2 × NSC_2	23.05 ± 0.32 a	1.37 ± 0.10 b	4.32 ± 0.07 a	5.86 ± 0.16 b

Cont. **Table 1**

	Total Fresh Weight	Leaf-to-Stem Ratio	Node Number	Stem Diameter
	g Plant^{-1}		n° Plant^{-1}	mm
CV × NSC				
Eleonora × NSC_1	21.01 ± 0.31	1.17 ± 0.01 c	4.07 ± 0.03 b	6.12 ± 0.05 a
Eleonora × NSC_2	21.03 ± 0.78	1.18 ± 0.02 c	4.29 ± 0.08 a	5.93 ± 0.08 b
Italiano Classico × NSC_1	19.78 ± 0.53	1.46 ± 0.01 b	3.98 ± 0.08 bc	5.72 ± 0.07 c
Italiano Classico × NSC_2	20.03 ± 0.67	1.56 ± 0.03 a	3.89 ± 0.08 c	5.31 ± 0.06 d
Significance				
CV	***	***	***	***
BT	***	***	***	***
NSC	ns	***	*	***
CV × BT	**	ns	***	ns
BT × NSC	***	***	***	***
CV × NSC	ns	***	***	***

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student t-test for cultivar and nutrient solution concentration mean effect and according to Tukey–Kramer HSD test for the rest.

3.2. Physiological Parameters

Except for A_{CO2}, no significant differences were observed between the two cultivars for the main physiological parameters reported in **Table 2**. The NSC factor significantly affected the SPAD, A_{CO2}, gs, and E. The BT factor increased all parameters as a function of the biostimulant dose compared with the control.

In the CV × BT interaction, the biostimulant, regardless of dose, increased leaf area (+19.8%) and A_{CO2} (+17.3%) of Eleonora compared with the control. The highest WUEi was obtained from the Eleonora × BT1 combination. In Italiano Classico, the dose of biostimulant BT2 determined the highest leaf area (351.75 cm^2 $plant^{-1}$), A_{CO2} (28.16 μmol CO_2 m^{-2} s^{-1}), and gs (1.38 mol H_2O m^{-2} s^{-1}).

Relative to the BT × NSC, when plants were grown in NSC_1, the leaf area and SPAD increased as the dose of biostimulant increased. The same trend was also observed with NSC_2 but exclusively for the leaf area, while SPAD increased, on average, by 8.2%, compared with the control. Furthermore, in relation to the more concentrated nutrient solution, the highest gs (1.32 mol H_2O m^{-2} s^{-1}) and E (6.92 mol H_2O m^{-2} s^{-1}) were obtained with the BT_2 dose.

As reported in **Table 2**, the CV × NSC interaction did not result in significant differences for SPAD, Fv/Fm, and A_{CO2}. Specifically, in Eleonora, the NSC_2 decreased leaf area and gs by 4.5 and 7.6%, respectively, compared with the NSC_1. In contrast, in Italiano Classico, no significant differences were observed for the above parameters between the different concentrations of nutrient solutions. In Eleonora, E and WUEi did not show significant differences between the different nutrient solutions used. In contrast, in Italiano Classico, the use of the most concentrated solution increased E by 7.7% and decreased WUEi by 6.2%.

Table 2. Analysis of variance and mean comparisons for leaf area, SPAD index, Fv/fm, net CO_2 assimilation rate (A_{CO2}), stomatal conductance (gs), transpiration (E), and instantaneous water use efficiency (WUEi) of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Leaf Area	SPAD Index	Fv/Fm	ACO_2	gs	E	WUE_i
	cm^2 $Plant^{-1}$			μmol CO_2 m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	mol CO_2 mol H_2O^{-1}
Cultivar (CV)							
Eleonora	309.09 ± 7.00	35.86 ± 0.39 a	0.80 ± 0.00	28.22 ± 0.54 a	1.27 ± 0.03	6.18 ± 0.10	4.57 ± 0.07
Italiano Classico	304.47 ± 9.27	34.37 ± 0.38 b	0.79 ± 0.00	25.85 ± 0.46 b	1.24 ± 0.03	6.16 ± 0.12	4.21 ± 0.06
Biostimulant Treatment (BT)							
Control	266.96 ± 5.42 c	33.22 ± 0.31 c	0.79 ± 0.00 c	24.69 ± 0.31 c	1.18 ± 0.01 c	5.80 ± 0.12 c	4.27 ± 0.08 b
BT_1	313.08 ± 4.30 b	35.75 ± 0.40 b	0.80 ± 0.00 b	27.37 ± 0.65 b	1.26 ± 0.04 b	6.19 ± 0.08 b	4.42 ± 0.11 ab
BT_2	340.30 ± 4.10 a	36.37 ± 0.27 a	0.81 ± 0.00 a	29.04 ± 0.37 a	1.32 ± 0.02 a	6.52 ± 0.13 a	4.47 ± 0.10 a
Nutrient Solution Concentration (NSC)							
NSC_1	308.34 ± 7.60	34.45 ± 0.40 b	0.79 ± 0.00	26.79 ± 0.59 b	1.28 ± 0.03 a	6.01 ± 0.11 b	4.46 ± 0.07
NSC_2	305.23 ± 8.81	35.77 ± 0.39 a	0.80 ± 0.00	27.28 ± 0.55 a	1.24 ± 0.02 b	6.34 ± 0.10 a	4.31 ± 0.08
CV × BT							
Eleonora × Control	273.01 ± 9.26 d	33.91 ± 0.31	0.79 ± 0.00	25.30 ± 0.49 c	1.19 ± 0.02 b	5.81 ± 0.11	4.36 ± 0.04 bc
Eleonora × BT_1	325.41 ± 3.88 b	36.60 ± 0.46	0.80 ± 0.00	29.44 ± 0.28 ab	1.36 ± 0.06 a	6.24 ± 0.12	4.72 ± 0.11 a
Eleonora × BT_2	328.86 ± 2.70 b	37.06 ± 0.33	0.81 ± 0.00	29.92 ± 0.21 a	1.26 ± 0.01 b	6.50 ± 0.18	4.62 ± 0.15 ab
Italiano Classico × Control	260.92 ± 5.36 d	32.53 ± 0.37	0.78 ± 0.00	24.08 ± 0.19 c	1.17 ± 0.01 b	5.80 ± 0.22	4.18 ± 0.15 c
Italiano Classico × BT_1	300.75 ± 2.32 c	34.90 ± 0.46	0.80 ± 0.00	25.31 ± 0.29 c	1.17 ± 0.01 b	6.14 ± 0.10	4.12 ± 0.05 c
Italiano Classico × BT_2	351.75 ± 3.76 a	35.68 ± 0.15	0.80 ± 0.00	28.16 ± 0.49 b	1.38 ± 0.01 a	6.55 ± 0.20	4.32 ± 0.12 bc
BT × NSC							
Control × NSC_1	272.69 ± 9.78 c	32.53 ± 0.39 d	0.78 ± 0.00	24.08 ± 0.24	1.19 ± 0.01 b	5.46 ± 0.11 d	4.41 ± 0.08 abc
BT_1 × NSC_1	316.52 ± 7.15 b	34.81 ± 0.42 b	0.80 ± 0.00	27.46 ± 0.81	1.31 ± 0.08 a	6.43 ± 0.05 b	4.27 ± 0.10 bc
BT_2 × NSC_1	335.80 ± 4.95 a	36.02 ± 0.26 a	0.80 ± 0.00	28.83 ± 0.67	1.32 ± 0.03 a	6.13 ± 0.09 c	4.71 ± 0.13 a
Control × NSC_2	261.24 ± 4.51 c	33.91 ± 0.30 c	0.79 ± 0.00	25.30 ± 0.47	1.17 ± 0.02 b	6.14 ± 0.06 bc	4.13 ± 0.11 c
BT_1 × NSC_1	309.64 ± 5.03 b	36.69 ± 0.42 a	0.80 ± 0.00	27.28 ± 1.10	1.21 ± 0.01 b	5.95 ± 0.03 c	4.58 ± 0.18 ab
BT_2 × NSC_2	344.81 ± 6.42 a	36.72 ± 0.45 a	0.81 ± 0.00	29.26 ± 0.36	1.32 ± 0.03 a	6.92 ± 0.04 a	4.23 ± 0.07 c

Cont. **Table 2**

	Leaf Area	SPAD Index	Fv/Fm	ACO_2	gs	E	WUE_i
	cm^2 $Plant^{-1}$			$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	$mol\ H_2O\ m^{-2}\ s^{-1}$	$mol\ H_2O\ m^{-2}\ s^{-1}$	$mol\ CO_2\ mol\ H_2O^{-1}$
CV × NSC							
Eleonora × NSC_1	316.28 ± 6.33 a	35.12 ± 0.48	0.80 ± 0.00	27.91 ± 0.93	1.32 ± 0.05 a	6.08 ± 0.14 bc	4.59 ± 0.10 a
Eleonora × NSC_2	301.91 ± 12.46 b	36.59 ± 0.54	0.80 ± 0.01	28.53 ± 0.59	1.22 ± 0.02 b	6.28 ± 0.15 ab	4.55 ± 0.11 a
Italiano Classico × NSC_1	300.40 ± 13.78 b	33.78 ± 0.58	0.79 ± 0.01	25.67 ± 0.56	1.23 ± 0.04 b	5.93 ± 0.17 c	4.34 ± 0.10 a
Italiano Classico × NSC_2	308.55 ± 13.10 ab	34.95 ± 0.42	0.80 ± 0.00	26.02 ± 0.75	1.25 ± 0.03 ab	6.39 ± 0.15 a	4.07 ± 0.06 b
Significance							
CV	ns	***	ns	***	ns	ns	***
BT	***	***	***	***	***	***	*
NSC	ns	***	ns	*	**	***	**
CV × BT	***	ns	ns	***	***	ns	**
BT × NSC	**	**	ns	ns	*	***	***
CV × NSC	***	ns	ns	ns	***	*	*

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student *t*-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

3.3. Mineral profile

As shown in **Table 3**, the mineral profile was significantly affected by all factors considered in the experiment (CV, BT, and NSC). Except for calcium and magnesium, the CV × BT interaction resulted in significant differences for all the reported parameters (**Table 3**). The application of biostimulants at both doses did not result in significant differences for nitrate and S compared with the control for both cultivars. However, in Eleonora, the dose of BT_2 increased P (+17.1%) and K (+15%) compared with the control, while in Italiano Classico, it increased only P (+41.6%). Regarding the interaction between BT and NSC, the nitrate of plants grown in NSC_1 showed a significant increase with the dose of BT_2 compared with the corresponding control condition.

The use of the biostimulant in NSC_1 increased K and decreased Ca, compared with the control, while no significant differences were recorded for either macroelement in NSC_2. On the contrary, when plants were grown in NSC_2, a decrease in nitrate concentration (–12.4%) was observed with the BT_1 dose compared with the control. Regardless of the NSC, the highest p values were obtained at the dose BT_2. The CV × NSC interaction significantly influenced K and P accumulation only. Specifically, in Eleonora, an increase in K (+13.1%) was observed when NSC_2 was used. On the other hand, the same solution determined the highest p values (6.82 g kg^{-1} d.m.) in Italiano Classico.

Table 3. Analysis of variance and mean comparisons for the mineral concentration of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Nitrate	P	K	Ca	Mg	S
	(mg kg^{-1} fw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)
Cultivar (CV)						
Eleonora	2988.16 ± 122.67 a	6.56 ± 0.15 b	53.17 ± 1.43 a	10.27 ± 0.35 b	3.32 ± 0.06 a	0.95 ± 0.02 b
Italiano Classico	2584.55 ± 92.73 b	7.58 ± 0.37 a	48.51 ± 0.81 b	13.22 ± 0.54 a	3.16 ± 0.05 b	1.46 ± 0.04 a
Biostimulant Treatment (BT)						
Control	2766.18 ± 181.42 b	6.36 ± 0.13 b	47.48 ± 1.88 c	13.13 ± 0.82 a	3.32 ± 0.07 a	1.20 ± 0.08 ab
BT_1	2583.26 ± 123.40 c	6.61 ± 0.27 b	51.13 ± 1.14 b	10.96 ± 0.56 b	3.21 ± 0.06 ab	1.17 ± 0.07 b
BT_2	3009.64 ± 97.30 a	8.24 ± 0.40 a	53.91 ± 1.00 a	11.13 ± 0.56 b	3.18 ± 0.07 b	1.24 ± 0.10 a
Nutrient Solution Concentration (NSC)						
NSC_1	2434.36 ± 90.26 b	6.6 ± 0.18 b	48.34 ± 1.26 b	12.25 ± 0.62 a	3.37 ± 0.03 a	1.26 ± 0.06 a
NSC_2	3138.35 ± 75.61 a	7.55 ± 0.36 a	53.34 ± 1.00 a	11.23 ± 0.51 b	3.11 ± 0.06 b	1.14 ± 0.07 b
CV × BT						
Eleonora × Control	2901.45 ± 336.83 ab	6.24 ± 0.18 c	48.93 ± 3.83 bc	11.92 ± 0.33	3.43 ± 0.05	1.00 ± 0.02 b
Eleonora × BT_1	2837.60 ± 142.44 b	6.14 ± 0.09 c	54.32 ± 0.61 ab	9.44 ± 0.45	3.29 ± 0.11	0.94 ± 0.03 b
Eleonora × BT_2	3225.43 ± 49.60 a	7.31 ± 0.10 b	56.25 ± 0.15 a	9.44 ± 0.36	3.23 ± 0.11	0.91 ± 0.05 b
Italiano Classico × Control	2630.90 ± 155.07 bc	6.48 ± 0.18 bc	46.03 ± 0.16 c	14.35 ± 1.50	3.22 ± 0.13	1.40 ± 0.10 a
Italiano Classico × BT_1	2328.92 ± 144.35 c	7.07 ± 0.48 bc	47.94 ± 1.12 c	12.47 ± 0.51	3.13 ± 0.03	1.40 ± 0.03 a
Italiano Classico × BT_2	2793.84 ± 143.40 b	9.18 ± 0.59 a	51.57 ± 1.49 abc	12.82 ± 0.30	3.14 ± 0.08	1.56 ± 0.04 a
BT × NSC						
Control × NSC_1	2226.11 ± 69.60 d	6.28 ± 0.21 cd	43.34 ± 1.33 c	14.35 ± 1.48 a	3.41 ± 0.05	1.31 ± 0.13 a
BT_1 × NSC_1	2270.56 ± 117.17 d	6.02 ± 0.09 d	49.30 ± 1.66 b	11.16 ± 0.36 b	3.30 ± 0.08	1.22 ± 0.11 ab
BT_2 × NSC_1	2806.42 ± 150.99 c	7.50 ± 0.20 b	52.39 ± 1.81 ab	11.23 ± 0.48 b	3.38 ± 0.04	1.24 ± 0.11 ab
Control × NSC_2	3306.25 ± 152.67 a	6.45 ± 0.16 cd	51.62 ± 2.63 ab	11.92 ± 0.39 ab	3.23 ± 0.14	1.09 ± 0.05 b
BT_1 × NSC_1	2895.95 ± 119.00 bc	7.20 ± 0.42 bc	52.96 ± 1.26 ab	10.75 ± 1.11 b	3.11 ± 0.07	1.12 ± 0.10 ab
BT_2 × NSC_2	3212.85 ± 48.35 ab	8.99 ± 0.67 a	55.43 ± 0.49 a	11.03 ± 1.07 b	2.99 ± 0.04	1.23 ± 0.19 ab

Cont. **Table 3**

	Nitrate	P	K	Ca	Mg	S
	(mg kg^{-1} fw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)
CV × NSC						
Eleonora × NSC$_1$	2606.97 ± 148.52	6.31 ± 0.21 b	49.91 ± 2.43 b	10.62 ± 0.19	3.41 ± 0.04	1.01 ± 0.01
Eleonora × NSC$_2$	3369.35 ± 74.66	6.82 ± 0.18 b	56.43 ± 0.29 a	9.91 ± 0.68	3.22 ± 0.09	0.89 ± 0.03
Italiano Classico × NSC$_1$	2261.76 ± 71.54	6.88 ± 0.28 b	46.78 ± 0.48 b	13.88 ± 0.96	3.32 ± 0.05	1.51 ± 0.03
Italiano Classico × NSC$_2$	2907.35 ± 73.34	8.27 ± 0.63 a	50.25 ± 1.34 b	12.55 ± 0.46	3.00 ± 0.04	1.40 ± 0.07
Significance						
CV	***	***	***	***	***	***
BT	***	***	***	***	*	**
NSC	***	***	***	***	***	***
CV × BT	*	***	***	ns	ns	***
BT × NSC	***	***	***	**	ns	***
CV × NSC	ns	***	***	ns	ns	ns

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student t-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

3.4. Pigments Accumulation

The data presented in **Table 4** show that the BT factor significantly affected all parameters. Regarding the CV effect, except for chlorophyll a/b, the highest values for all parameters were obtained in Eleonora. On the other hand, the NSC factor exclusively influenced chlorophyll b, total chlorophylls, and carotenoids. Regarding the CV × BT interaction, in Eleonora, compared with the control, a dose-dependent increase in chlorophyll a and total chlorophylls was observed. In Italiano Classico, the biostimulant, regardless of dose, significantly increased chlorophyll b and total chlorophylls compared with the control. The biostimulant did not significantly change the chlorophyll a/b ratio for both cultivars. The Italian Classico × BT_2 combination recorded the lowest carotenoids value (0.30 mg g^{-1} fw).

Regarding the BT × NSC interaction, regardless of the concentration of the nutrient solution, the use of biostimulant at both doses resulted in higher values of chlorophyll b and total chlorophylls compared with control conditions. In contrast, the use of biostim-ulants in NSC_1 reduced carotenoids compared with the control. In Eleonora, the more concentrated solution reduced carotenoids (–10.2%) compared with what was obtained from the same cultivar grown in NSC_1.

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Table 4. Analysis of variance and mean comparisons for pigments concentration of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Chlorophyll a	Chlorophyll b	Total Chlorophylls	Carotenoids	Chlorophyll a/b
	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	
Cultivar (CV)					
Eleonora	1.13 ± 0.02 a	0.67 ± 0.02 a	1.75 ± 0.03 a	0.37 ± 0.01 a	1.703 ± 0.04
Italiano Classico	1.07 ± 0.02 b	0.63 ± 0.02 b	1.68 ± 0.03 b	0.34 ± 0.01 b	1.742 ± 0.06
Biostimulant Treatment (BT)					
Control	1.02 ± 0.01 b	0.55 ± 0.02 b	1.55 ± 0.02 c	0.36 ± 0.02 a	1.875 ± 0.07 a
BT_1	1.11 ± 0.02 a	0.68 ± 0.01 a	1.74 ± 0.01 b	0.36 ± 0.01 a	1.645 ± 0.03 b
BT_2	1.16 ± 0.03 a	0.71 ± 0.03 a	1.84 ± 0.03 a	0.34 ± 0.01 b	1.647 ± 0.07 b
Nutrient Solution Concentration (NSC)					
NSC_1	1.10 ± 0.02	0.62 ± 0.02 b	1.68 ± 0.03 b	0.37 ± 0.01 a	1.806 ± 0.06
NSC_2	1.10 ± 0.02	0.68 ± 0.02 a	1.74 ± 0.04 a	0.34 ± 0.01 b	1.639 ± 0.04
CV × BT					
Eleonora × Control	1.05 ± 0.01 c	0.60 ± 0.01 b	1.61 ± 0.01 c	0.37 ± 0.01 a	1.740 ± 0.03 ab
Eleonora × BT_1	1.11 ± 0.04 b	0.68 ± 0.02 ab	1.73 ± 0.01 b	0.36 ± 0.01 a	1.637 ± 0.04 b
Eleonora × BT_2	1.23 ± 0.02 a	0.73 ± 0.05 a	1.91 ± 0.04 a	0.37 ± 0.01 a	1.732 ± 0.13 b
Italiano Classico × Control	1.00 ± 0.02 c	0.51 ± 0.03 c	1.50 ± 0.03 d	0.36 ± 0.03 a	2.009 ± 0.13 a
Italiano Classico × BT_1	1.11 ± 0.02 b	0.67 ± 0.01 ab	1.76 ± 0.02 b	0.35 ± 0.02 a	1.653 ± 0.04 b
Italiano Classico × BT_2	1.09 ± 0.02 bc	0.70 ± 0.01 a	1.78 ± 0.02 b	0.30 ± 0.01 b	1.562 ± 0.01 ab
BT × NSC					
Control × NSC_1	1.03 ± 0.01	0.53 ± 0.04 d	1.54 ± 0.04 c	0.41 ± 0.01 a	1.976 ± 0.13
BT_1 × NSC_1	1.13 ± 0.03	0.68 ± 0.01 b	1.74 ± 0.01 b	0.34 ± 0.02 bc	1.663 ± 0.04
BT_2 × NSC_1	1.15 ± 0.05	0.65 ± 0.01 bc	1.78 ± 0.02 b	0.35 ± 0.02 bc	1.779 ± 0.10
Control × NSC_2	1.02 ± 0.02	0.58 ± 0.01 cd	1.57 ± 0.03 c	0.32 ± 0.01 c	1.774 ± 0.04
BT_1 × NSC_1	1.10 ± 0.03	0.68 ± 0.02 b	1.75 ± 0.02 b	0.37 ± 0.01 ab	1.627 ± 0.04
BT_2 × NSC_2	1.17 ± 0.03	0.78 ± 0.03 a	1.91 ± 0.04 a	0.32 ± 0.01 c	1.515 ± 0.06

Cont. **Table 4**

	Chlorophyll a	Chlorophyll b	Total Chlorophylls	Carotenoids	Chlorophyll a/b
	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	
CV × NSC					
Eleonora × NSC_1	1.15 ± 0.03 a	0.64 ± 0.01	1.73 ± 0.03	0.39 ± 0.01 a	1.793 ± 0.06
Eleonora × NSC_2	1.10 ± 0.03 ab	0.69 ± 0.04	1.77 ± 0.06	0.35 ± 0.00 b	1.614 ± 0.06
Italiano Classico × NSC_1	1.05 ± 0.02 b	0.59 ± 0.04	1.64 ± 0.05	0.34 ± 0.02 b	1.819 ± 0.12
Italiano Classico × NSC_2	1.09 ± 0.03 ab	0.66 ± 0.03	1.72 ± 0.05	0.33 ± 0.02 b	1.664 ± 0.05
Significance					
CV	***	**	***	***	ns
BT	***	***	***	***	***
NSC	ns	***	***	***	***
CV × BT	**	*	***	***	***
BT × NSC	ns	***	*	***	ns
CV × NSC	**	ns	ns	**	ns

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student *t*-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

4. Discussion

Hydroponic systems are increasingly popular and widely used to improve the yield of leafy vegetables such as basil (*Ocimum basilicum* L.) and, not least, are valuable tools for understanding how the combined action of preharvest factors affects the leaves' characteristics. For this purpose, we evaluated the supplementation of plant-derived protein hydrolysate in two nutrient solutions with different macronutrient concentrations to understand and improve the yield and physiological response of two Genovese basil cultivars grown in a floating raft system.

The lower unit yields of Genovese basil grown in open fields recorded by Nicoletto et al.[33] and Formisano et al.[34] show that hydroponics, thanks to the higher planting density, the lower abiotic and biotic pressure, and the potentially unlimited availability of nutrients and water, maximizes the production of this leafy vegetable, confirming the results of Ciriello et al.[30].

Furthermore, the better growth conditions of our system also affected leaf dry matter, which was significantly lower compared with the authors' findings mentioned above. Additionally, the leaf-to-stem ratio recorded by Formisano et al.[34] in the open field of the same basil cultivars (Eleonora and Italiano Classico) was lower. The leaf-to-stem ratio is a crucial quality parameter for the industrial production of "pesto sauce", as an excessive fibrousness of the stem extends the processing time needed to process the leaves (increased temperature), triggering oxidative processes resulting in the blackening of the green sauce with negative impacts on the quality of the final product[28].

Regardless of the NSC and BT factors, the measured parameters significantly depended on the genetic material, although both basil cultivars belonged to the 'Genovese' type. These findings are not surprising since it is well established in the literature that different basil cultivars can have distinct productive and physiological properties[33,35-37]. In particular, Eleonora had a higher fresh yield compared with Italiano Classico. This result is probably attributable to the stem component (> stem diameter and > node number) since the leaf area did not vary significantly among the two cultivars. It should be noted that Eleonora was characterized by higher pigments (chlorophyll a, b, and total and carotenoids), which, in addition to affecting net CO_2 assimilation, resulted in a higher SPAD index. An increase in the SPAD index is often correlated with higher greenness, which is a valuable quality characteristic for leafy vegetables such as basil[38].

In soilless hydroponic systems, in addition to genetic material, the careful management of macronutrients is also crucial to achieving high production and better physiological response. Although no ideal electrical conductivity value is known for different environmental conditions[39], overconcentrated or under concentrated nutrient solutions negatively affect the nutritional status and growth of vegetables[1,39]. However,

total fresh weight and ACO_2 were not significantly affected by the NSC at both levels (1 and 2 dS m^{-1}) in either cultivar. Our results probably reflect the fact that the macronutrient concentration in the nutrient solutions was optimal for basil, which was also confirmed by the maximum quantum efficiency of the PSII photochemistry (Fv/Fm) that did not vary significantly in the NSC treatment 40. Similarly, Hosseini et al.[1] observed a yield reduction at NSC of less than 0.9 ds/m, while Walters and Currey[41] did not show significant differences in Sweet, Holy, and Lemon basil up to 4 ds/m.

Although no production differences were observed for the NSC factor, regardless of CV and BT, the nitrate increased by 28.9% when the nutrient solution concentration was doubled (2 ds/m; **Table 3**). The higher transpiration (E) of plants grown in 2 ds/m nutrient solutions probably accounts for the observed luxury consumption of nitrate (**Tables 2 and 3**). Similar results were reported in previous work on basil[42], pakchoi (*Brassica campestris* L. ssp. Chinensis)[39], and lettuce (*Lactuca sativa* L.)[43]. However, as reported by Colla et al.[44], it should be noted that nitrate accumulation in leaves is also strongly dependent on the genetic aspect. Eleonora and Italiano Classico showed different responses to nitrate ac-cumulation in the leaves, with the latter having the lowest value (**Table 3**). Similar to nitrate, the use of a more concentrated nutrient solution resulted in the luxury consumption of potassium, as evidenced by Walters and Currey[41]. Regardless of the effect of the CV, the increase in potassium in the leaves was coupled with a reduction in magnesium and calcium as the nutrient solution concentration increased, probably because of the well-recognized antagonism between these macroelements[41].

Similar to what was observed by Hosseini et al.[1], our study shows a positive cor-relation between the concentration of macronutrients in the nutrient solution and the chlorophylls (**Table 4**). Regardless of CV and BT, the higher chlorophylls a, b, and total chlorophylls obtained in plants grown at EC 2 dS m^{-1} were probably attributable to the higher nitrogen values. Nitrogen is one of the critical constituents of chlorophyll as it is involved in its biosynthesis and the structure of the porphyrin ring[45]. Chenard et al.[46] reported a concomitant increase in carotenoids and chlorophylls in hydroponically grown parsley (*Petroselinum crispum* L. cv. Dark Green Italian), a finding that is not in line with our results. To confirm the unclear dependence of carotenoids on nutrient solution con-centration, Fallovo et al.[43] reported that the use of nutrient solutions with different concentrations did not affect the amount of these pigments in lettuce.

Soilless systems, such as the floating graft system, are highly dependent on external chemical input, making them not always environmentally sustainable. For this reason, biostimulant supplementation in the nutrient solution could be a valuable tool to increase the sustainability of hydroponic systems[7,8]. In the present study, regardless of CV and NSC, total fresh weight, leaf area, leaf-to-stem ratio, node number, and stem

diameter increased with protein hydrolysate biostimulant (PHs) in the nutrient solution (**Tables 1 and 2**). The yield improvements obtained are consistent with what has been reported by several authors on horticultural crops[16,47,48]. The increased percentage of dry matter does not entirely agree with the literature reviewed. Caruso et al.[47], Rouphael et al.[48], and El-Nakhel et al.[32] reported contrasting results after application of PH in arugula (*Diplotaxis tenuifolia* (L.) DC), spinach, and two different species of microgreens (*Daucus carota* L. and *Anethum graveolens* L.), respectively, underlining how interactions between physiologically active compounds of PH are highly dependent on plant species, environmental and growth conditions, dose, and application time[49]. However, interestingly, supplementation of PHs in the less concentrated nutrient solution ensured higher fresh yield than what was obtained from control x NSC_2, confirming in part that the use of amino acids to replace a part of nitrogen fertilization can support the yield and growth of leafy vegetables in hydroponics[50].

Although the physiological and biochemical mechanisms underlying the effects of biostimulants are still unclear, we hypothesize that the improved fresh production achieved is attributable to the bioactive molecules in Trainer®. Bioactive compounds such as readily absorbed amino acids and PH signaling peptides provide a plethora of ben-eficial effects that influence the growth and upregulation of N and C metabolism[51]. Specifically, the main effects of PHs would seem to be attributable to the "hair growth-promoting peptide", which can modify the root architecture (increased length, density, and the number of lateral roots), leading to increased nutrient uptake[52,53]. Furthermore, biostimulants would also act as physiological primers that can promote indol-3-acetic acid (IAA) and abscisic acid (ABA) biosynthesis and enhance photosynthetic activity[16,55,56].

Similar to the findings of Cristofano et al.[51] on two *Lactuca sativa* L. cultivars (Ballerina and Canasta) grown hydroponically, the use of biostimulant in nutrient solution at dose BT_2 significantly increased A_{CO2} in both cultivars, justifying the increase in yield (**Tables 1 and 2**). As hypothesized by Rouphael et al.[21], the improvement in net CO_2 assimilation rate could be reasoned to be the beneficial effect of the biostimulant on stomatal conductance. Furthermore, the improved photosynthetic performance and the higher value of water use efficiency (WUEi) obtained at the dose of BT_2, compared with the control condition, were accompanied by an increase in K (**Table 3**). This element plays a crucial role in osmotic balance and turgor-dependent processes such as stomatal opening and thus CO_2 diffusion; its increase could have induced better stomatal reactivity by stimulating growth and yield[54,55]. The improved functioning of the photosynthetic machinery after the application of PHs in the nutrient solution could have been related to a higher amount of pigment and a higher Fv/Fm, compared with the control (**Tables 2 and 4**). Indeed, as suggested by Yakhin and collaborators[56], the

biostimulant would improve light utili-zation efficiency, dissipate excitation energy in photosystem II antennae, and increase the photosynthetic pigment biosynthesis.

Moreover, the reviewed literature shows a clear effect of biostimulants on reducing the nitrate concentration in leafy vegetables such as arugula, lettuce, chard, spinach, peppermint, spearmint, and pakchoi[4,57]. As suggested by Colla et al.[58] and Calvo et al.[59], PHs would regulate metabolic pathways involved in nitrogen metabolism by over-loading the phloem with amino acids, thereby limiting nitrate uptake and storage. However, our results on nitrate storage in basil tissues at different doses of biostimulants in nutrient solution are contradictory. At the BT_1 dose, the nitrate decreased significantly (−6.6%) compared with the control, consistent with the results reported by the authors above. Nitrate reduction was even more evident when plants were grown in NSC_2 (**Table 3**). On the contrary, the BT_2 dose, regardless of the NSC and CV, caused an increase in nitrate (+8.8%), probably attributable to increased plant transpiration activity (**Tables 2 and 3**). This discrepancy in the results recorded in our experiment further emphasizes how the effects of the biostimulant on the storage of nitrate in basil are strongly influenced by the dose used.

5. Conclusions

Reducing chemical inputs is vital, especially in hydroponics, which uses nutrient solutions that often exceed the real needs of plants. From this perspective, the integration of biostimulants into nutrient solutions is an environmentally sustainable strategy for horticultural production. Our results showed a different physiological and productive response between Eleonora and Italiano Classico. Particularly, Eleonora provided the highest fresh production but the lowest leaf-to-stem ratio, an essential parameter for transforming leaves into "pesto" sauce. Italiano Classico recorded the lowest nitrate values. Surprisingly, the concentration of the nutrient solution did not affect the fresh production in either cultivar, while nitrate, phosphorus, potassium, and total chlorophyll increased as the concentration of macronutrients increased. Supplementation of Trainer® into the nutrient solution improved physiological parameters (ACO_2, gs, E, Fv/Fm) in a dose-dependent manner, thus increasing fresh production. In particular, the BT_1 × NSC_1 combination compared with the control condition with double the fertilizer concentration in the nutrient solution (NSC_2) showed that a significant yield increase could be achieved in a sustainable manner, and it demonstrated the feasibility of filling in the reduction in fertilizer input, with interesting economic implications to consider. Not least, regardless of cultivar and nutrient solution, using the biostimulant at the BT_1 dose significantly reduced nitrate, the antinutritional compound par excellence in leafy vegetables.

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Chapter 10
Zinc biofortification of hydroponically grown basil: stress physiological responses and impact on antioxidant secondary metabolites of genotypic variants

Abstract: *Ocimum basilicum* L. is an aromatic plant rich in bioactive metabolites beneficial to human health. The agronomic biofortification of basil with Zn could provide a practical and sustainable solution to address Zn deficiency in humans. Our research appraised the effects of biofortification implemented through nutrient solutions of different Zn concentration (12.5, 25.0, 37.5, and 50 μM) on the yield, physiological indices (net CO_2 assimilation rate, transpiration, stomatal conductance, and chlorophyll fluorescence), quality, and Zn concentration of basil cultivars 'Aroma 2' and 'Eleonora' grown in a floating raft system. The ABTS, DPPH, and FRAP antioxidant activities were determined by UV-VIS spectrophotometry, the concentrations of phenolic acids by mass spectrometry using a Q Extractive Orbitrap LC-MS/MS, and tissue Zn concentration by inductively coupled plasma mass spectrometry. Although increasing the concentration of Zn in the nutrient solution significantly reduced the yield, this reduction was less evident in 'Aroma 2'. However, regardless of cultivar, the use of the maximum dose of Zn (50 μM) increased the concentration of carotenoids, polyphenols, and antioxidant activity on average by 19.76, 14.57, and 33.72%, respectively, compared to the Control. The significant positive correlation between Zn in the nutrient solution and Zn in plant tissues underscores the suitability of basil for soilless biofortification programs.

Keywords: *Ocimum basilicum* L.; Floating raft system; Zn agronomic biofortification; Pigments; UHPLC; Phenolics

1. Introduction

A diversified and well-balanced diet based on high nutritional quality foods is prerequisite to good health and, according to the World Health Organization (WHO), it is dependent on the development of sustainable agricultural systems[1,2]. Insidious and invisible, the "hidden hunger" associated with micronutrient malnutrition (Fe, Zn, I, and Se) affects more than two billion people in underdeveloped areas even in the most industrialized countries[1,3]. The impact is so dramatic that, according to the World Bank, the economic cost of dealing with the problem is estimated to about 5% of a country's gross domestic product[4]. Although the role of Zn in human nutrition has been known since 1961, its deficiency is widespread[1]. Given its vital function in critical phases of growth, development, and reproduction, inadequate Zn intake jeopardizes the mental and physical well-being of adults and children by altering the immune, nervous, visual, gastrointestinal, and skeletal systems and increasing the incidence of infections and cancer[5-9]. Although Zn deficiency is associated with overconsumption of processed foods and grains high in phytates, it should be noted that agricultural soils often limit bioaccumulation of this valuable mineral in agricultural products due to low phyto-availability or total deficiency[10,11]. The link between agriculture and nutrition highlights how the mineral enrichment process of agricultural products, known as biofortification, is a practical and sustainable solution to Zn deficiency in humans, since most of the human diet is plant-based[4,12]. Through agronomic practice, genetic improvement, and genetic engineering strategies, biofortification can increase the bioavailability of essential trace elements in the edible parts of plants[4,13]. The agronomic approach, based on crop management and fertilization practices to improve the mobilization and uptake of microelements by plants, has been recognized as the most practical and user-friendly biofortification strategy[2,3]. Agronomic biofortification of staple crops is often ineffective in providing adequate Zn intake due to the presence of antinutritional compounds (*e.g.*, tannins and phytates) that suppress intestinal assimilation. In this regard, greater interest should be given to the biofortification of leafy vegetables because it facilitates higher Zn concentrations transported mainly through the xylem[14]. However, the results achievable by ordinary agronomic soil biofortification programs, either through fertilization or by foliar application, are severely influenced by interactions between genotype, environment, and soil characteristics, as well as nutrient interactions during uptake[2].

From this point of view, the limitations of agronomic biofortification in soil cultivation can be overcome by using hydroponic growing systems where nutrient solutions with ad hoc Zn concentrations would allow a standardized, fine control of leafy vegetable quality as already observed in *Lactuca sativa* L., *Thlaspi caerulescens*, and *Brassica oleracea*[10,15,16]. Furthermore, soilless growing systems could ensure efficient high-yield and high-quality production even under land-limiting (such as growing in urban

areas) or prohibitive (contaminated soils and scarce water resources) environments[3,17-19.] A successful hydroponic biofortification program could also be implemented on aromatic herbs such as basil, to increase the concentration of desirable secondary metabolites (such as phenolic acids and volatile aroma compounds) that characterize the flavor of tender green leaves and constitute traits of premium quality that consistently attract the interest of producers and consumers[20]. Zn is also essential for plants to perform crucial metabolic functions. This micronutrient is an integral component of enzymes, involved in the synthesis and degradation of sugars, lipids, and nucleic acids, it regulates the translation and transcription of DNA, stabilizes proteins, repairs photosystems, and regulates the function of chloroplasts, oxidoreductases, and hydrolytic enzymes[3,4,11,12]. Roots take Zn primarily as Zn^{2+} through ZIP transporters or chelated with low molecular weight compounds (phytosiderophores), a mechanism typical only of *Poaceae*[4,21]. In the plant, Zn is carried through the xylem either symplastically or apoplastically in its ionic form or bound with organic acids, histidine or nicotianamine[22] and the differences in concentration in the edible parts may depend both on the mode of uptake and on the distribution among the plant organs but especially on the species[10]. The hyperaccumulative *Brassicaceae*, *Caryophyllaceae*, *Polygonaceae*, and *Dichapetalaceae* can bioaccumulate up to 3,000 mg kg^{-1} dry weight of Zn (on average)[21]. In general, to support vital and metabolic functions, most plants need foliar Zn concentrations greater than 15-30 mg kg^{-1} dry weight, under which inhibition of photosynthesis and respiration rate, disruption of plasma membranes, increase in reactive oxygen species (ROS), and reduction in yield are observed[1,3,4,11]. However, in non-hyperaccumulative species, foliar concentrations of Zn over 100-700 mg kg^{-1} dry weight are toxic[23], causing growth reduction and yield suppression, chlorosis and leaf necrosis, reduced shoot and root development, reduced stomatal conductance and net carbon dioxide fixation, reduced and structural changes in chlorophyll, altered mitotic activity and membrane permeability, oxidative stress and lipid peroxidation, thus constraining biofortification programs[24,25]. Although several authors have reported critical ranges of Zn in cabbage (74-1,201 mg kg^{-1}), lettuce (20-60 mg kg^{-1}), broccoli (117-1,666 mg kg^{-1}), and leafy greens (up to 700 mg kg^{-1})[11,26], to our knowledge, no research has studied the effects of Zn biofortification on basil (*Ocimum basilicum L.*). The scientific literature has not classified basil as either a hyperaccumulator or non-hyperaccumulator species. In any case, in light of the encouraging results obtained with Selenium and Iodine biofortification programs[27-29] we hypothesize that conditions dictated by hydroponics (floating raft system) and the use of biofortified nutrient solutions at different concentrations of Zinc (12.5, 25.0, 37.5, and 50 μM) would help to understand the relationships between Zinc and basil. Based on the above, our study aimed to evaluate the impact of biofortification on the yield, physiological responses, quality, and Zn bioaccumulation in two basil cultivars (Aroma 2 and Eleonora) grown in a floating

raft system. The current work constitutes an important continuation of our earlier work (recently submitted for publication) that examines Zn biofortification of Genovese basil concerning the crop's mineral profile and the implications of biofortification applications on estimated daily intake of adults and children.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

The experimental trial was conducted at the Department of Agriculture, Federico II University, (Portici, NA, Italy; 43° 10′ N; 14° 58′ E, 60 m a.s.l.) in an unheated greenhouse from May 3 to 26, 2021. Genovese basil seedlings (*Ocimum basilicum* L.) 'Aroma 2' (Fenix, Belpasso, CT, Italy) and 'Eleonora' (Enza Zaden, Enkhuizen, NL-NH, The Netherlands) were sown at a density of 317 pt m^{-2} on April 13, 2021 in peat and vermiculite (1:2 *v*/*v*) in 54-hole polystyrene trays (52 × 32 × 6 cm; volume 0.06 L) and grown in a floating raft system (FRS) in individual plastic trays filled with 35 L of nutrient solution (NS). As indicated by Ciriello et al.[30], a control nutrient solution with osmotic water was prepared using the following concentration of macro and micronutrients: 14 mM N-NO_3^-, 1.5 mM P, 1.75 mM S, 3.0 mM K, 4.5 mM Ca, 1.5 mM Mg, 1.0 mM NH_4^+, 15 µM Fe, 9 µM Mn, 1.6 µM Zn, 0.3 µM Cu, 20 µM B, and 0.3 µM Mo. The trial was carried out in a randomized design with three replicates in a factorial arrangement (2 × 5), with two basil cultivars (Aroma 2 and Eleonora) and four biofortification treatments plus control. The latter consisted of four doses of Zn (12.5, 25, 37.5, and 50 µM) using $ZnSO_4 \times 7H_2O$ (Sigma-Aldrich, St. Louis, MO, USA) as a Zn source in the nutrient solution. Each experimental unit consisted of 54 plants. Biofortified nutrient solutions were provided twenty days after planting (at the phenological stage of two true leaves). The biofortified treatment lasted for 23 days. Plants were grown under natural light conditions. During the growing cycle, temperature and relative air humidity were recorded continuously with an interval of 10 minutes with dedicated WatchDog A150 dataloggers (Spectrum Technologies Inc., Aurora, IL, USA) placed at canopy level. Specifically, the day/night average air temperature and relative humidity were 27/18 °C and 50/70%, respectively.

2.2. Sampling and Determination of Biometric and Yield Parameters

Before flowering (43 days after sowing), twenty plants per replicate were sampled for the determination of height (cm), number of leaves, and fresh biomass (g plant−1). Leaf area was quantified using ImageJ v1.52a software (U.S. National Institutes of Health of the United States, Bethesda, USA). The epigeal parts of each plant were dried in a ventilated oven at 70 °C for 3 days to determine the dry biomass of the shoots and roots (g $plant^{-1}$) and their percent ratio. The dry matter (%) of the shoots was calculated as follows:

$$\frac{Shoots\ dry\ biomass}{Shoots\ fresh\ biomass} \times 100$$

The dry plant material was ground and sieved using an MF10.1 Wiley laboratory mill equipped with an MF0.5 sieve (IKA®, Staufen im Breisgau, BW, Germany) for mineral concentration determination. A representative plant sample was collected for each experimental unit and stored in liquid nitrogen for further qualitative analysis.

2.3. Leaf Color Assessment

Leaf color assessment according to human vision was performed using the CIELab color space defined by the CIE (International Commission on Illumination), which separates greyscale (L*) information more clearly from color (a* and b*) information. The color coordinates were measured on twenty young fully expanded leaves per replicate using a Minolta CR-300 colorimeter (Minolta Co. Ltd, Osaka, Japan). Chroma and hue angle were calculated from the equations reported by Ciriello, et al.[30]

2.4. Physiological Parameters, SPAD Index, and Pigments Measurement

Leaf gas exchange measurements were performed with a Li-6400 hand-held analyzer (LI-COR Biosciences, Lincoln, NE, USA). The measured parameters of interest were the net assimilation of CO^2 (A_{CO2}), the transpiration rate (E), and the stomatal conductance (gs). Relative humidity (RH) and CO^2 concentration of the leaf gas exchange analyzer were set at ambient values, while photosynthetically active radiation (PAR) and airflow rate were constant at 2,000 μmol m^{-2} s^{-1} and 500 mL s^{-1}, respectively. Chlorophyll fluorescence (Fv/Fm) measurements were made using a fluorometer Fv/Fm meter (Opti-Sciences, Hudson, NH, USA). The SPAD index was assessed using a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan). All physiological measurements were performed between 09:30 and 12:00 am on five fully expanded healthy young leaves for each replicate.

Chlorophyll a and b concentrations were determined by UV-Vis spectrophotometry (ONDA V-10 Plus, Giorgio Bormac Srl, Carpi, Italy) with an absorbance of 647 and 664, respectively, as described by Wellburn[31]. Total chlorophyll was calculated as chlorophyll a + chlorophyll b and was expressed as mg g^{-1} fresh weight (fw).

The β-carotene and lutein concentrations were quantified by high-performance liquid chromatography with diode array detection (HPLC-DAD) after extraction according to Salomon, et al.[32]. External standards of β-carotene and lutein (Sigma-Aldrich, Milan, Italy) were used to create the respective calibration curves. The results were expressed as μg g^{-1} dw.

2.5. Determination of Zn Concentration

According to the method described by Volpe, et al.[33] the Zn concentration in basil [μg g^{-1} dry weight (dw)] was determined by an inductively coupled plasma mass spectrometer (ICP-OES Spectroblue, Spectro Ametek, Berwyn, PA, USA) after digestion

with a mixture of HCl (37%) and HNO3 (65%) (3:9, *v*/*v*). An appropriate calibration curve was prepared using a standard solution with 1.0 to 100 µg L^{-1} Zn concentrations.

2.6. ABTS, DPPH, and FRAP Antioxidant Activities Determination

The antioxidant activities ABTS+ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reduction antioxidant potency) were determined by UV-VIS spectrophotometry (Shimadzu, Japan) according to the protocols described by Formisano, et al.[34]. Results were expressed as mmol Trolox equivalents kg^{-1} dw. Phenolic Concentration Determination

One hundred milligrams of freeze-dried basil were used to quantify and determine polyphenols using a UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a thermo-stated column (T=25 °C, 100 × 2.1 mm, Kinetex 1.7 µm biphenyl, Phenomenex, Torrance, CA, USA) and a quaternary pump (Ultimate 3000, Dionex, Sunnyvale, CA, USA). Mass spectrometry analysis was facilitated by a Q Exactive Orbitrap LC-MS/MS system (Thermo Fisher Scientific, Waltham, MA, USA). Phenolic compound sampling was performed according to the protocol detailed by Pannico, et al.[35]. The accuracy and calibration of the instruments used were set and checked using a mixture of reference standards (Thermo Fisher Scientific, Waltham, MA, USA). Data processing and analysis were performed using Xcalibur software, version 3.0.63 (Thermo Fisher Scientific, Waltham, MA, USA), and the results were expressed as µg g^{-1} dw.

2.7. Statistics

Data were subjected to analysis of variance (ANOVA) and means for cultivar treatment (CV) were compared by the Student's t-test, whereas the means for the biofortification treatment (Zn) and the two-way interaction (CV × Zn) were compared using the Tukey-Kramer HSD test at the $p < 0.05$ level. SPSS 20 software package (IBM Corp., Armonk, NY, USA) was used. Data represent mean ± standard error of 3 replicates ($n = 3$).

3. Results

3.1. Biometric and Yield Parameters

Plant height, number of leaves, fresh biomass, total dry biomass, and dry matter content for the control treatment were higher for 'Aroma 2' than 'Eleonora' (**Table 1**). On the other hand, the plant root dry weight of 'Eleonora' was higher than 'Aroma 2'. Significant Cultivar × Zn biofortification interaction was observed for all the parameters presented in **Table 1**, since Zn biofortification treatments did not always have the same effect on both cultivars. In particular, while both cultivars' plant height was negatively

affected by only one Zn solution treatment compared to the control, this treatment differed between 'Aroma 2' (12.5 μM) and 'Eleonora' (37.5 μM). Leaf area was reduced on average by 17.1% in 'Aroma 2' and by 25.2% in 'Eleonora'. It is noteworthy that the 25 μM Zn treatment did not have a significant effect on the leaf area of 'Aroma 2'. In addition, the plant leaf number did not appear to be as affected by Zn concentration treatments in 'Aroma 2' compared to 'Eleonora', in which it decreased analogously to increasing Zn concentration. Similarly, while 'Eleonora' fresh biomass declined almost uniformly with increasing Zn concentration in the nutrient solution, 'Aroma 2' fresh biomass was not affected by all Zn treatments. On the other hand, while Zn treatments affected both cultivars' total dry biomass negatively, Zn treatments overall reduced the dry biomass of 'Aroma 2' almost double (19%) that of 'Eleonora' (10%), compared in either case to the control. Cultivar differentiation with Zn biofortification was also observed in relation to dry matter content. The latter increased in 'Eleonora' in comparison to the control with all Zn concentration levels applied, while in 'Aroma 2' an increase was observed only after the application of 50.0 μM Zn in the nutrient solution. However, the application of the highest Zn (50 μM) concentration in the nutrient solution resulted in the highest content of dry matter in both cultivars. Dissimilar was also the behavior of the two cultivars concerning root dry weight, as 'Aroma 2' root dry weight decreased only with the application of 12.5 μM Zn in the nutrient solution, while all Zn treatments, except for 50 μM Zn, reduced the root dry weight of 'Eleonora' compared to the Control.

Table 1. Analysis of variance and mean comparisons for height, leaf number, leaf area, plant fresh biomass, total dry biomass, root dry weight, and dry matter of Aroma 2 and Eleonora basil cultivars grown hydroponically under different Zn treatments: 0 = Control; 12.5; 25; 37.5; 50 µmol of Zn].

Treatment	Height	Leaf number	Leaf area	Plant fresh biomass	Total dry biomass	Root dry weight	Dry matter
	cm	n°	cm²	%			
Cultivar (CV)							
Aroma 2	40.02 ± 0.32a	41.65 ± 0.91a	405.524 ± 9.017	23.41 ± 0.22a	2.31 ± 0.06a	0.221 ± 0.003b	10.4 ± 0.09a
Eleonora	35.48 ± 0.28b	35.98 ± 1.24b	393.971 ± 14.935	19.17 ± 0.53b	1.86 ± 0.03b	0.249 ± 0.007a	9.64 ± 0.13b
Zinc (Zn)							
Control	38.01 ± 1.02a	45.92 ± 0.86a	477.471 ± 10.989a	23.23 ± 0.66a	2.38 ± 0.16a	0.256 ± 0.012a	9.52 ± 0.24d
12.5 µM	38.40 ± 1.39a	38.88 ± 1.74b	398.609 ± 5.555b	21.78 ± 0.89b	1.96 ± 0.08c	0.218 ± 0.004c	9.99 ± 0.17b
25 µM	36.94 ± 0.57b	38.60 ± 0.80b	398.794 ± 10.659b	21.74 ± 0.78b	2.01 ± 0.09c	0.213 ± 0.003c	9.74 ± 0.16c
37.5 µM	36.59 ± 1.27b	35.98 ± 1.36c	360.941 ± 5.043c	20.52 ± 1.09c	1.97 ± 0.08c	0.236 ± 0.004b	10.17 ± 0.18b
50 µM	38.80 ± 0.90a	34.69 ± 1.83c	362.924 ± 9.613c	19.19 ± 1.39d	2.12 ± 0.10b	0.251 ± 0.010a	10.67 ± 0.13a
CV × Zn							
Aroma 2 × Control	40.27 ± 0.33ab	47.67 ± 0.36a	461.225 ± 8.046ab	24.68 ± 0.20a	2.73 ± 0.01a	0.229 ± 0.002bc	10.06 ± 0.07de
Aroma2 × 12.5	41.46 ± 0.16a	42.76 ± 0.17b	397.804 ± 5.086cd	23.74 ± 0.13ab	2.14 ± 0.02c	0.212 ± 0.002cd	10.36 ± 0.01bcd
Aroma2 × 25	38.19 ± 0.19c	40.25 ± 0.25c	419.435 ± 3.633bc	23.44 ± 0.08bc	2.21 ± 0.03c	0.205 ± 0.003d	10.08 ± 0.12cde
Aroma2 × 37.5	39.40 ± 0.30bc	38.83 ± 0.98cd	366.158 ± 4.701de	22.92 ± 0.06bcd	2.15 ± 0.03c	0.228 ± 0.004bc	10.56 ± 0.04b
Aroma2 × 50	40.79 ± 0.02a	38.75 ± 0.47cd	383.000 ± 3.704cde	22.28 ± 0.09cd	2.34 ± 0.01b	0.232 ± 0.003bc	10.92 ± 0.14a
Eleonora × Control	35.75 ± 0.10de	44.17 ± 0.68b	493.718 ± 16.586a	21.78 ± 0.11d	2.03 ± 0.03d	0.282 ± 0.006a	8.98 ± 0.03h
Eleonora × 12.5	35.33 ± 0.43e	35.00 ± 0.17ef	399.415 ± 11.303cd	19.82 ± 0.30e	1.78 ± 0.01f	0.224 ± 0.001bcd	9.62 ± 0.02fg
Eleonora × 25	35.69 ± 0.10de	36.96 ± 0.65de	378.153 ± 11.349cde	20.04 ± 0.35e	1.81 ± 0.02ef	0.222 ± 0.004cd	9.39 ± 0.03g
Eleonora × 37.5	33.79 ± 0.40f	33.13 ± 0.38fg	355.724 ± 8.823de	18.12 ± 0.37f	1.79 ± 0.01f	0.245 ± 0.002b	9.78 ± 0.02ef
Eleonora × 50	36.81 ± 0.29d	30.63 ± 0.14g	342.849 ± 6.728e	16.09 ± 0.33g	1.90 ± 0.01e	0.271 ± 0.010a	10.42 ± 0.08bc
Significance							
CV	***	***	ns	***	***	***	***
Zn	***	***	***	***	***	***	***
CV × Zn	***	***	**	***	***	***	*

* Significant effect at the 0.05 level, ** 0,01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

3.2. Colorimetric Parameters

Significant CV × Zn interactions were observed for all leaf colorimetric parameters analyzed (L*, a*, C* and h°; **Table 2**), which indicates a cultivar-dependent response to Zn biofortification in terms of leaf colorimetry. Leaf coloration of 'Aroma 2' was darker in the control compared to all Zn biofortification treatments, whereas 'Eleonora' was non-responsive at any level of Zn biofortification. The intensity of green color was minimally reduced in 'Aroma 2' only in response to the 50 µM Zn application, as denoted by lower negative values of a*; contrarily, all Zn treatments increased the intensity of green color in 'Eleonora' compared to the control, with saturation observed at 25 µM Zn or higher. Cultivar behavior was also dissimilar with respect to hue angle (h°). In 'Aroma 2', hue angle decreased at Zn level 25.0 µM or higher, denoting a tendency for yellower hue, whereas in 'Eleonora' all Zn levels except 50.0 µM resulted in greener hue than the control.

Table 2. Analysis of variance and mean comparisons for leaf colorimetric components L*, a*, b*, Chroma, and Hue angle of Aroma 2 and Eleonora basil cultivars grown hydroponically under different Zn treatments: 0 = Control; 12.5; 25; 37.5; 50 µmol of Zn].

Treatment	L*	a*	b*	Chroma	Hue Angle (°)
Cultivar (CV)					
Aroma 2	44.51 ± 0.29	−9.22 ± 0.15b	21.99 ± 0.34b	24.00 ± 0.31b	112.75 ± 0.40a
Eleonora	44.64 ± 0.08	−8.84 ± 0.29a	24.61 ± 0.65a	26.43 ± 0.73a	110.62 ± 0.19b
Zinc (Zn)					
Control	43.85 ± 0.48c	−8.16 ± 0.53a	20.09 ± 0.27d	21.71 ± 0.39c	112.01 ± 1.14a
12.5 µM	44.04 ± 0.13c	−8.97 ± 0.29b	23.66 ± 0.93bc	25.75 ± 0.98ab	112.67 ± 0.69a
25 µM	45.00 ± 0.20ab	−9.54 ± 0.06c	24.28 ± 0.61ab	26.38 ± 0.68ab	112.01 ± 0.43a
37.5 µM	45.23 ± 0.15a	−9.47 ± 0.04c	24.85 ± 0.45a	26.64 ± 0.46a	110.96 ± 0.10b
50 µM	44.76 ± 0.17b	−9.01 ± 0.39b	23.63 ± 1.08c	25.58 ± 0.98b	110.78 ± 0.16b
CV × Zn					
Aroma 2 × Control	42.79 ± 0.18f	−9.34 ± 0.06c	20.34 ± 0.07de	22.39 ± 0.15de	114.52 ± 0.24a
Aroma2 × 12.5	43.77 ± 0.10e	−9.60 ± 0.03c	21.59 ± 0.04c	23.64 ± 0.12cd	114.13 ± 0.41ab
Aroma2 × 25	45.41 ± 0.06qa	−9.60 ± 0.09c	22.95 ± 0.26b	24.91 ± 0.3bc	112.92 ± 0.17b
Aroma2 × 37.5	45.46 ± 0.25a	−9.41 ± 0.08c	23.86 ± 0.12b	25.66 ± 0.08b	111.13 ± 0.11c
Aroma2 × 50	45.12 ± 0.12ab	−8.14 ± 0.17b	21.22 ± 0.20cd	23.41 ± 0.05cd	111.05 ± 0.16c
Eleonora × Control	44.91 ± 0.01abcd	−6.99 ± 0.15a	19.84 ± 0.54e	21.04 ± 0.55e	109.49 ± 0.37d
Eleonora × 12.5	44.32 ± 0.05de	−8.35 ± 0.20b	25.73 ± 0.12a	27.87 ± 0.53a	111.2 ± 0.24c
Eleonora × 25	44.59 ± 0.16bcd	−9.48 ± 0.06c	25.62 ± 0.16a	27.85 ± 0.20a	111.1 ± 0.25c
Eleonora × 37.5	45.00 ± 0.03abc	−9.53 ± 0.01c	25.84 ± 0.11a	27.61 ± 0.35a	110.79 ± 0.08c
Eleonora × 50	44.40 ± 0.01cd	−9.87 ± 0.06c	26.03 ± 0.05a	27.75 ± 0.17a	110.5 ± 0.19cd
Significance					
CV	n.s.	***	***	***	***
Zn	***	***	***	***	***
CV × Zn	***	***	***	**	***

***Significant effect at the 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test

3.3. Physiological Parameters and Pigments

Cultivar physiology under control conditions was similar for all the parameters examined, except for CO_2 assimilation rate (A_{CO2}), since 'Aroma 2' exhibited a higher A_{CO2} than 'Eleonora' (**Table 3**). In general, adding Zn to the nutrient solution appeared to stress both cultivars based on their physiological parameters. However, cultivar response to Zn treatment levels was not uniform, as indicated by the significant CV × Zn interaction. The interaction had an impact on all physiological attributes except for A_{CO2}, which was reduced on average by 13% for both cultivars under supplemental Zn treatments in the nutrient solution. Stomatal conductance was reduced on average by 16.3% and 21.3% for 'Aroma 2' and 'Eleonora, respectively. The lowest Zn treatment did not have an effect on 'Eleonora' stomatal function. Although transpiration rate (E) of 'Aroma 2' was not affected, transpiration of 'Eleonora' was reduced on average by 19.9% for treatments exceeding 12.5 μM Zn in the nutrient solution. Zinc treatments decreased SPAD index of 'Eleonora' by 12.3% on average, while only the intermediate Zn treatments (25 and 37.5 μM) reduced the chlorophyll concentration of 'Aroma 2' by 4.3%, on average. Also. Zn treatments negatively affected the maximum quantum yield of photosystem II (Fv/Fm) of both cultivars, namely by 4.2% and 3.9% on average, for 'Aroma 2' and 'Eleonora', respectively. However, while 'Eleonora' Fv/Fm appeared to decrease proportionally with increasing Zn concentration, 'Aroma 2' Fv/Fm decreased up to 25μM concentration and showed no further change thereafter.

Under control conditions, lutein and β-carotene values were higher in 'Aroma 2' than 'Eleonora', although the latter had a higher total chlorophyll concentration (**Table 3**). Zn application had a significant effect on all basil plant pigments when compared to the control. However, CV × Zn interaction was observed for all basil pigments since Zn treatments did not affect uniformly the two cultivars. For instance, the addition of Zn to the nutrient solution had a much greater effect overall on 'Eleonora' plant total chlorophyll concentration, which decreased on average by 79.1% more than that of 'Aroma 2'. Furthermore, the lutein concentration of 'Eleonora' was reduced only when plants were treated at the lowest Zn concentration (12.5 μM), whereas the lutein concentration of 'Aroma 2' increased (on average by 27.8 %) only when plants were exposed to the highest Zn treatments (37.5-50 μM). Finally, the β-carotene concentration of the two cultivars increased only when plants of 'Aroma 2' were exposed to the highest Zn treatments, 37.5 and 50 μM, and when 'Eleonora' plants were exposed to 25 and 50 μM Zn.

Table 3. Analysis of variance and mean comparisons for net CO_2 assimilation rate (ACO_2), stomatal conductance (gs), transpiration (E), SPAD index, Fv/Fm, total chlorophyll, and carotenoids of Aroma 2 and Eleonora basil cultivars grown hydroponically under different Zn treatments: 0 = Control; 12.5; 25; 37.5; 50 µmol of Zn].

Treatment	ACO_2	gs	E	SPAD index	Fv/Fm	Total Chlorophyll	Lutein	β-Carotene
	µmol CO_2 m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}			mg g^{-1} fw		
Cultivar (CV)								
Aroma 2	19.16 ± 0.38a	0.212 ± 0.01a	4.12 ± 0.06	37.43 ± 0.18a	0.796 ± 0.004b	1.73 ± 0.04	992.35 ± 34.06a	410.71 ± 8.30a
Eleonora	18.54 ± 0.35b	0.190 ± 0.01b	4.06 ± 0.12	34.28 ± 0.62b	0.803 ± 0.005a	1.69 ± 0.07	890.77 ± 21.70b	401.09 ± 11.51b
Zinc (Zn)								
Control	21.03 ± 0.28a	0.231 ± 0.01a	4.57 ± 0.09a	38.31 ± 0.24a	0.826 ± 0.003a	2.03 ± 0.05a	907.24 ± 12.70c	370.14 ± 4.00d
12.5 µM	19.73 ± 0.19b	0.213 ± 0.01b	4.25 ± 0.09ab	36.02 ± 0.58b	0.809 ± 0.003b	1.76 ± 0.01b	838.14 ± 33.38c	394.94 ± 5.82c
25 µM	17.82 ± 0.21c	0.188 ± 0.01c	3.82 ± 0.10c	35.78 ± 0.53b	0.794 ± 0.004c	1.70 ± 0.02b	879.66 ± 12.43c	388.88 ± 3.47c
37.5 µM	18.08 ± 0.24c	0.187 ± 0.01c	3.93 ± 0.11bc	34.78 ± 0.98c	0.788 ± 0.003c	1.57 ± 0.02c	987.06 ± 44.81b	419.93 ± 17.66b
50 µM	17.59 ± 0.15c	0.184 ± 0.01c	3.88 ± 0.10c	34.40 ± 1.38c	0.779 ± 0.002d	1.47 ± 0.03d	1095.7 ± 47.46a	455.60 ± 13.69a
CV × Zn								
Aroma 2 × Control	21.38 ± 0.50	0.243 ± 0.01a	4.47 ± 0.03ab	38.60 ± 0.44a	0.824 ± 0.002ab	1.93 ± 0.02b	886.18 ± 12.18d	377.65 ± 4.34de
Aroma2 × 12.5	20.04 ± 0.23	0.211 ± 0.01b	4.08 ± 0.01bc	37.24 ± 0.20ab	0.804 ± 0.002cd	1.78 ± 0.02c	911.11 ± 7.97cd	407.27 ± 4.10cd
Aroma2 × 25	18.26 ± 0.16	0.203 ± 0.01b	4.03 ± 0.03bc	36.94 ± 0.07b	0.786 ± 0.001ef	1.72 ± 0.01cd	898.72 ± 18.96cd	381.86 ± 1.97de
Aroma2 × 37.5	18.52 ± 0.16	0.207 ± 0.01b	4.07 ± 0.15bc	36.97 ± 0.01b	0.783 ± 0.001ef	1.61 ± 0.02de	1080.21 ± 36.33ab	458.09 ± 10.11ab
Aroma2 × 50	17.61 ± 0.28	0.194 ± 0.01bc	3.93 ± 0.06bc	37.40 ± 0.18ab	0.783 ± 0.002ef	1.54 ± 0.02e	1185.52 ± 53.64a	428.65 ± 4.09bc
Eleonora × Control	20.68 ± 0.16	0.219 ± 0.01ab	4.67 ± 0.18a	38.02 ± 0.07ab	0.829 ± 0.004a	2.13 ± 0.04a	928.30 ± 14.63cd	362.63 ± 2.13e
Eleonora × 12.5	19.42 ± 0.19	0.214 ± 0.01b	4.42 ± 0.10ab	34.80 ± 0.42c	0.813 ± 0.004bc	1.75 ± 0.02c	765.17 ± 13.50e	382.61 ± 0.78de
Eleonora × 25	17.39 ± 0.13	0.173 ± 0.01cd	3.61 ± 0.03c	34.61 ± 0.18c	0.803 ± 0.002cd	1.69 ± 0.04cd	860.60 ± 7.07de	395.89 ± 2.68d
Eleonora × 37.5	17.64 ± 0.25	0.168 ± 0.01d	3.78 ± 0.14c	32.59 ± 0.17d	0.794 ± 0.002de	1.53 ± 0.02e	893.91 ± 6.66cd	381.77 ± 0.79de
Eleonora × 50	17.58 ± 0.19	0.174 ± 0.01cd	3.83 ± 0.20c	31.39 ± 0.72d	0.777 ± 0.003f	1.40 ± 0.01f	1005.88 ± 17.83bc	482.55 ± 13.92a
Significance								
CV	***	***	n.s.	***	***	n.s.	***	*
Zn	***	***	***	***	***	***	***	***
CV × Zn	n.s.	**	*	***	**	***	***	***

* Significant effect at the 0.05 level, ** 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD

3.4. Plant Root and Shoot Zn Accumulation

Significant cultivar differentiation was observed with respect to Zn accumulation in the root and shoot of plants as 'Aroma 2' grown in the control solution accumulated on average more Zn in their roots (31.4%) and shoots (19.9%) than the 'Eleonora' control plants. Increasing Zn concentration in the nutrient solution resulted in both cultivars showing a relative increase in root and shoot Zn concentration. Overall, supplementing the nutrient solution with Zn increased root Zn concentration in 'Aroma 2' by 13.4% and 'Eleonora' by 12.8%, when compared to the control. Notable was also the differentiation in Zn concentration by the shoots of the two cultivars compared to the control, as Zn accumulation in the shoots increased on average by 23.1% in 'Aroma 2' compared to 9.7% in 'Eleonora'. This disproportional Zn accumulation potential in the shoots of the two cultivars was manifested as significant CV × Zn interaction. In fact, Zn addition to the nutrient solution had a greater effect on 'Aroma 2' plant shoot Zn levels compared to the control, which was overall approximately 138.8% higher than that of 'Eleonora' (**Figure 1A**). Interaction was observed also concerning Zn root levels as the behavior of the two cultivars differed in terms of root Zn concentration following the gradual Zn concentration increase in the nutrient solution. Specifically, raising Zn solution concentration from 12.5 to 25 μM increased root Zn accumulation of 'Aroma 2' by 116.7% more than that of 'Eleonora'. Conversely, the shift from 37.5 to 50 μM of Zn in the nutrient solution increased 'Eleonora' root Zn upload by 43.7% more than that of 'Aroma 2' (**Figure 1B**).

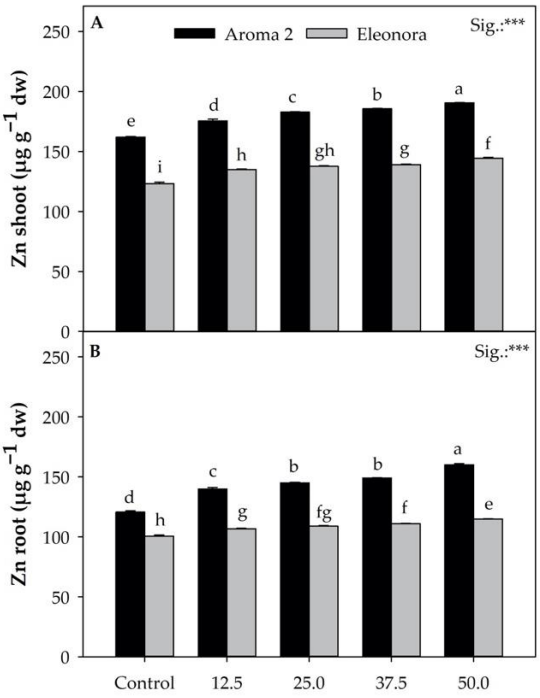

Figure 1. Interaction plots among Cultivar × Zn treatment for Zn accumulation in shoots (A) and roots (B) of 'Aroma 2' and 'Eleonora' basil cultivars grown hydroponically under different Zn treatments (Zn): 0 = Control; 12.5; 25; 37.5; 50 µM of Zn. Data represent means of 3 replicates (n=3). Different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

3.5. Antioxidant Activity

The antioxidant activity as assessed by the DPPH, FRAP, and ABTS assays, were higher in the control treatment of 'Aroma 2' than that of 'Eleonora'. Both cultivars' antioxidant activity (DPPH, FRAP, and ABTS) was altered by supplemental Zn in the nutrient solution (**Table 4).** The antioxidant activities determined by DPPH, FRAP, and ABTS assays were all significantly affected by CV × Zn interaction as the addition of Zn affected the antioxidant activity of 'Eleonora' to a much greater extent than that of 'Aroma 2', moreover several Zn levels did not have the same effect on the two cultivars. Overall, the increase of supplemental Zn from 12.5 to 50 µM increased the antioxidant activity of 'Eleonora' by 159.7%, 229%, and 188.8% more than that recorded for 'Aroma 2' in terms of DPPH, FRAP, and ABTS, respectively. Notable was the fact that the DPPH activity of 'Aroma 2' was reduced even by the lowest Zn treatment while the 12.5 and 25 µM Zn levels did not have any effect on the same cultivar's ABTS activity.

Table 4. Analysis of variance and mean comparisons for DPPH, FRAP, and ABTS antioxidant activities of Aroma 2 and Eleonora basil cultivars grown hydroponically under different Zn treatments: 0 = Control; 12.5; 25; 37.5; 50 µmol of Zn].

Treatment	DPPH	FRAP	ABTS
	mmol trolox kg^{-1} dw		
Cultivar (CV)			
Aroma 2	218.23 ± 9.60a	186.29 ± 4.34a	233.87 ± 3.43a
Eleonora	206.34 ± 11.53b	154.18 ± 6.83b	209.67 ± 9.70b
Zinc (Zn)			
Control	162.05 ± 13.07e	140.33 ± 12.27e	192.61 ± 13.01e
12.5 µM	188.93 ± 7.00d	165.15 ± 5.70c	208.23 ± 7.80d
25 µM	206.62 ± 1.28c	159.85 ± 7.35d	221.65 ± 2.26c
37.5 µM	234.94 ± 4.79b	189.07 ± 4.59b	228.23 ± 11.47b
50 µM	268.90 ± 1.61a	196.77 ± 6.39a	258.13 ± 6.40a
CV × Zn			
Aroma 2 × Control	191.23 ± 0.51e	167.70 ± 1.33d	221.66 ± 1.31c
Aroma2 × 12.5	173.51 ± 1.21f	177.24 ± 1.50c	225.40 ± 3.03c
Aroma2 × 25	208.98 ± 0.64d	176.27 ± 0.48c	224.90 ± 2.02c
Aroma2 × 37.5	245.45 ± 0.78b	199.27 ± 0.90b	253.51 ± 1.09b
Aroma2 × 50	271.98 ± 1.66a	210.98 ± 0.94a	243.90 ± 0.94b
Eleonora × Control	132.87 ± 1.68g	112.97 ± 1.34g	163.55 ± 0.36f
Eleonora × 12.5	204.34 ± 2.36d	153.06 ± 3.74e	191.07 ± 0.26e
Eleonora × 25	204.25 ± 1.48d	143.43 ± 0.51f	218.41 ± 3.30c
Eleonora × 37.5	224.42 ± 1.94c	178.88 ± 0.80c	202.96 ± 4.22d
Eleonora × 50	265.81 ± 0.87a	182.55 ± 1.04c	272.35 ± 1.37a
Significance			
CV	***	***	***
Zn	***	***	***
CV × Zn	***	***	***

*** Significant effect 0.001 level. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

3.6. Phenolic Acids

Ten phenolic acids were identified in both basil cultivars (**Table 5**). Chicoric acid, was the most abundant phenolic acid in the control solution for both cultivars with a value of 4872.7 μg g^{-1} dw and 4319 μg g^{-1} dw for 'Aroma 2' and 'Eleonora' respectively, while rosmarinic acid was the second most abundant phenolic acid (523.8 μg g^{-1} dw for 'Aroma 2' and 358.5 μg g^{-1} dw for 'Eleonora'). The ranking of the remaining phenolic acids was also similar in the two cultivars except for salvianic and caffeic acid. Salvianic and caffeic acids ranked 7th and 9th for 'Aroma 2', while it was the other way around for 'Eleonora'. Under control conditions, 'Aroma 2' total phenolic acids concentration was higher by 12.8 % than 'Eleonora', since half of the individual phenolic acids concentration (rosmarinic acid, salvianolic acid A, salvianolic acid K, chlorogenic acid, and salvianic acid A) was higher in 'Aroma 2' than in 'Eleonora'. No differences among the two cultivars were observed for the rest of the phenolic acids (caftaric acid, caffeic acid, feruloyl tartaric acid, salvianolic acid L, and cichoric acid) in the control solution. Zinc additional quantity to the nutrient solution altered the individual phenolic acids concentration and the total phenolic concentration of both cultivars. An exception was recorded for 'Eleonora' salvianolic acid L concentration which was not affected by Zn treatments. The effect of Zinc, though, was subjected to significant CV × Zn interaction for all the individual phenolic acids and for the total phenolic acids concentration. In all the phenolic profile parameters examined, interaction was significant because Zn treatments did not have the same effect on the two cultivars. For example, all Zn levels influenced the total phenolic concentration of 'Eleonora' and 'Aroma 2', except the lowest Zn treatment which had no effect on 'Aroma 2'. Furthermore, the addition of Zn to the nutrient solution overall, had a much greater effect on 'Eleonora' plant total phenolic concentration as the latter increased by 47.8% more than that of the 'Aroma 2' plants, compared to their respective controls. The CV × Zn interaction for chicoric acid concentration was also significant because while Zn levels up to 37.5 μM appeared to affect the two cultivars similarly, the highest Zn level (50 μM) increased the chicoric acid of 'Aroma 2' by 47.4% more than 'Eleonora', compared to the control. Regarding the rest of the phenolic acids and depending on the cultivar, some Zn levels had a positive or negative effect on one or the other or both cultivars while others had no effect at all. Specifically, certain levels of Zn (what is presented next in parentheses is the average value) increased the concentration compared to the control of salvianic acid A ('Aroma 2'= 27.4%, 'Eleonora'= 120.3%), caftaric acid ('Aroma 2'= 20.4%, 'Eleonora'= 22.1%), caffeic acid ('Eleonora'= 42.3%), chlorogenic acid ('Eleonora'= 13.2%), feruloyl tartaric acid ('Aroma 2'= 15%, 'Eleonora'= 24.9%), salvianolic acid K ('Aroma 2'= 14.4%, 'Eleonora'= 136%), salvianolic acid A ('Aroma 2'= 21.1%, 'Eleonora'= 149.9%) , salvianolic acid L ('Aroma 2'= 26.2%) and rosmarinic acid ('Aroma 2'= 13.1%, 'Eleonora'=

23.5%). Several Zn treatments had also a negative effect on 'Aroma 2' salvianic A (7.8%), caffeic (19%), chlorogenic (21.2%) and rosmarinic (13.1%) acids concentration.

Table 5. Analysis of variance and mean comparisons for phenolic profile of Aroma 2 and Eleonora basil cultivars grown hydroponically under different Zn treatments: 0 = Control; 12.5; 25; 37.5; 50 µmol of Zn].

Treatment	Salvianic acid A	Caftaric acid	Caffeic acid	Chlorogenic acid	Feruloyl tartaric acid
			µg g^{-1} dw		
Cultivar (CV)					
Aroma	55.40 ± 2.41a	51.12 ± 1.15	48.85 ± 1.51b	9.59 ± 0.30a	58.57 ± 0.96b
Eleonora	11.04 ± 1.16b	50.53 ± 1.40	61.27 ± 3.10a	8.76 ± 0.16b	63.43 ± 2.07a
Zinc (Zn)					
Control	28.50 ± 10.23b	47.19 ± 0.81c	54.06 ± 0.85b	9.83 ± 0.64a	52.60 ± 0.90c
12.5 µM	27.78 ± 8.86b	50.91 ± 0.63b	45.21 ± 1.73c	8.89 ± 0.18b	64.26 ± 1.20a
25 µM	29.27 ± 8.00b	53.49 ± 2.48a	51.09 ± 1.83b	9.62 ± 0.28a	62.20 ± 1.44ab
37.5 µM	41.15 ± 13.14a	48.80 ± 0.46bc	60.92 ± 7.11a	7.99 ± 0.09c	59.42 ± 0.36b
50 µM	39.40 ± 9.41a	53.73 ± 3.02a	64.03 ± 4.29a	9.54 ± 0.08a	66.53 ± 3.86a
CV × Zn					
Aroma 2 × Control	51.38 ± 0.15c	48.94 ± 0.38bcd	55.33 ± 1.39b	11.21 ± 0.40a	52.29 ± 1.38d
Aroma2 × 12.5	47.59 ± 0.27d	51.18 ± 1.38b	41.52 ± 1.02f	8.85 ± 0.36cde	62.40 ± 0.51bc
Aroma2 × 25	47.12 ± 0.96d	58.94 ± 0.98a	47.71 ± 1.90def	10.22 ± 0.19ab	60.76 ± 0.11bc
Aroma2 × 37.5	70.53 ± 0.73a	49.40 ± 0.72bc	45.20 ± 1.43ef	8.13 ± 0.10de	59.08 ± 0.46bcd
Aroma2 × 50	60.40 ± 1.30b	47.14 ± 0.37cd	54.49 ± 0.72bc	9.53 ± 0.17bc	58.30 ± 0.59cd
Eleonora × Control	5.62 ± 0.18g	45.44 ± 0.32d	52.79 ± 0.25bcd	8.45 ± 0.08de	52.91 ± 1.42d
Eleonora × 12.5	7.97 ± 0.39g	50.64 ± 0.08bc	48.89 ± 0.57cde	8.92 ± 0.17cd	66.11 ± 1.89b
Eleonora × 25	11.42 ± 0.31f	48.05 ± 0.13bcd	54.48 ± 1.31bc	9.02 ± 0.04cd	63.64 ± 2.87bc
Eleonora × 37.5	11.77 ± 0.54f	48.20 ± 0.43bcd	76.64 ± 1.99a	7.85 ± 0.08e	59.75 ± 0.58bcd
Eleonora × 50	18.41 ± 0.22e	60.33 ± 1.43a	73.57 ± 0.75a	9.56 ± 0.03bc	74.75 ± 2.55a
Significance					
Cultivar (CV)	***	n.s.	***	***	***
Zinc (Z)	***	***	***	***	***
CV × Z	***	***	***	***	***

Cont. **Table 5**

Treatment	Salvianolic acid K	Salvianolic acid A	Salvianolic acid L	Rosmarinic acid	Cichoric acid	Total Phenolic
	μg g^{-1} dw					
Cultivar (CV)						
Aroma	224.84 ± 4.89a	88.04 ± 3.23a	102.77 ± 2.80a	491.42 ± 8.62a	4041.92 ± 60.70a	5172.60 ± 69.59a
Eleonora	99.58 ± 9.27b	81.24 ± 7.32b	82.68 ± 2.84b	426.00 ± 11.73b	3900.97 ± 40.73b	4785.51 ± 73.36b
Zinc (Zn)						
Control	129.00 ± 37.27d	56.13 ± 8.63d	84.71 ± 6.90	441.12 ± 37.04c	3692.51 ± 38.47d	4595.83 ± 127.65d
12.5 μM	150.64 ± 26.86c	85.77 ± 3.80b	96.79 ± 1.44	432.83 ± 9.15c	3913.77 ± 28.3c	4876.83 ± 72.57c
25 μM	156.94 ± 23.47bc	77.79 ± 1.77c	93.77 ± 5.35	440.51 ± 9.41c	3978.15 ± 25.15bc	4952.84 ± 55.01c
37.5 μM	177.72 ± 31.33ab	103.15 ± 3.46a	97.29 ± 9.62	471.34 ± 12.63b	4021.93 ± 26.43b	5089.69 ± 86.17b
50 μM	196.75 ± 22.58a	100.38 ± 8.12a	91.09 ± 4.49	507.74 ± 8.62a	4250.88 ± 79.96a	5380.06 ± 103.97a
CV × Zn						
Aroma 2 × Control	210.31 ± 0.14b	75.32 ± 1.54e	93.66 ± 4.74bc	523.74 ± 3.18a	3750.14 ± 63.13de	4872.67 ± 65.06def
Aroma2 × 12.5	210.61 ± 0.15b	93.94 ± 1.47cd	97.01 ± 2.88abc	449.47 ± 11.79c	3974.62 ± 12.84bc	5037.2 ± 22.85cd
Aroma2 × 25	209.38 ± 0.30b	80.31 ± 0.61e	103.91 ± 6.21ab	460.94 ± 5.05c	3985.45 ± 52.14bc	5064.73 ± 49.08c
Aroma2 × 37.5	246.93 ± 4.23a	108.05 ± 3.74ab	118.18 ± 5.00a	499.21 ± 1.95ab	4075.55 ± 14.55b	5280.26 ± 9.11b
Aroma2 × 50	246.98 ± 1.35a	82.59 ± 1.85de	101.10 ± 0.53ab	523.75 ± 1.05a	4423.84 ± 43.98a	5608.12 ± 40.71a
Eleonora × Control	47.68 ± 18.21e	36.94 ± 1.17f	75.77 ± 11.66c	358.51 ± 4.96e	3634.88 ± 9.58e	4319.00 ± 24.46g
Eleonora × 12.5	90.67 ± 3.27d	77.59 ± 1.79e	96.57 ± 1.41abc	416.19 ± 1.55d	3852.91 ± 11.67cd	4716.46 ± 9.44f
Eleonora × 25	104.51 ± 2.26d	75.27 ± 2.99e	83.63 ± 1.28bc	420.09 ± 0.05d	3970.85 ± 19.73	4840.96 ± 14.25ef
Eleonora × 37.5	108.50 ± 9.92d	98.25 ± 4.67bc	76.40 ± 1.09c	443.47 ± 4.11cd	3968.3 ± 20.13	4899.12 ± 26.93de
Eleonora × 50	146.51 ± 5.00c	118.17 ± 3.12a	81.07 ± 0.57bc	491.72 ± 10.67b	4077.92 ± 10.84	5152.00 ± 19.47bc
Significance						
Cultivar (CV)	***	***	***	***	***	***
Zinc (Z)	***	***	n.s.	***	***	***
CV × Z	**	***	**	***	***	***

** Significant effect at the 0.01 level, *** 0.001 level, ns=non-significant effect. Data represent means ± standard error of 3 replicates (n=3). Treatment means within each column followed by different letters denote significant differences ($P < 0.05$) according to Tukey-Kramer HSD test.

4. Discussion

In soilless growth systems, biofortification of the nutrient solution can augment the concentration, translocation and accumulation of trace elements in the edible plant organs due to the enhanced availability of trace elements, such as Zn, but also due to the absence of soil × root interaction[36]. In this study, results showed that basil is characterized by high genetic variability in Zn accumulation capacity, as control plants of 'Aroma 2' accumulated more Zn in their roots and shoots by 31.4% and 19.9% than 'Eleonora', respectively. Furthermore, our results demonstrated that by biofortifying the nutrient solution with Zn it is possible to further increase its concentration in the edible part of basil cultivars. Indeed, supplementing the nutrient solution with Zn increased Zn shoot accumulation of 'Aroma 2' by 23.1% and 'Eleonora' by 9.7%, as overall average of Zn treatments, compared to the control. Similar studies have reported analogous results with other plant species (*Lactuca sativa* L., *Brassica oleracea* L. var. capitata, *Brassica oleracea* L. var. italica, and *Beta vulgaris* L.) when were exposed to Zn biofortified solutions[12,23,37]. However, in our study the increase in Zn concentration in basil tissues (root, shoot) was not proportional to the Zn concentration increase in the nutrient solution. In addition, Zn bioaccumulation in the roots of both cultivars was on average 26% higher than in the shoots (overall average of Zn treatments and the Control). This behavior has been cited for other Zn non-hyperaccumulative plants such as *Oryza sativa* and *Beta vulgaris* L.[11,37]. It seems that this behavior is regulated by non-hyperaccumulative plant genes such as the ZIP, HMA, MTP, ZIF1, and FRD3. These genes, which are involved in Zn concentration, sequestration and redistribution in the plant, are up-regulated only under Zn deficiency, a condition that was not observed in this trial[22]. Conversely, concentration, translocation and tissue bioaccumulation of Zn are maximized when hyperaccumulator species are exposed to Zn surplus conditions due to the substantially higher expression of the abovementioned genes[11]. Concerning basil, our results showed that basil Zn biofortification potential is also highly influenced by cultivar. Specifically, in this study, supplementing the nutrient solution with additional Zn had a much greater effect on 'Aroma 2' plant shoot Zn levels, which were approximately 138.8% more than those of 'Eleonora', compared to the control. Given this and the fact that after the application of the maximum Zn level (50 μM), accumulation in the shoots of 'Eleonora' was lower even than the shoots of 'Aroma 2' control plants, underlines the crucial role of cultivar selection in the expected results for biofortification programs of non-hyperaccumulative species. This conclusion is in agreement with other studies concerning basil biofortification trials with selenium and three lettuce genotypes biofortified with Zn in soil trials[2,38]. It is worth mentioning that Zn concentrations achieved in the two basil cultivars used in this study were similar to those obtained from three lettuce cultivars biofortified in soil cultivation with 30 mg kg^{-1} of $ZnSO_4$[38]. This aspect highlights how, compared to soil cultivation, closed hydroponic

systems offer a more rational management of key macro-micronutrients, while at the same time being able to achieve the intended Zn concentrations (15-30 mg kg^{-1} dw)[11,14].

Although both cultivars belong to the Genovese basil type, under control conditions 'Aroma 2' plants were more robust than 'Eleonora' plants, growing taller, with a greater number of leaves, higher plant leaf area, fresh biomass, total dry biomass, and dry matter than 'Eleonora' plants. It is worth noting that, under control conditions, the observed cultivar differentiation in terms of morphological characteristics (fresh biomass, number of leaves, and leaf area) could have a decisive effect on the Zn concentration and accumulation capacity of the two basil cultivars[39-42]. The latter becomes particularly important since it is reported that Zn distribution across Zn sinks could be regulated by plant morphological characteristics and Zn accumulation could be decreased under conditions that biomass is reduced[39]. Nevertheless, both basil varieties appeared to be susceptible when were exposed to the exceeding levels of Zn. Basil response to additional Zn was manifested by the alteration of morphological traits (plant height, leaf number, leaf area, fresh biomass), leaf CIELAB color, physiological traits (total chlorophyll, A_{CO2}, gs, E, SPAD index, and Fv/Fm) and antioxidant activity (DPPH, FRAP, ABTS). The previous response of basil plants after Zn application denotes a typical reaction of plants under heavy metal stress[25]. Typical visual symptoms of altered plant growth due to Zn toxicity are growth inhibition, chlorosis of young leaves and cell death probably as a consequence of inhibition of DNA synthesis and inevitably cell mitosis[43]. As it turns out, the Zn stress conditions plants were subjected to in this study were mild since no such severe visual symptoms were observed in any of the two cultivars. Furthermore, under these mild stress conditions, depending on the Zn level, a decrease in fresh biomass from 3.80-9.72% for 'Aroma 2' to 8.9-29.9% for 'Eleonora' could be observed, although the plants of both cultivars remained marketable without particular problems in their appearance. What was observed visually was that Zn surplus made 'Eleonora' plants appear more greenish compared to the control (−a*, +h°) while Zn treatments (above 25 μM) lessened the greenness of 'Aroma 2' plants (+a*, −h°). However, it is noted that the 'Aroma 2' cultivar was more tolerant to Zn surplus since plant leaf area, leaf number, and fresh biomass decreased on average by 32.3%, 38.6%, and 57.18%, respectively, less than the plants of 'Eleonora' compared to the control and overall Zn treatments. Most likely, as in the control solution, the higher number of leaves and fresh biomass of 'Aroma 2', acted as Zn sinks and could also account for the higher Zn accumulation in this cultivar compared to 'Eleonora' under Zn excess[43].

In our study, the observed variation in yield attributes of basil under Zn stress is probably related to the efficiency of the photosynthetic mechanisms of the two cultivars. Photosynthetic efficiency of plants is reduced under abiotic stresses, such as heavy metal stress, due to their negative effect on photosystems performance, electron transport mechanisms, gas exchange parameters, chlorophyll, and other photosynthetic

pigments biosynthesis[44-45]. Specifically, Zn can inactivate chlorophyll by replacing Mg from the porphyrin head of the chlorophyll molecule during photosynthesis[44]. Furthermore, Zn exposure has been found to disturb the energy migration from the antenna complexes to the chlorophyll of the PSII reaction centers and could be responsible for inactivation of a part of the reaction centers[45]. The above can explain to a certain degree the decline of total chlorophyll content, SPAD index, and Fv/Fm of both cultivars in our experiment under Zn stress. Noteworthily, in our study and under control conditions, photosynthesis related features of the two cultivars were similar for almost all the parameters examined (except for total chlorophyll content and A_{CO2}). However, adding Zn to the nutrient solution stressed 'Eleonora' plants physiology to a greater extent than 'Aroma 2' plants when compared to the control. Total chlorophyll concentration, stomatal conductance, and SPAD index of 'Eleonora' were reduced to a greater extent than 'Aroma 2', by 44.2%, 23.8% and 63.9%, respectively overall Zn treatments. In addition, transpiration rate of 'Eleonora' was reduced on average by 19.9% (above 12.5 μM Zn) and Fv/Fm appeared to decrease with increasing Zn concentration while transpiration rate of 'Aroma 2' was not affected and Fv/Fm decreased only up to the 25μM Zn level and thereafter remained the same. It must be noted though, that Fv/Fm of both cultivars fell within the optimal range (~0.80) for basil[46].

A factor related to photosynthesis that could provide an explanation for the different responses of the two cultivars is the production of reactive oxygen species (ROS) under Zn surplus. Excessive ROS production under heavy metal stress conditions, such as Zn stress, has been associated with photosynthetic rate limitation and thus plant biomass reduction[47-49]. On the other hand, ROS play an important role as signaling molecules by regulating numerous biological processes including response to abiotic stresses[50-51]. In this study, cultivar tolerance to Zn surplus was probably related to their antioxidant system efficiency and therefore the ability of each cultivar to balance the cellular ROS level, maintaining the essential redox homeostasis thus preventing extreme oxidative stress conditions[51]. Indeed, our results showed that the antioxidant activity of both cultivars increased at almost all Zn levels compared to the control. Likewise, studies on *Pisum sativum* L., *Brassica oleracea* L. var. botrytis, *Brassica oleracea* L. var. italica, *Solanum lycopersicum* L., *Brassica nigra* L., and *Phaseolus vulgaris* L. demonstrated increased plant antioxidant activity under Zn surplus regardless of the cultivar used[52-55]. It was suggested that increased Zn bioaccumulation in plant tissues could act as an activator of antioxidant cofactor enzymes and thereby increase plant antioxidant capacity[56-57]. However, in this study the fact that the addition of Zn increased the antioxidant activity of 'Eleonora' much more than that of 'Aroma 2' (159.7%, 229%, and 188.8% for DPPH, FRAP, and ABTS, respectively) without any yield benefits for

'Eleonora' confirms its greater sensitivity and consequently lower adaptability to excess Zn.

To prevent ROS oxidative damage, plants have several antioxidant mechanisms with overlapping functions at their disposal[58]. The latter include highly efficient antioxidant carotenoid or phenolic compounds that could provide enormous flexibility in redox control[58]. Carotenoids, such as lutein and β-carotene, are effective chloroplast antioxidants located in close proximity to chlorophylls. At the same time, however, their bioactive oxidized products can induce changes in gene expression that lead to acclimatization to abiotic stress conditions[59]. In our study, carotenoid production by the two cultivars was not always similar under Zn treatments. When plants were exposed to the highest Zn treatments (37.5-50 μM) both lutein and β-carotene content of 'Aroma 2' increased while only β-carotene (at 25 and 50 μM) concentration of 'Eleonora' improved when compared to the control. The latter could relate to the increased SPAD index and therefore the photosynthetic efficiency of 'Aroma 2' compared to 'Eleonora', especially at the higher Zn concentrations. Polyphenolic compounds may have also played an important role in controlling ROS over-production under Zn stress in our study. Chlorophyll concentration reduction under Zn excess and especially the increased production of ROS could have signaled the production of phenolic compounds[50] since both basil cultivars' total phenolic concentration increased in comparison to the control. An important role of phenols is their action as scavengers of free radicals to protect the plant from oxidative stress[60]. In our study total phenolic content demonstrated a positive correlation with DPPH ($r= 0.84$, $p<0.001$), FRAP ($r= 0.94$, $p<0.001$), and ABTS ($r= 0.82$, $p<0.001$) antioxidant activities, while it appeared to have a negative correlation with total chlorophyll concentration ($r= -0.71$, $p<0.001$). Similar studies on lettuce[61,62] and basil[29,63] have reported an increase in total phenolics after biofortification with iodine in soilless systems. More support for our results is provided by the role of Zn as a cofactor of crucial amino acids in secondary metabolic pathways such as shikimate dehydrogenase (SKDH), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO). Recent studies on *Brassica oleracea* L. cv. Bronco and *Coriandrum sativum* L. with Zn integration have observed an increase in aromatic amino acids such as phenylalanine, tryptophan, and tyrosine, which are essential precursors of auxin and salicylate and enzyme regulators of the secondary metabolic pathway[13,24].

It is remarkable that under the highest Zn biofortification treatment (50 μM) both carotenoid (lutein and β-carotene) and phenolic concentration of 'Eleonora' were enhanced and reached higher levels to those of 'Aroma 2' control. The latter is very crucial because it could enable us to bio-enhance commercial cultivars whose low concentration of certain compounds beneficial to human health is determined genetically. The health benefits of carotenoids for humans include their general role as antioxidants and the prevention of degenerative macular diseases[64]. Polyphenols have

also a well-known antioxidant activity and play an important role in human nutrition. Studies on basil widely support the impact of genotype on the biosynthesis and bioaccumulation of individual phenolic acids. In a recent study, Ciriello, et al.[30] found a higher concentration of rosmarinic acid in basil cultivars 'Aroma 2' and 'Italiano Classico' and of chicoric acid in 'Eleonora'. In a similar study the same authors reported comparable results in Genovese basil cultivars, showing a significant cultivar-dependent response to cichoric and rosmarinic acid genotype[30]. Kwee and Niemeyer[65] reported lower concentrations of chicoric acid in *Ocimum basilicum* × *Ocimum americanum*, in contrast to what was observed in *Ocimum basilicum* var. thyrsiflorum. The investigation of phenolic profiles in our study confirmed the influence of genotype on the biosynthesis of phenolic compounds, revealing preferential bioaccumulation of chicoric acid regardless of the cultivar. Chicoric acid is crucial for plants' protection against insects, viruses, bacteria, fungi, and nematodes, and for humans in exerting antitumor, anti-obesity, antiviral, antidiabetic, and inhibitory functions of HIV integrase by inhibiting its replication[66].

5. Conclusions

In this study, we evaluated the potential of producing Genovese basil biofortified with Zn by adopting an agronomic strategy of increasing Zn integration in the nutrient solution (12.5, 25, 37.5, and 50 μM). The management of Zn in the floating raft system increased the concentration of Zn in the 'Aroma 2' and 'Eleonora' basil cultivars while negatively impacting their yield and physiology. However, increasing Zn in the nutrient solution significantly increased antioxidant activity, carotenoid, and polyphenol concentrations. Specifically, the best compromise between yield, phytochemical quality and zinc accumulation in leaves was observed in Aroma 2 biofortified with the highest zinc doze (50 μM). The highest tissue zinc levels in Aroma 2 × 50 (160.12 mg g^{-1} dw) resulted in a yield reduction of less than 10%, but a 15% increase in total phenolics. However, the strong cultivar-dependent response observed in the present study suggests that in biofortification programs for non-hyperaccumulative crops, the varietal choice is crucial in order to maximize the accumulation of this essential micronutrient and minimize yield loss. However, the consumption of biofortified Genovese basil with Zn can increase the intake of Zn by consumers while providing a product enriched concomitantly in valuable phytochemicals such as carotenoids and polyphenols.

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Chapter 11

Zinc biofortification of Genovese basil: influence on mineral profile and estimated daily intake in adults and children

Abstract: Despite the well-known beneficial function of Zn in human health, its deficiency is an increasingly recognized worldwide concern. In this work, we evaluated the agronomic biofortification of two basil (*Ocimum basilicum* L.) cultivars ('Aroma 2' and 'Eleonora') using nutrient solutions with different Zn concentrations (0, 12.5, 25, 37.5, and 50 μM). We focused on the impact of biofortification on the mineral profile quantified by ICP OES. Compared to the control, biofortification treatments increased Zn concentration by 22.03% (on average). Consumption of one serving of 50 μM of Zn biofortified basil 'Aroma 2' guarantees an estimated daily intake (EDI) of 275.746 and 91.915 μg day^{-1} in adults and children, respectively. Furthermore, Zn biofortification positively affected the mineral profile of the leaves. Compared to the control, the B_{50} dose of Zn (50 μM of Zn) increased the EDI of macro and microelements in adults and children. This aspect highlights how biofortified basil consumption would improve consumers' nutritional status.

Keywords: *Ocimum basilicum* L; Biofortification; Hydroponics; Nutrient management; Trace elements; EDI; Hazard quotient

1. Introduction

A On January 19, 2022, the world population peaked at 8 billion and is predicted to reach 10 billion over the next decades[1], introducing new challenges to the food supply. In this fast-changing scenario, agriculture will be called upon to guarantee food safety for future generations with yield maximization as its primary goal[2]. Unsurprisingly, achieving the goal of "zero hunger" clashes with the imperative of contrasting "hidden hunger," which affects more than a third of the world's population[3]. Regular intake of microelements, due to their antioxidant and metabolic function, could reduce the incidence of anaemia, diabetes mellitus, blindness, cognitive and mental retardation, premature mortality, and stunted growth, mainly in the crucial phases of development, pregnancy, and senility[4]. Additionally, strengthens the immune system by protecting the human body from viral infections, an increasingly newsworthy topic[5]. Probably, nutritional deficiencies depend on an unbalanced and unvaried diet and overeating of poor-quality cereals and processed foods that manifest developing social needs[6,7]. What could be the key? Today, the pharmaceutical industry offers supplements to manage nutritional deficiencies in the diet. However, while the consumption of "ready-to-eat" food supplements would bring benefits, on the other hand it is an "unnatural" and economically unsustainable solution. The cheapest and most sustainable solution would be to adopt food schemes based on a prevailing consumption of vegetables, natural carriers of fiber, minerals, and bioactive compounds, without demonizing cereals, meat, and fish[8]. However, in industrialized countries, the appreciation and consumption of vegetables are low, especially among children, due to the bitter taste of some nutritional compounds and the green color of chlorophyll; on the contrary, in non-industrialized countries, the consumption of vegetables is limited by their low availability and the need to consume energy-dense foods[9]. Increasing vegetable consumption could boost the economy of poorer countries, in contrast to industrialized ones where the imperative is to increase liking, especially among young people[10-13]. Spices and herbs could provide valuable aid, appreciated natural flavor enhancers that can "mask" the bitter flavors of some vegetables, increasing their liking and consumption. A prime example is given by basil, whose aromatic, tender, and unmistakable leaves have elected it among the most popular leafy vegetables in international gastronomy and the processing industry, as it is always used both as a garnish for soups and salads and as a dressing (e.g., the pesto sauce). Research has focused on characterizing the aromatic and phenolic profile that distinguishes this versatile leafy vegetable, revealing low interest in the mineral composition. Like other leafy vegetables, basil is low in calories and has a good mineral composition[14] that would guarantee a natural supply of nutrients in the daily diet. At present, just the antinutrient nitrate is under the magnifying glass of researchers, even though there are no regulatory thresholds for basil under European Union Regulation No. 1258/2011[15]. Agronomic and genetic approaches are among the different strategies

to produce biofortified vegetables[15]. Although it is the best long-term solution, the latter approach is currently held back by high costs and restrictive regulations that prohibit the use of GMOs in some countries around the world. In light of this, agronomic biofortification programs are the best option as they would allow increased concentrations of essential nutrients (e.g., iodine, silicon, calcium, iron, zinc, magnesium, selenium, and copper) in edible parts of plants[8]. As suggested by the same authors, biofortified products, in addition to being a "natural" solution to counteract nutritional deficiencies related to unbalanced diets and new dietary habits (such as vegan eating), could provide premium nutritional characteristics typical of "superfoods" enhancing the economic sustainability of this practice. However, appropriate biofortification programs can improve mineral concentration in short-cycle leafy vegetables as observed already in mizuna (*Brassica rapa* var. nipposinica), tatsoi (*Brassica rapa* subsp. narinosa), chicory (*Cichorium intybus*), basil (*Ocimum basilicum* L.), purslane (*Portulaca oleracea*), and lettuce (*Lactuca sativa* L.)[8]. Bioenriched vegetables through agronomic biofortification could be a tangible long-term solution to assure nutritional security because, unlike staple crops, these can be effortlessly grown hydroponically. The independence from agricultural soil, associated with continuous imbibition of roots in nutrient solutions biofortified with macro and/or microelements, would boost the buildup of minerals in the edible parts of plant, more than can be achieved by the open field growing. At the same time, it would also increase this practice's economic and environmental sustainability. In recent years, researchers have assessed the effects of hydroponic biofortification of basil with Se and I, focusing on the influence of this practice on the nutraceutical profile[17-19]. Unlike the investigations on many leafy vegetables such as lettuce, *Brassicaceae* microgreens, purslane, and broccoli (*Brassica oleracea* var. italica)[1,6,20-22], little attention has been given to biofortification with Zn in basil, although nearly 20% of the world's population is at risk of Zn deficiency. Although this problem involves more non-industrialized countries, it is worth noting that even in industrialized ones, Zn deficiency occurs more frequently in children, pregnant women, and the elderly[16,23]. A fundamental element of proteins and several enzymes, Zn plays a crucial role in maintaining reproductive, sensory, digestive, and neurocognitive biological functions[24,25]. Based on the above, the research objectives of the present work were a) to increase the Zn concentration in the edible parts of two basil cultivars ('Aroma 2' and 'Eleonora') grown hydroponically and biofortified with five doses of Zn (0, 12.5, 25, 37.5, and 50 μM) added in nutrient solution; (b) to define the mineral profile of basil considering the well-known synergistic and antagonistic relationships between Zn and macro and microelements; c) calculate the estimated daily intake (EDI) of macro and microelements and nitrate hazard quotient in children and adults (males and females).

2. Materials and Methods

2.1. Plant Materials and Experimental Condition

The Zn-biofortified basil plants were grown in the Department of Agriculture of Federico II University of Naples, located in Portici (Naples, Italy; lat. 40°51'N, long. 14°34'E; 60 m a.s.l.) under a passive ventilation greenhouse. On 13 April 2021, basil (*Ocimum basilicum* L.) Genovese cultivars 'Aroma 2' (Fenix, Belpasso, Italy) and 'Eleonora' (Enza Zaden, Enkhuizen, The Netherlands) were sown in 54-hole polystyrene trays filled with a substrate of 2/3 vermiculite (Perlite Italiana, Corsico, Italy) and 1/3 peat (Vigorplant, Fombio, Italy) (*v*/*v*). At the 2-3 true leaves stage (May 1, 2021), the trays with plants were placed on a soilless floating raft system (FRS). The FRS system consisted of polypropylene tanks (52×32×6 cm) containing a control nutrient solution complete with macro and microelements as follows: 14.0 mM N-NO_3^-, 1.75 mM S, 1.50 mM P, 5.0 mM K, 4.5 mM Ca, 1.5 mM Mg, 1.0 mM N-NH_4^+, 20.0 μM B, 15.0 μM Fe, 9.0 μM Mn, 1.6 μM Zn, 0.3 μM Cu, 0.3 μM Mo. The pH was maintained at 5.8 (±0.2) while immersion pumps provided oxygenation (Aquaball 60; Eheim, Stuttgart, BW, Germany). The experimental biofortification program consisted of four nutrient solutions supplemented with increasing doses of zinc (12.5, 25, 37.5, and 50 μM, henceforth $B_{12.5}$, B_{25}, $B_{37.5}$ and B_{50}) using $ZnSO_4 \times 7H_2O$ (Sigma-Aldrich, St. Louis, MO, USA) as a Zn source plus a control nutrient solution (B_0). Biofortification factors (B) and cultivar (CV) were arranged according to a randomized factorial experimental design with three replications. At harvest, the fresh and dry weight and dry matter percentage of leaves were determined.

2.2. Nitrate Determination

According to Formisano et al.[26], leaf nitrate was quantified by ion chromatography coupled to an electrical conductivity detector (ICS3000, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA). An aliquot of 0.25 g of dried leaves was finely ground by a grinding mill (IKA, Staufen im Breisgau, BW, Germany), extracted in 50 mL of ultrapure water (Arium® Advance EDI pure water system; Sartorius, Goettingen, LS, Germany), homogenized (R-10M centrifuge; 6000 rpm, 10 min; Remi Elektrotechnik Ltd., Mumbai, Maharashtra, India) and finally frozen in liquid nitrogen. Then, the samples were placed in thermostated water bath at 80 °C for 10 min (SW22 shaking water bath; Julabo, Seelbach, BW, Germany) centrifuged for a further 10 min at 6000 rpm. After filtration to 0.45 μm by syringe filter (Whatman International Ltd., Maidstone, Kent, UK), 25 μL of supernatant was analyzed by ion chromatography. Equipment and materials were purchased from Thermo Scientific™ Dionex™ (Sunnyvale, CA, USA). NO_3^- separation was performed using an IonPac® ATC-HC anion trap (4 × 50 mm), an IonPac® AG11-HC guard column (9 × 75 mm), an IonPac® AG11-HC IC column (4 × 50 mm), and a self-regenerating suppressor (DRS600; 4 mm) in gradient mode (5 mM-30 mM potassium

hydroxide with a flow of 1.5 mL min^{-1}). The integration and quantification of NO_3^- was performed using Chromeleon™ 6.8 Chromatography Data System software and results were expressed as mg g^{-1} fw. Each treatment was analyzed in triplicate (n=3).

2.3. Determination of macro and microelements

2.3.1. Reagents and standards

All reagents and standards used for macro and microelements determination by inductively coupled plasma optical emission spectrometric analysis (ICP OES) were purchased from Sigma-Aldrich (Milan, Italy). Calibration standards were prepared from ICP multi-element standard solution ICP Calibration mix EH61 PrimAg (Romil, Cambridge, UK). For the determination of the accuracy, the Standard Reference Material (SRM) 1570a (NIST) (Sigma-Aldrich) for trace elements in spinach leaves was prepared and analysed in triplicate in the same way as the samples. All laboratory glassware used was decontaminated by soaking it in sulfuric acid solution (10 %, *v*/*v*, Merck, Milan, Italy) overnight and then rinsed several times with deionized water and with ultra-pure water, respectively (Merck Millipore, Darmstadt, Germany).

2.3.2. Sample preparation

Basil leaves were washed with ultra-pure water and placed in forced-ventilation oven at 65 °C until a constant weight was reached and then finely ground. Before ICP OES analysis (Spectroblue, Spectro Ametek, Berwyn, PA, USA), 0.5 g of the dried plant sample, weighted into PTFE vessels, was processed by microwave-assisted digestion (MLS-1200, Microwave Laboratory Systems, Milestone, Shelton, CT, USA) in a mixure of 9 mL nitric acid (70%, for trace analysis), 3 mL hydrochloric acid (30%, for trace analysis), and 0.5 mL H_2O_2 (≥30%, for trace analysis). The microwave heating program was from 20 °C to 180 °C in 15 min and hold at 180 °C for 10 min, with 800 W power setting. After cooling, each fully digested sample was transferred to volumetric flask and the volume made up to 50 mL with ultra-pure water[27].

2.3.3. Analysis of macro- and micro-elements by ICP OES

Analysis of macro- and micro-elements of the digested samples was made by ICP OES. The plasma source was supplied by argon of 99.999% purity (SOL, Marcianise, CE, Italy). The instrumental parameters are shown in **Table 1**. The instrument was daily optimized for maximum signal and tuned with a solution called ICAL specific for the wavelength range investigated. For non-alkaline elements (Fe, Mn, Cu, Se, Zn, and P), the calibration curve was built in the 1.0–100 mg L^{-1} interval and the quantity of the minerals expressed in mg g^{-1} dw. For alkaline elements (K, Ca, Mg, and Na), the calibration curve was built within the 2 mg L^{-1} to 1000 mg L^{-1} range, and the quantity of

the minerals expressed in mg g^{-1} dw. Macro and micro-elements were expressed as mg g^{-1} fw and μg g^{-1} fw, respectively, based on each basil sample's original dw.

Table 1. Instrumental parameters for the inductively coupled plasma optical emission spectrometry (ICP OES) in this study

Parameters	**Value**
Radio frequency generator (MHz)	40
Radio frequency power (W)	1350
Plasma gas rate (Argon, L min^{-1})	14.5
Auxiliary gas rate (Argon, L min^{-1})	0.3
Nebulizer flow rate (L min^{-1})	0.7
Nebulizer	Cross-flow
Injector tube diameter (mm)	2.0
Scan regions dwell time (ms)	200
Detection mode	Pulse counting
Element	**wavelength (nm)**
K	404.7
Ca	315.9
Mg	279.1
Na	589.6
Fe	238.2
Mn	257.6
Cu	324.7
Se	196.0
Zn	206.2
P	214.9

2.3.4. Sample analysis and method accuracy

Accuracy was determined using the Standard Reference Material (SRM) 1570a (NIST) (Sigma-Aldrich, Milan, Italy). The recovery was in the 87-98 % interval (**Table 2**). The LoD was calculated based on the minimum detectable concentration characterized by a signal to noise ratio equal to or greater than 3, while the LoQ was calculated based on the minimum concentration of the element considered, which is characterized by a signal to noise ratio equal to or greater than 10.

Table 2. Results observed for the certified reference materials NIST 1570a. Statistically significant differences between certified and measured values were not observed ($p > 0.05$; Student's t-test).

Macro-element	Certified value (mg kg^{-1} dw)	Measured value
Potassium (K)	2.903 ± 0.026	2.890 ± 0.320
Calcium (Ca)	1.526 ± 0.066	1.411 ± 0.100
Magnesium (Mg)	0.890*	0.819 ± 0.050
Sodium (Na)	1.821 ± 0.023	1.696 ± 0.230
Phosphorus (P)	0.519 ± 0.007	0.491 ± 0.050
Micro-element	**Certified value (mg kg^{-1} dw)**	**Measured value**
Iron (Fe)	-	170 ± 2.3
Manganese (Mn)	76.0 ± 1.2	68.5 ± 2.0
Copper (Cu)	12.22 ± 0.86	11.6 ± 0.8
Selenium (Se)	0.115 ± 0.004	0.108 ± 0.006
Zinc (Zn)	82.3 ± 3.9	76.0 ± 3.8

Data are mean ± standard deviation; * Values are expressed as mass fraction in percent (%). ** Value is expressed.

2.3.5. Validation of the analytical procedure

We used the NIST SRM 1570a (Trace Elements in Spinach Leaves) certified reference material to ensure trueness and precision of the analytical procedures (**Table 2**). The obtained values were always within the certified range, proving the accuracy of the analytical procedures used. The limit of detection (LoD) and limit of quantification (LoQ) based on background equivalent concentration (BEC) are reported in **Table 3**.

Table 3. Limit of detection (LoD) and limit of quantification (LoQ) of macro- and microelements.

Macro-element	LoD (mg kg^{-1} dw)	LoQ (mg kg^{-1} dw)
K	0.048	0.231
Ca	0.039	0.101
Mg	0.033	0.159
Na	0.052	0.545
Micro-element	**LoD (mg kg^{-1} dw)**	**LoQ (mg kg^{-1} dw)**
Fe	0.002	0.010
Mn	0.001	0.004
Cu	0.002	0.005
Se	0.001	0.004
Zn	0.031	0.111
P	0.538	1.785

2.4. Estimated Daily Intake, Nutrient Contribution, and Nitrate Hazard Quotient

Leaf Estimated daily intake (EDI) for each nutrient (K, Ca, Mg, Na and P, Fe, Mn, Cu, Se, and Zn) was calculated with the following equation.

$$EDI = Nutrient\ content \times DC_{basil}$$

where:

The nutrient content represents the concentration of each macro (mg g^{-1} fw) and micro (μg g^{-1} fw) element for the two biofortified basil cultivars at different levels of Zn.

DC is the daily consumption of fresh basil expressed in g. Because there is no DC reference value available for fresh basil, we considered a daily consumption of 5 g for children and 15 g for adults.

The percentage Nutritional Contribution (NC %) per serving for children, adults (males and females) and the elderly of Ca, Mg, P, Fe, Cu, Se, and Zn was calculated using the following equation:

$$NC\ (\%) = \frac{EDI}{RDA}$$

The percentage nutritional contribution (NC) to meal for children, adults (males and females) and the elderly of K, Na, and Mn was calculated with the following equation:

$$NC\ (\%) = \frac{EDI}{AI}$$

Where:

EDI = the estimated daily intake for each nutrient analyzed

RDA and AI = recommended dietary allowances and adequate intakes, respectively, for children, adults (males and females) and the elderly[28-33].

For children and adults, the hazard quotient (HQ) of nitrate in basil was calculated according to the following equation:

$$HQ = \frac{EDI_{nitrate}}{RfD}$$

Where RfD is the tolerable upper intake level of nitrate in the diet, calculated from EFSA values (EFSA, 2017) as follows:

$$RfD = 3.7\ mg\ kg^{-1}\ of\ body\ weight\ day^{-1}$$

considering a body weight of 15 kg for children and 70 kg for adults.

HQ values less than 1.00 indicate that the meal is safe for human consumption.

2.5. Statistics

Statistical analysis was performed using the SPSS 20 software package (SPSS Inc., Chicago, IL, USA), and data were reported as mean ± standard deviation. Student's t-test was used to identify significant differences in the mean Cultivar (CV) effect, while Biofortification (B) effects and the interaction between B and CV were subjected to analysis of variance (ANOVA) and then evaluated by Tukey's test (HDS).

3. Results and Discussion

3.1. Mineral concentration of Genovese basil

Tables 4, **5**, and **Figure 1** show the macro and microelements profile of basil biofortified in FRS with different doses of Zn. The CV × B interactions were significant for all minerals analyzed by ion chromatography and ICP OES, except for K and P, which showed a significant difference only for mean effects. Specifically, the use of the B_{50} biofortified solution resulted in a significant increase in Ca (20.42%), Mg (23.01%), Mn (15.80%), Cu (19.16%), and Zn (45.38%) concentration for both cultivars compared to control (B_0). In contrast, the opposite trend was recorded for nitrate where the absolute highest values were obtained from the 'Eleonora' × B_{50} while the lowest values were obtained from the 'Aroma' × $B_{37.5}$.

Table 4. Macroelement concentration of Genovese basil cultivars 'Aroma 2' and 'Eleonora' at different doses of zinc added in nutrient solution.

Treatment	K	Na	Ca	Mg	P	Nitrate
	mg g^{-1} fw					
Cultivar (CV)						
Aroma 2	6.326±0.385	0.303±0.039	3.657±0.169	0.944±0.082	1.353±0.034	0.763±0.173
Eleonora	5.277±0.411	0.152±0.011	2.801±0.383	0.803±0.053	1.103±0.039	1.946±0.579
Biofortification (B)						
B_0	5.302±0.641d	0.194±0.062d	2.987±0.695c	0.782±0.073e	1.203±0.158b	1.839±0.875a
$B_{12.5}$	5.608±0.528c	0.213±0.077cd	2.965±0.612c	0.836±0.044d	1.201±0.128b	1.241±0.568c
B_{25}	5.770±0.551bc	0.230±0.086bc	3.264±0.267b	0.877±0.071c	1.224±0.141b	1.423±0.811b
$B_{37.5}$	5.890±0.618b	0.238±0.086b	3.331±0.472b	0.912±0.107b	1.234±0.143b	1.373±0.899bc
B_{50}	6.439±0.566a	0.263±0.105a	3.597±0.328a	0.962±0.100a	1.278±0.121a	0.896±0.111d
CV × B						
Aroma 2 × B_0	5.885±0.067	0.248±0.031d	3.619±0.059bc	0.848±0.016cd	1.346±0.021	1.043±0.097d
Aroma 2 × $B_{12.5}$	6.088±0.089	0.284±0.003c	3.522±0.071cd	0.873±0.028c	1.317±0.022	0.726±0.038fg
Aroma 2 × B_{25}	6.273±0.018	0.309±0.003bc	3.506±0.053cd	0.942±0.003b	1.354±0.003	0.686±0.027fg
Aroma 2 × $B_{37.5}$	6.453±0.037	0.317±0.002b	3.762±0.044ab	1.009±0.022a	1.365±0.007	0.555±0.025g
Aroma 2 × B_{50}	6.934±0.260	0.359±0.008a	3.877±0.187a	1.050±0.044a	1.383±0.060	0.805±0.037ef
Eleonora × B_0	4.719±0.058	0.140±0.002e	2.355±0.094f	0.715±0.003e	1.059±0.008	2.635±0.056a
Eleonora × $B_{12.5}$	5.129±0.037	0.142±0.004e	2.408±0.044f	0.799±0.008d	1.085±0.005	1.755±0.113c
Eleonora × B_{25}	5.267±0.049	0.152±0.006e	3.022±0.027e	0.812±0.010d	1.095±0.011	2.160±0.127b
Eleonora × $B_{37.5}$	5.326±0.040	0.158±0.004e	2.901±0.033e	0.814±0.007d	1.103±0.006	2.191±0.105b
Eleonora × B_{50}	5.945±0.047	0.167±0.002e	3.317±0.017d	0.875±0.008c	1.172±0.002	0.988±0.066de
Significance						
CV	***	***	***	***	***	***
B	***	***	***	***	***	***
CV × B	n.s.	***	***	***	n.s.	***

Data are mean values ± standard deviation, n = 3. Different letters within columns indicate significant mean differences according to Tukey HSD test (p = 0.05). CV factor was compared according to t-test. n.s. and *** denote non-significant or significant effects at $p \leq 0.001$, respectively.

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Table 5. Microelement concentration of Genovese basil cultivars 'Aroma 2' and 'Eleonora' at different doses of zinc added in nutrient solution.

Treatment	Mn	Fe	Cu	Se
	μg g^{-1} fw			
Cultivar (CV)				
Aroma 2	21.282±1.284	25.392±0.823	2.832±0.159	0.233±0.026
Eleonora	16.356±1.250	20.877±0.549	3.114±0.246	0.244±0.032
Biofortification (B)				
B_0	17.827±2.806cd	23.032±2.380b	2.766±0.086d	0.221±0.022b
$B_{12.5}$	17.595±2.229d	22.600±1.951b	2.937±0.127bc	0.215±0.011b
B_{25}	18.480±3.079c	22.867±2.869b	2.854±0.170cd	0.225±0.017b
$B_{37.5}$	19.549±3.074b	23.283±2.934ab	3.014±0.219b	0.258±0.025a
B_{50}	20.644±2.419a	23.891±2.419a	3.296±0.250a	0.273±0.019a
CV × B				
Aroma 2 × B_0	20.370±0.298cd	25.194±0.331ab	2.710±0.068d	0.202±0.008e
Aroma 2 × $B_{12.5}$	19.614±0.417de	24.359±0.467b	2.838±0.104cd	0.215±0.015de
Aroma 2 × B_{25}	21.288±0.046bc	25.485±0.074ab	2.705±0.039d	0.239±0.004bcde
Aroma 2 × $B_{37.5}$	22.356±0.033ab	25.960±0.135a	2.821±0.083cd	0.235±0.007cde
Aroma 2 × B_{50}	22.782±0.869a	25.963±1.324a	3.086±0.101b	0.274±0.013ab
Eleonora × B_0	15.284±0.459g	20.871±0.187cd	2.821±0.069cd	0.240±0.011bcd
Eleonora × $B_{12.5}$	15.573±0.093g	20.840±0.118cd	3.035±0.025bc	0.216±0.007de
Eleonora × B_{25}	15.673±0.223fg	20.249±0.161d	3.003±0.063bc	0.210±0.009de
Eleonora × $B_{37.5}$	16.742±0.053f	20.607±0.077cd	3.207±0.038b	0.280±0.004a
Eleonora × B_{50}	18.507±0.416e	21.818±0.049c	3.505±0.120a	0.272±0.028abc
Significance				
CV	***	***	***	*
B	***	***	***	***
CV × B	**	*	*	***

Data are mean values ± standard deviation, n = 3. Different letters within columns indicate significant mean differences according to Tukey HSD test (p = 0.05). CV factor was compared according to t-test. *; **, and *** denote non-significant or significant effects at $p \leq$ 0.05, 0.01, and 0.001, respectively.

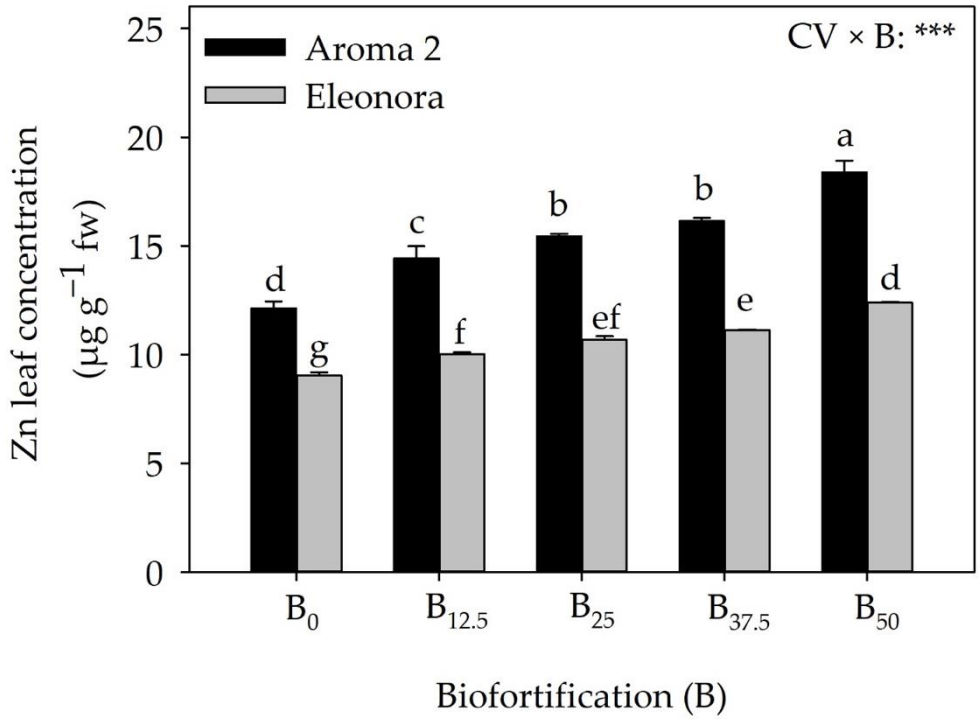


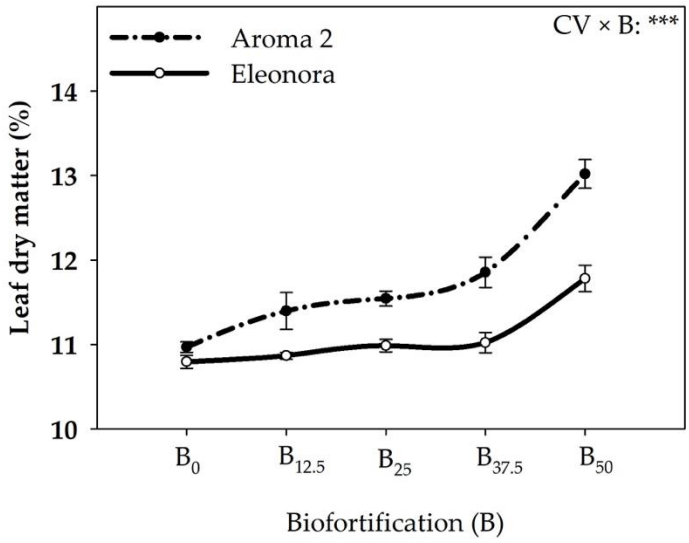
Figure 1. Analysis of variance and mean comparisons for Zn leaf concentration of 'Aroma 2' and 'Eleonora' basil under different Zn treatments: B_0 = control; $B_{12.5}$; B_{25}; $B_{37.5}$; B_{50} μM of Zn. ***significant effect at $p \leq 0.001$. Data represent means ± standard deviation (n = 3). Different letters within columns indicate significant mean differences according to Tukey HSD test (p = 0.05).

Although leafy vegetables are recognized contributors to minerals in the human diet, the scientific community has always neglected the mineral composition of basil in favor of its phenolic and aromatic composition. This gap is even more exacerbated by the vast genetic diversity of the genus *Ocimum*, as highlighted in several studies, regardless of the system and growing conditions[34-36]. According to the literature, the results reported in **Table 4**, **Table 5**, and **Figure 1** show that, although the two basil cultivars belonged to the Genovese type, they had a significant difference in mineral profile ($p < 0.001$), in contrast to Se ($p < 0.05$). 'Aroma 2' had higher concentrations of K, Na, Ca, Mg, P, Mn, Fe, and Zn, while 'Eleonora' of Cu, Se, and nitrate. The significant difference in the tissue concentration of nitrate, the quintessential antinutritional compound in leafy vegetables, can again be attributed to genetics. The values recorded in 'Aroma 2' (0.763 mg g^{-1} fw) and 'Eleonora' (1.946 mg g^{-1} fw) were within the ranges (0.240 to 4.280 mg g^{-1} fw) reported by Muráriková and Neugebauerová[37] in seven

different basil cultivars ('Ohře', 'Lettuce Leaf', 'Purple Opal', 'Dark Green', 'Mammolo Genovese', 'Mánes', and 'Red Rubin'). On average, macroelements of basil cultivars (**Table 4**) accounted for 99.6% of the mineral concentration, similar to Xiao et al.[38] in 30 microgreen varieties. As also reported by Alibas et al.[39], Burducea et al.[40], and Ciriello et al.[34], the most abundant macroelement in the basil cultivars was K with an average value of 5.801 mg g^{-1} fw, slightly higher than the USDA[14] values (1.28-5.65 mg g^{-1} fw), followed by Ca, P, Mg, and Na. For the microelements (**Table 5** and **Figure 1**), our results confirmed the findings of Pandeyet al.[41], in which Fe was the most abundant microelement, followed by Mn, Zn, Cu, and Se. Based on our results, it is evident that 'Aroma 2' and 'Eleonora', regardless of biofortification treatments, had higher mean concentrations of K, Ca, and Mg compared to the findings of a recent study on minor leafy vegetables such as brassica mizuna, tatsoi, and endive (*Cichorium endivia* L.)[42]. The same trend was observed by Kim et al.[43] in a detailed review on lettuce, one of the most widely used and consumed leafy vegetables. Specifically, our mean values of K, P, Mg, and Zn were higher than those reported for different lettuce cultivars (about ten times higher). However, it should be noted that the observed results, in addition to depending on the genotype, could result from the different growth conditions, environmental conditions and analytical procedures used.

As observed by Barrameda-Medina et al.[20], de Almeida et al.[1], and D'Imperio et al.[6] in broccoli, in lettuce, and purslane, respectively, Zn supplementation in nutrient solution increased Zn concentration in Aroma 2 and Eleonora (**Figure 1**). These results underscore how the soilless growing conditions (stable pH, absence of agricultural soil, and close contact of the roots with a biofortified nutrient solution) were crucial for the expected results. However, basil is not considered a hyperaccumulator of Zn, unlike *Brassicaceae*, *Caryophyllaceae*, *Polygonaceae*, and *Dichapetalaceae*[8]. 'Aroma 2' and 'Eleonora' recorded the maximum bioaccumulation of Zn in the B_{50} treatment. Specifically, between the control (B_0) and B_{50} treatment, was observed a Zn increase of 51.47 and 37.22% in 'Aroma 2' and 'Eleonora', respectively. Despite this, the Zn concentration in 'Aroma 2' × B_{50} was 48.32% higher than that obtained at the same dose in 'Eleonora'. The difference between cultivars can be attributed to genetics, as observed in comparable studies on lettuce and purslane[1,6]. The better results obtained by 'Aroma 2' could probably be due to a better constitutive transpiration rate that would have favored xylem transport of Zn into tissues[1]. Many works on basil biofortification have evaluated its effectiveness only in tissue accumulation of the element targeted for biofortification, not focusing on its potential effects on mineral profile concentration[44-46]. Although cross-talks between macro and microelements in plants have long been studied, the molecular basis of these nutritional interactions remains poorly understood[47]. Compared to Zn, the state of the art shows significant antagonism between macro (K, Ca, Na, and P) and microelements (Fe and Cu) since many of them share the same membrane transporters[48,49]. However,

our results disagree with the above. Supplementation of the maximum dose of Zn in the nutrient solution, regardless of the cultivar, increased, compared to the control, the concentration of all macro and microelements, except for nitrate. The explanation for these contradictory results can be attributed to several aspects, such as a) use of a hydroponic growing system (absence of agricultural soil and pH fluctuations)[50]; b) biofortification in nutrient solution and not by leaf spray (better regulation of nutrient uptake, transport, and partitioning)[51]; (c) short growth cycle compared to staple crops ordinarily grown on agricultural soils[21]; (d) exclusively xylem transport, unlike *Graminaceae*; and (e) the use of Zn in the nutrient solution, which resulted in a significant increase in leaf dry matter percentage for both cultivars (**Supplementary Figure 1**). As previously reported, an opposite trend was observed for nitrate. The use of the maximum dose of Zn, compared to the control, reduced the concentration of this antinutrient by 22.81 and 62.50% in 'Aroma 2' and 'Eleonora', respectively. In support of the above, Barrameda-Medina et al.[20] correlated NO_3^- reduction with increased zinc concentration (between 63 and 97%) in lettuce. This result is not surprising because Zn is involved in nitrogen metabolism in plants, as high concentrations of Zn can inhibit NO_3^- assimilation and N incorporation into amino acids and proteins[52]. Similar results were observed by Siddiqui et al.[53] in *Cicer arietinum*.



Supplementary Figure 1. Analysis of variance and mean comparisons for leaf dry matter (%) of 'Aroma 2' and 'Eleonora' basil under different Zn treatments: B_0 =control; $B_{12.5}$; B_{25}; $B_{37.5}$; B_{50} µM of Zn. ***significant effect at p≤0.001. Data represent means ± standard error (n=3).

3.2. Estimated Daily Intake and Nitrate Hazard Quotient in Children and Adults

Based on the results obtained after biofortification with zinc on the macro- and micro-nutrient profile (**Table 4**, **5** and **Figure 1**), the EDI of all macro (K, Na, Ca, Mg, and P) and micro-nutrients (Mn, Fe, Zn, Cu, and Se) was calculated assuming a per serving consumption of 5 and 15 g of fresh basil in children and adults, respectively (**Table 6**, **Table 7**, and **Figure 2**).

Table 6. Estimated daily intake (EDI) of macro- and micro-nutrients and nitrate Hazard Quotient (HQ_n) for children.

Treatment	K	Na	Ca	Mg	P
	mg day^{-1}				
Cultivar (CV)					
Aroma 2	31.634±1.928	1.518±0.199	18.288±0.847	4.723±0.414	6.767±0.176
Eleonora	26.388±2.057	0.761±0.055	14.005±1.917	4.017±0.266	5.515±0.198
Biofortification (B)					
B_0	26.511±3.206d	0.973±0.312d	14.939±3.479c	3.910±0.367e	6.015±0.791b
$B_{12.5}$	28.044±2.642c	1.066±0.389cd	14.828±3.062c	4.181±0.222d	6.005±0.640b
B_{25}	28.851±2.759bc	1.153±0.430c	16.322±1.339b	4.386±0.358c	6.123±0.709b
$B_{37.5}$	29.450±3.092b	1.190±0.434b	16.659±2.362b	4.560±0.537b	6.172±0.718b
B_{50}	32.199±2.832a	1.318±0.525a	17.986±1.644a	4.813±0.500a	6.390±0.608a
CV × B					
Aroma 2 × B_0	29.427±0.337	1.243±0.156d	18.099±0.298bc	4.242±0.082cd	6.734±0.105
Aroma 2 × $B_{12.5}$	30.440±0.448	1.421±0.019c	17.613±0.358cd	4.365±0.144c	6.586±0.110
Aroma 2 × B_{25}	31.366±0.090	1.545±0.016bc	17.532±0.269cd	4.712±0.015b	6.770±0.019
Aroma 2 × $B_{37.5}$	32.268±0.187	1.556±0.010b	18.810±0.221b	5.046±0.111a	6.827±0.037
Aroma 2 × B_{50}	34.670±1.303	1.797±0.043a	19.386±0.935a	5.251±0.222a	6.917±0.301
Eleonora × B_0	23.595±0.290	0.703±0.012e	11.779±0.470f	3.578±0.015e	5.296±0.040
Eleonora × $B_{12.5}$	25.648±0.186	0.712±0.021e	12.043±0.221f	3.998±0.042d	5.425±0.028
Eleonora × B_{25}	26.337±0.249	0.760±0.034e	15.112±0.136e	4.060±0.053d	5.476±0.056
Eleonora × $B_{37.5}$	26.631±0.201	0.794±0.021e	14.508±0.165e	4.074±0.038d	5.516±0.034
Eleonora × B_{50}	29.729±0.238	0.839±0.011e	16.587±0.089d	4.376±0.044c	5.863±0.014
Significance					
CV	***	***	***	***	***
B	***	***	***	***	***
CV × B	n.s.	***	***	***	n.s

Cont. **Table 6**

Treatment	Mn	Fe	Cu	Se	HQ_n
			μg day^{-1}		
Cultivar (CV)					
Aroma 2	106.412±6.421	126.964±4.119	14.162±0.797	1.168±0.134	0.068±0.015
Eleonora	81.782±6.252	104.387±2.748	15.574±1.231	1.220±0.160	0.175±0.052
Biofortification (B)					
B_0	89.137±14.034dc	115.164±11.902b	13.830±0.433d	1.109±0.113b	0.165±0.078a
$B_{12.5}$	87.975±11.145d	113.007±9.757b	14.686±0.637bc	1.079±0.055b	0.111±0.051c
B_{25}	92.404±15.395c	114.338±14.349b	14.272±0.850cd	1.125±0.088b	0.128±0.073bc
$B_{37.5}$	97.746±15.374b	116.419±14.670ab	15.072±1.098b	1.291±0.126a	0.123±0.080b
B_{50}	103.222±12.098a	119.456±12.099a	16.480±1.250a	1.367±0.099a	0.080±0.010d
CV × B					
Aroma 2 × B_0	101.851±1.492cd	125.973±1.658ab	13.552±0.344d	1.014±0.042e	0.094±0.008d
Aroma 2 × $B_{12.5}$	98.074±2.085de	121.799±2.335b	14.194±0.522cd	1.077±0.078de	0.065±0.003fg
Aroma 2 × B_{25}	106.443±0.233bc	127.427±0.371ab	13.526±0.195d	1.199±0.022bcde	0.061±0.002fg
Aroma 2 × $B_{37.5}$	111.780±0.166ab	129.804±0.675a	14.105±0.417cd	1.178±0.039cde	0.050±0.002g
Aroma 2 × B_{50}	113.910±4.348a	129.818±6.620a	15.433±0.508b	1.374±0.065ab	0.072±0.003ef
Eleonora × B_0	76.423±2.295g	104.355±0.935cd	14.107±0.346cd	1.204±0.056bcd	0.237±0.05a
Eleonora × $B_{12.5}$	77.876±0.466g	104.202±0.593cd	15.179±0.126bc	1.081±0.038de	0.158±0.010c
Eleonora × B_{25}	78.366±1.119fg	101.249±0.806d	15.018±0.318bc	1.051±0.048de	0.194±0.011b
Eleonora × $B_{37.5}$	83.712±0.266f	103.035±0.388cd	16.039±0.109b	1.404±0.021a	0.197±0.009b
Eleonora × B_{50}	92.535±2.083e	109.094±0.249c	17.527±0.603a	1.360±0.141abc	0.089±0.005de
Significance					
CV	***	***	***	n.s.	***
B	***	***	***	***	***
CV × B	**	*	*	***	***

Data are mean values ± standard deviation, n = 3. Different letters within columns indicate significant mean differences according to Tukey HSD test (p = 0.05). CV factor was compared according to t-test. n.s., *, and *** denote non-significant or significant effects at $p \leq$ 0.05 and 0.001, respectively.

Table 7. Estimated daily intake (EDI) of macro- and micro-nutrients and nitrate Hazard Quotient (HQ_n) for adults.

Treatment	K	Na	Ca	Mg	P
	mg day^{-1}				
Cultivar (CV)					
Aroma 2	94.903±5.786	4.556±0.599	54.865±2.541	14.170±1.244	20.302±0.515
Eleonora	79.165±6.172	2.285±0.167	42.017±5.753	12.052±0.800	16.546±0.595
Biofortification (B)					
B_0	79.534±9.619d	2.919±0.936d	44.818±10.439c	11.731±1.102e	18.046±2.373b
$B_{12.5}$	84.132±7.927c	3.200±1.167cd	44.485±9.188c	12.544±0.677d	18.017±1.921b
B_{25}	86.555±8.279bc	3.460±1.291bc	48.966±4.017b	13.159±1.075c	18.336±2.128b
$B_{37.5}$	88.350±9.277b	3.571±1.302b	49.977±7.088b	13.682±1.612b	18.516±2.155b
B_{50}	96.599±8.498a	3.954±1.577a	53.960±4.932a	14.440±1.500a	19.172±1.824a
CV × B					
Aroma 2 × B_0	88.282±1.011	3.729±0.470d	54.298±0.896bc	12.727±0.247cd	20.204±0.317
Aroma 2 × $B_{12.5}$	91.320±1.345	4.264±0.059c	52.841±1.076cd	13.095±0.434c	19.760±0.332
Aroma 2 × B_{25}	94.099±0.270	4.637±0.049bc	52.596±0.809cd	14.136±0.047b	20.310±0.059
Aroma 2 × $B_{37.5}$	96.805±0.561	4.759±0.030b	56.430±0.665ab	15.140±0.333a	20.482±0.111
Aroma 2 × B_{50}	104.010±3.911	5.392±0.131a	58.159±2.805a	15.753±0.668a	20.753±0.905
Eleonora × B_0	70.787±0.871	2.109±0.037e	35.337±1.410f	10.736±0.047e	15.889±0.122
Eleonora × $B_{12.5}$	76.94±0.559	2.136±0.063e	36.129±0.663f	11.994±0.126d	16.275±0.086
Eleonora × B_{25}	79.011±0.747	2.282±0.103e	45.336±0.408e	12.182±0.160d	16.428±0.168
Eleonora × $B_{37.5}$	79.894±0.604	2.383±0.065e	43.524±0.495e	12.224±0.114d	16.550±0.102
Eleonora × B_{50}	89.189±0.716	2.517±0.035e	49.761±0.268d	13.128±0.132c	17.591±0.042
Significance					
CV	***	***	***	***	***
B	***	***	***	***	***
CV × B	n.s.	***	***	***	n.s.

Cont. **Table 7**

Treatment	Mn	Fe	Cu	Se	HQ nitrate
	μg day^{-1}				
Cultivar (CV)					
Aroma 2	319.236±19.263	380.893±12.357	42.487±2.393	3.506±0.404	0.044±0.010
Eleonora	245.347±18.756	313.162±8.246	46.723±3.693	3.660±0.481	0.112±0.033
Biofortification (B)					
B_0	267.412±42.104cd	345.493±35.706b	41.490±1.300d	3.328±0.339b	0.106±0.050a
$B_{12.5}$	263.926±33.435d	339.002±29.273b	44.060±1.913bc	3.238±0.166b	0.071±0.032c
B_{25}	277.214±46.187c	343.015±43.048b	42.816±2.551cd	3.376±0.264b	0.082±0.046b
$B_{37.5}$	293.238±46.124b	349.259±44.010ab	45.218±3.294b	3.874±0.380a	0.079±0.052bc
B_{50}	309.668±36.295a	358.370±36.299a	49.440±3.752a	4.102±0.297a	0.051±0.006d
CV × B					
Aroma 2 × B_0	305.554±4.478cd	377.921±4.976ab	40.658±1.034cd	3.043±0.126e	0.060±0.005d
Aroma 2 × $B_{12.5}$	294.223±6.255de	365.397±7.006b	42.582±1.568cd	3.233±0.236de	0.042±0.002fg
Aroma 2 × B_{25}	319.331±0.699bc	382.282±1.113ab	40.579±0.587d	3.599±0.068bcde	0.039±0.001fg
Aroma 2 × $B_{37.5}$	335.340±0.498ab	389.412±2.027a	42.317±1.252cd	3.535±0.117cde	0.0321±0.001g
Aroma 2 × B_{50}	341.731±13.044a	389.456±19.862a	46.299±1.525b	4.123±0.195ab	0.0466±0.002ef
Eleonora × B_0	229.270±6.886g	313.065±2.805cd	42.321±1.040cd	3.613±0.169bcd	0.152±0.003a
Eleonora × $B_{12.5}$	233.629±1.399g	312.607±1.781cd	45.538±0.379bc	3.243±0.116de	0.101±0.06c
Eleonora × B_{25}	235.098±3.357fg	303.747±2.418d	45.054±0.955bc	3.153±0.144de	0.125±0.07b
Eleonora × $B_{37.5}$	251.136±0.799f	309.105±1.164cd	48.119±0.571b	4.213±0.063a	0.126±0.006b
Eleonora × B_{50}	277.605±6.250e	327.284±0.748c	52.582±1.811a	4.081±0.425abc	0.057±0.003de
Significance					
CV	***	***	***	n.s.	***
B	***	***	***	***	***
CV × B	**	*	*	***	***

Data are mean values ± standard deviation, n = 3. Different letters within columns indicate significant mean differences according to Tukey HSD test (p = 0.05). CV factor was compared according to t-test. n.s., *, **, and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

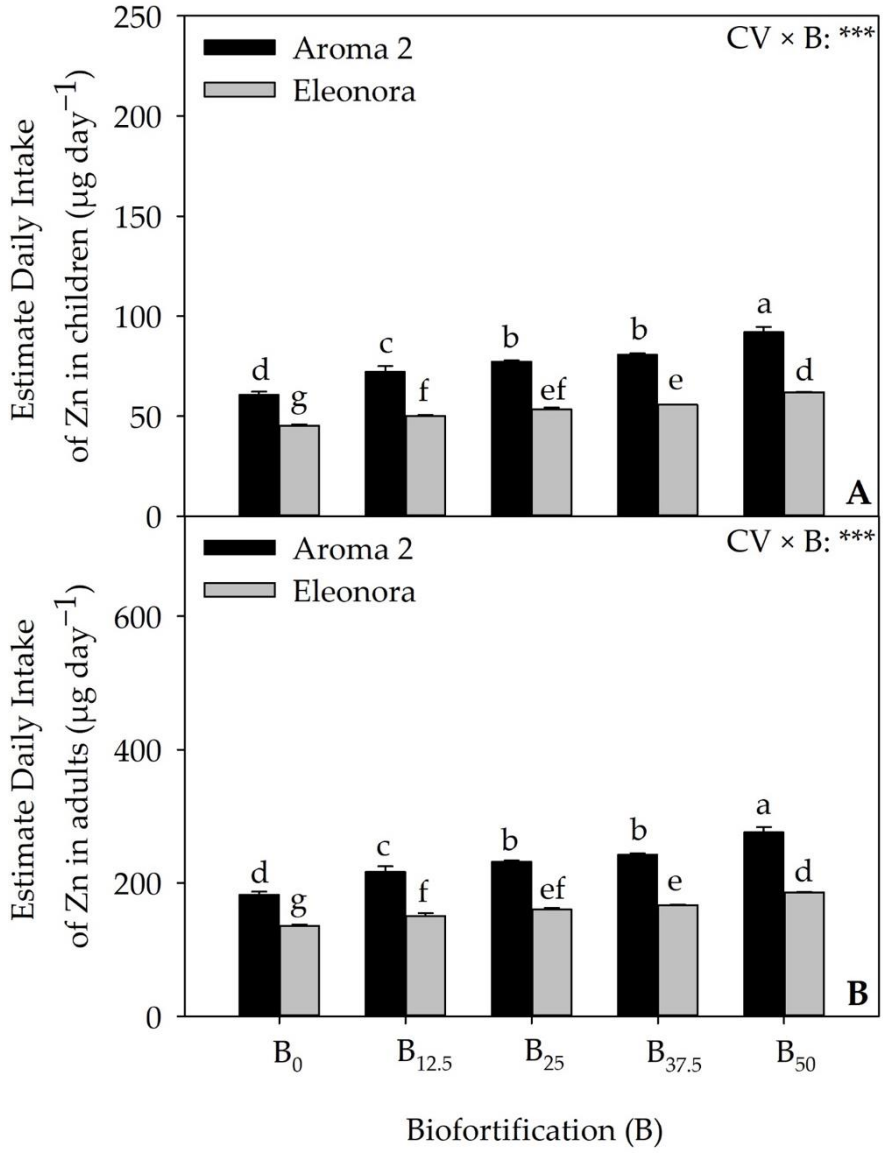


Figure 1: Analysis of variance and mean comparisons for Estimate Daily Intake in children (**A**) and adults (**B**) of 'Aroma 2' and 'Eleonora' basil under different Zn treatments: B_0 = control; $B_{12.5}$; B_{25}; $B_{37.5}$; B_{50} µM of Zn. ***significant effect at $p \leq 0.001$. Data represent means ± standard deviation (n=3). Different letters within columns indicate significant mean differences according to Tukey HSD test ($p = 0.05$).

Statistical analysis shows that regardless of cultivar, biofortification significantly ($p < 0.001$) increased all EDI values in both adults and children. Although the importance of Zn intake was established as early as the 1960s, today, nearly a third of the world's population (2.5 billion) does not compensate for Zn deficiency through a daily diet[7]. The clinical incidence of this deficiency is more severe as its magnitude increases and age decreases, with greater criticality highlighted in children[7,54]. In light of the above, the significant increase in EDI-Zn ($p < 0.001$) in children and adults with the intake of biofortified basil, corroborates the importance of extending biofortification programs to leafy crops that are well suited for soilless cultivation, as previously reported by D'Imperio et al.[6] in purslane and by de Almeida et al.[1] in lettuce. Regardless of age, the highest EDI-Zn was obtained from the 'Aroma 2' × B_{50} interaction (91.915 and 275.746 μg day^{-1} in children and adults, respectively: **Figures 2A** and **2B**), while the lowest from 'Aroma 2' × B_0 and 'Eleonora' × B_0 interactions. Based on the recommended dietary allowances (RDA) and adequate intakes (AI) for macro and microelements reported in **Table 8**, regardless of the cultivar, the B_{50} treatment increased, compared to the control, the Zn nutrient contribution (NC-Zn) by 45.36% in children and adults and 45.55% in the elders (**Supplementary Table 1**).

Table 8. Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for macro- and micro-element for children, adults (males and females), and elders. Macroelements (K, Na, Ca, Mg, and P) were expressed as mg day^{-1} while microelements (Mn, Fe, Zn, Cu, and Se) as μg day^{-1}.

Element	Children	Adults		Elders
		Males	Females	
K	**3800**	**4700**	**4700**	**4700**
Na	**1200**	**1433**	**1433**	**1200**
Ca	1000	1000	1066	1200
Mg	130	413	316	370
P	500	700	700	700
Mn	**1500**	**2300**	**1800**	**2050**
Fe	10000	8000	14600	8000
Zn	5000	11000	8000	9500
Cu	440	900	900	900
Se	30	55	55	55

RDAs in ordinary type and AIs in bold type.

Supplementary Table 1. Nutritional Contribution (NC) per meal for children, adults (males and females), and elders of Mn, Fe, Zn, Cu, and Se. Values were expressed as percentage.

Treatment	Mn				Fe			
	Children	Adults		Elders	Children	Adults		Elders
		Male	Female			Male	Female	
Cultivar (CV)								
Aroma 2	7.094±0.428	13.879±0.838	17.735±1.070	15.572±0.940	1.269±0.041	4.761±0.154	2.608±0.085	4.761±0.154
Eleonora	5.452±0.417	10.667±0.816	13.630±1.042	11.968±0.915	1.043±0.027	3.914±0.103	2.144±0.056	3.914±0.103
Biofortification (B)								
B_0	5.942±0.936cd	11.626±1.831cd	14.856±2.339cd	13.044±2.054cd	1.151±0.119b	4.318±0.446b	2.366±0.245b	4.318±0.446b
$B_{12.5}$	5.865±0.743d	11.475±1.454d	14.662±1.858d	12.874±1.631d	1.130±0.098b	4.237±0.366b	2.321±0.201b	4.237±0.366b
B_{25}	6.160±1.026c	12.052±2.008c	15.400±2.566c	13.522±2.253c	1.143±0.143b	4.287±0.538b	2.349±0.295b	4.287±0.538b
$B_{37.5}$	6.516±1.025b	12.749±2.005b	16.291±2.562b	14.304±2.250b	1.164±0.147ab	4.365±0.55ab	2.392±0.301ab	4.365±0.55ab
B_{50}	6.881±0.807a	13.463±1.578a	17.203±2.016a	15.105±1.770a	1.194±0.121a	4.479±0.454a	2.454±0.249a	4.479±0.454a
CV × B								
Aroma 2 × B_0	6.79±00.100cd	13.284±0.195cd	16.975±0.249cd	14.905±0.218cd	1.259.017ab	4.724±0.062ab	2.588±0.034ab	4.724±0.062ab
Aroma 2 × $B_{12.5}$	6.538±0.139de	12.792±0.272de	16.345±0.348de	14.352±0.305de	1.217±0.023b	4.567±0.088b	2.502±0.048b	4.567±0.088b
Aroma 2 × B_{25}	7.092±0.016bc	13.883±0.030bc	17.740±0.039bc	15.577±0.034bc	1.274±0.004ab	4.778±0.014ab	2.618±0.008ab	4.778±0.014ab
Aroma 2 × $B_{37.5}$	7.452±0.011ab	14.580±0.022ab	18.630±0.028ab	16.358±0.024ab	1.298±0.007a	4.867±0.025a	2.667±0.014a	4.867±0.025a
Aroma 2 × B_{50}	7.594±0.290a	14.857±0.567a	18.985±0.725a	16.669±0.636a	1.298±0.066a	4.868±0.248a	2.667±0.136a	4.868±0.248a
Eleonora × B_0	5.094±0.153g	9.968±0.299g	12.737±0.383g	11.183±0.336g	1.043±0.009cd	3.913±0.035cd	2.144±0.019cd	3.913±0.035cd
Eleonora × $B_{12.5}$	5.191±0.031g	10.157±0.061g	12.979±0.078g	11.396±0.068g	1.042±0.006cd	3.907±0.022cd	2.141±0.012cd	3.907±0.022cd
Eleonora × B_{25}	5.224±0.075fg	10.221±0.146fg	13.061±0.187fg	11.468±0.164fg	1.012±0.008d	3.796±0.030d	2.080±0.017d	3.796±0.030d
Eleonora × $B_{37.5}$	5.580±0.018f	10.918±0.035f	13.952±0.044f	12.250±0.039f	1.030±0.004cd	3.863±0.015cd	2.117±0.008cd	3.863±0.015cd
Eleonora × B_{50}	6.169±0.139e	12.069±0.272e	15.422±0.347e	13.541±0.305e	1.090±0.002c	4.091±0.009c	2.241±0.005c	4.091±0.009c
Significance								
CV	***	***	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
CV × B	**	**	**	**	*	*	*	*

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Cont. **Supplementary Table 1**

Treatment	Zn				Cu			
	Children	Adults		Elders	Children	Adults		Elders
		Males	Females			Males	Females	
Cultivar (CV)								
Aroma 2	1.530±0.215	2.087±0.293	2.870±0.402	2.416±0.339	3.218±0.181	4.720±0.266	4.720±0.266	4.720±0.266
Eleonora	1.064±0.116	1.452±0.159	1.996±0.218	1.681±0.184	3.539±0.280	5.191±0.410	5.191±0.410	5.191±0.410
Biofortification (B)								
B_0	1.058±0.171e	1.443±0.234e	1.984±0.322e	1.671±0.271e	3.143±0.099d	4.610±0.144d	4.610±0.144d	4.610±0.144d
$B_{12.5}$	1.222±0.244d	1.667±0.333d	2.292±0.458d	1.930±0.386d	3.337±0.145bc	4.895±0.213bc	4.895±0.213bc	4.895±0.213bc
B_{25}	1.305±0.261c	1.780±0.357c	2.448±0.490c	2.062±0.413c	3.243±0.193cd	4.757±0.284cd	4.757±0.284cd	4.757±0.284cd
$B_{37.5}$	1.363±0.275b	1.858±0.375b	2.557±0.515b	2.152±0.434b	3.425±0.250b	5.024±0.366b	5.024±0.366b	5.024±0.366b
B_{50}	1.538±0.330a	2.098±0.450a	2.885±0.618a	2.429±0.521a	3.745±0.284a	5.493±0.417a	5.493±0.417a	5.493±0.417a
CV × B								
Aroma 2 × B_0	1.213±0.032d	1.654±0.043d	2.275±0.059d	1.916±0.050d	3.080±0.078d	4.517±0.115d	4.517±0.115cd	4.517±0.115cd
Aroma 2 × $B_{12.5}$	1.443±0.056c	1.968±0.076c	2.706±0.105c	2.279±0.088c	3.225±0.119cd	4.731±0.174cd	4.731±0.174cd	4.731±0.174cd
Aroma 2 × B_{25}	1.544±0.012b	2.105±0.016b	2.895±0.022b	2.438±0.018b	3.074±0.044d	4.508±0.065d	4.508±0.065d	4.508±0.065d
Aroma 2 × $B_{37.5}$	1.613±0.015b	2.200±0.020b	3.025±0.027b	2.547±0.023b	3.205±0.095cd	4.701±0.139cd	4.701±0.139cd	4.701±0.139cd
Aroma 2 × B_{50}	1.838±0.054a	2.506±0.074a	3.446±0.101a	2.902±0.085a	3.507±0.116b	5.144±0.170b	5.144±0.170b	5.144±0.170b
Eleonora × B_0	0.903±0.016g	1.231±0.021g	1.693±0.029g	1.426±0.025g	3.206±0.079cd	4.702±0.116cd	4.702±0.116cd	4.702±0.116cd
Eleonora × $B_{12.5}$	1.001±0.009f	1.366±0.013f	1.878±0.018f	1.582±0.015f	3.449±0.029bc	5.059±0.042bc	5.059±0.042bc	5.059±0.042bc
Eleonora × B_{25}	1.067±0.018ef	1.455±0.024ef	2.001±0.033ef	1.685±0.028ef	3.413±0.072bc	5.006±0.106bc	5.006±0.106bc	5.006±0.106bc
Eleonora × $B_{37.5}$	1.112±0.004e	1.517±0.005e	2.085±0.007e	1.756±0.006e	3.645±0.043b	5.346±0.063b	5.346±0.063b	5.346±0.063b
Eleonora × B_{50}	1.239±0.004d	1.690±0.006d	2.323±0.008d	1.956±0.007d	3.983±0.137a	5.842±0.201a	5.842±0.201a	5.842±0.201a
Significance								
CV	***	***	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
CV × B	***	***	***	***	*	*	*	*

Cont. **Supplementary Table 1**

Treatment	Se			
	Children	Adults		Elders
		Males	Females	
Cultivar (CV)				
Aroma 2	3.896±0.450	6.376±0.736	6.376±0.736	6.376±0.736
Eleonora	4.067±0.535	6.656±0.875	6.656±0.875	6.656±0.875
Biofortification (B)				
B_0	3.698±0.377b	6.051±0.618b	6.051±0.618b	6.051±0.618b
$B_{12.5}$	3.598±0.185b	5.887±0.303b	5.887±0.303b	5.887±0.303b
B_{25}	3.751±0.293b	6.138±0.480b	6.138±0.480b	6.138±0.480b
$B_{37.5}$	4.305±0.423a	7.044±0.692a	7.044±0.692a	7.044±0.692a
B_{50}	4.557±0.330a	7.458±0.541a	7.458±0.541a	7.458±0.541a
CV × B				
Aroma 2 × B_0	3.381±0.141e	5.533±0.231e	5.533±0.231e	5.533±0.231e
Aroma 2 × $B_{12.5}$	3.592±0.262de	5.879±0.429de	5.879±0.429de	5.879±0.429de
Aroma 2 × B_{25}	3.998±0.076bcde	6.543±0.124bcde	6.543±0.124bcde	6.543±0.124bcde
Aroma 2 × $B_{37.5}$	3.928±0.130cde	6.428±0.213cde	6.428±0.213cde	6.428±0.213cde
Aroma 2 × B_{50}	4.581±0.218ab	7.496±0.356ab	7.496±0.356ab	7.496±0.356ab
Eleonora × B_0	4.015±0.188bcd	6.570±0.308bcd	6.570±0.308bcd	6.570±0.308bcd
Eleonora × $B_{12.5}$	3.603±0.129de	5.896±0.211de	5.896±0.211de	5.896±0.211de
Eleonora × B_{25}	3.503±0.161de	5.733±0.263de	5.733±0.263de	5.733±0.263de
Eleonora × $B_{37.5}$	4.681±0.071a	7.660±0.116a	7.660±0.116a	7.660±0.116a
Eleonora × B_{50}	4.534±0.473abc	7.420±0.774abc	7.420±0.774abc	7.420±0.774abc
Significance				
CV	n.s.	n.s.	n.s.	n.s.
B	***	***	***	***
CV × B	***	***	***	***

Data are mean values ± standard deviation, $n = 3$. Different letters within columns indicate significant mean differences according to Tukey HSD test (p=0.05). CV factor was compared according to t-Test. n.s., *, **, and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

However, although the observed percentage increases lead to an NC-Zn of only 3% higher in women and contribute marginally to the RDA, it should be noted that: a) our results, for the same serving of basil consumed, would ensure a daily intake of Zn almost 1.5 fold higher than that obtained with biofortified lettuce[1], driving research toward the biofortification of "minor" species[6]; b) biofortification should not aim to increase the RDA but the concentration of macro and microelements to counter Zn deficiency, a key element for several target consumer groups. Unsurprisingly, Zn performs essential functions for humans, ensuring the proper functioning of the immune, reproductive, sensory, digestive, and nervous systems and being a cofactor of proteins and enzymes[54]. The interaction CV × B significantly influenced the EDI of the other macro and microelements analyzed by ICP OES, except for K and P (**Table 6**). Their values were significantly influenced by the average effects of CV and B, with the highest values obtained from 'Aroma 2' and B_{50} treatment. Specifically, one serving of B_{50} biofortified basil ensured 0.847% NC-K in children and 2.055% in adults and the elderly, considering that Western dietary patterns are increasingly geared toward diets high in sodium and low in potassium[55]. Today, only 10% of children in the United States reach the adequate intake level of K. At the same time, adults do not exceed 3%, considering their prominent role in maintaining heart function[55]. As previously observed for Zn, 'Aroma 2' × $B_{37.5}$ and 'Aroma 2' × B_{50} interactions, compared to the control, ensured the highest EDI-Ca, -Mg, and -Na, in adults and children (**Table 6** and **Table 7**). Only 5 g of B_{50} biofortified basil covered almost 4% of the Mg requirement (**Supplementary Table 2**), whose intake has decreased in recent decades[56]. Similarly, in the elderly, 15 g of basil biofortified with the same dose of Zn provided almost 4.5% of the daily Mg requirement, which plays a crucial role in maintaining neuromuscular and cardiac function and glycemic control[8,56]. In 'Aroma 2' and 'Eleonora', the maximum dose of zinc in the nutrient solution (B_{50}) increased EDI-Ca by 20.39% in children and adults compared to the control (**Table 6** and **Table 7**), resulting in increased NC-Ca (**Supplementary Table 2**).

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Supplementary Table 2. Nutritional contribution (NC) per meal for children, adults (males and females), and elders of K, Na, Ca, Mg, and P. Values were expressed as percentage.

Treatment	K				Na			
	Children	Adults		Elders	Children	Adults		Elders
		Male	Female			Male	Female	
Cultivar (CV)								
Aroma 2	0.832±0.051	2.019±0.123	2.019±0.123	2.019±0.123	0.126±0.017	0.317±0.042	0.317±0.042	0.379±0.050
Eleonora	0.694±0.054	1.684±0.131	1.684±0.131	1.684±0.131	0.063±0.005	0.159±0.012	0.159±0.012	0.190±0.014
Biofortification (B)								
B_0	0.697±0.084d	1.692±0.205d	1.692±0.205d	1.692±0.205d	0.081±0.026d	0.203±0.065d	0.203±0.065d	0.243±0.078d
$B_{12.5}$	0.738±0.070c	1.790±0.169c	1.790±0.169c	1.790±0.169c	0.088±0.032cd	0.223±0.081cd	0.223±0.081cd	0.266±0.097cd
B_{25}	0.759±0.073bc	1.841±0.176bc	1.841±0.176bc	1.841±0.176bc	0.096±0.036bc	0.241±0.09bc	0.241±0.09bc	0.288±0.108bc
$B_{37.5}$	0.775±0.081b	1.879±0.197b	1.879±0.197b	1.879±0.197b	0.099±0.036b	0.249±0.091b	0.249±0.091b	0.297±0.109b
B_{50}	0.847±0.075a	2.055±0.181a	2.055±0.181a	2.055±0.181a	0.109±0.044a	0.275±0.110a	0.275±0.110a	0.329±0.131a
CV × B								
Aroma 2 × B_0	0.774±0.009	1.878±0.022	1.878±0.022	1.878±0.022	0.103±0.013d	0.260±0.033d	0.260±0.033d	0.310±0.039d
Aroma 2 × $B_{12.5}$	0.801±0.012	1.942±0.029	1.942±0.029	1.942±0.029	0.118±0.002c	0.297±0.004c	0.297±0.004c	0.355±0.005c
Aroma 2 × B_{25}	0.825±0.002	2.002±0.006	2.002±0.006	2.002±0.006	0.128±0.001bc	0.323±0.003bc	0.323±0.003bc	0.386±0.004bc
Aroma 2 × $B_{37.5}$	0.849±0.005	2.059±0.012	2.059±0.012	2.059±0.012	0.132±0.001b	0.332±0.002b	0.332±0.002b	0.396±0.003b
Aroma 2 × B_{50}	0.912±0.034	2.212±0.083	2.212±0.083	2.212±0.083	0.149±0.004a	0.376±0.009a	0.376±0.009a	0.449±0.011a
Eleonora × B_0	0.620±0.008	1.506±0.019	1.506±0.019	1.506±0.019	0.058±0.001e	0.147±0.003e	0.147±0.003e	0.175±0.003e
Eleonora × $B_{12.5}$	0.674±0.005	1.637±0.012	1.637±0.012	1.637±0.012	0.059±0.002e	0.149±0.004e	0.149±0.004e	0.178±0.005e
Eleonora × B_{25}	0.693±0.007	1.681±0.016	1.681±0.016	1.681±0.016	0.063±0.003e	0.159±0.007e	0.159±0.007e	0.190±0.009e
Eleonora × $B_{37.5}$	0.700±0.005	1.699±0.013	1.699±0.013	1.699±0.013	0.066±0.002e	0.166±0.005e	0.166±0.005e	0.198±0.005e
Eleonora × B_{50}	0.782±0.006	1.897±0.015	1.897±0.015	1.897±0.015	0.069±0.001e	0.175±0.002e	0.175±0.002e	0.209±0.003e
Significance								
CV	***	***	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
CV × B	n.s.	n.s.	n.s.	n.s.	***	***	***	***

Cont. **Supplementary Table 2.**

Treatment	Ca				Mg			
	Children	Adults		Elders	Children	Adults		Elders
		Male	Female			Man	Woman	
Cultivar (CV)								
Aroma 2	1.828±0.085	5.486±0.254	5.146±0.238	4.572±0.212	3.633±0.319	3.428±0.301	4.474±0.393	4.474±0.393
Eleonora	1.400±0.192	4.201±0.575	3.941±0.540	3.501±0.479	3.090±0.205	2.916±0.194	3.806±0.253	3.806±0.253
Biofortification (B)								
B_0	1.493±0.348c	4.481±1.044c	4.204±0.979c	3.734±0.870c	3.008±0.283e	2.838±0.267e	3.704±0.348e	3.704±0.348e
$B_{12.5}$	1.482±0.306c	4.448±0.919c	4.173±0.862c	3.707±0.766c	3.216±0.171d	3.035±0.162d	3.961±0.211d	3.961±0.211d
B_{25}	1.632±0.134b	4.896±0.402b	4.593±0.377b	4.080±0.335b	3.374±0.276c	3.183±0.260c	4.155±0.340c	4.155±0.340c
$B_{37.5}$	1.665±0.236b	4.997±0.709b	4.688±0.665b	4.164±0.591b	3.508±0.413b	3.310±0.390b	4.320±0.509b	4.320±0.509b
B_{50}	1.798±0.164a	5.396±0.493a	5.061±0.463a	4.496±0.411a	3.702±0.385a	3.493±0.363a	4.560±0.474a	4.560±0.474a
CV × B								
Aroma 2 × B_0	1.809±0.030bc	5.429±0.090bc	5.093±0.084bc	4.524±0.075bc	3.263±0.064cd	3.079±0.060cd	4.019±0.078cd	4.019±0.078cd
Aroma 2 × $B_{12.5}$	1.761±0.036cd	5.284±0.108cd	4.956±0.101cd	4.403±0.090cd	3.357±0.111c	3.168±0.105c	4.135±0.137c	4.135±0.137c
Aroma 2 × B_{25}	1.753±0.027cd	5.259±0.081cd	4.934±0.076cd	4.383±0.067cd	3.624±0.012b	3.420±0.011b	4.464±0.015b	4.464±0.015b
Aroma 2 × $B_{37.5}$	1.881±0.022ab	5.643±0.067ab	5.293±0.062ab	4.702±0.055ab	3.882±0.086a	3.662±0.081a	4.781±0.105a	4.781±0.105a
Aroma 2 × B_{50}	1.938±0.094a	5.815±0.281a	5.455±0.263a	4.846±0.234a	4.039±0.171a	3.811±0.162a	4.974±0.211a	4.974±0.211a
Eleonora × B_0	1.177±0.047f	3.533±0.141f	3.314±0.132f	2.944±0.118f	2.752±0.012e	2.597±0.011e	3.390±0.015e	3.390±0.015e
Eleonora × $B_{12.5}$	1.204±0.022f	3.612±0.066f	3.389±0.062f	3.010±0.055f	3.075±0.032d	2.901±0.031d	3.787±0.04d	3.787±0.04d
Eleonora × B_{25}	1.511±0.014e	4.533±0.041e	4.252±0.038e	3.778±0.034e	3.123±0.041d	2.947±0.039d	3.847±0.051d	3.847±0.051d
Eleonora × $B_{37.5}$	1.450±0.017e	4.352±0.050e	4.082±0.046e	3.627±0.041e	3.134±0.029d	2.957±0.028d	3.860±0.036d	3.860±0.036d
Eleonora × B_{50}	1.658±0.009d	4.976±0.027d	4.668±0.025d	4.146±0.022d	3.366±0.034c	3.176±0.032c	4.145±0.042c	4.145±0.042c
Significance								
CV	***	***	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
CV × B	***	***	***	***	***	***	***	***

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Cont. **Supplementary Table 2.**

Treatment	P			
	Children	Adults		Elders
		Man	Woman	
Cultivar (CV)				
Aroma 2	1.353±0.034	2.900±0.074	2.900±0.074	2.900±0.074
Eleonora	1.103±0.040	2.363±0.085	2.363±0.085	2.363±0.085
Biofortification (B)				
B_0	1.203±0.158b	2.578±0.339b	2.578±0.339b	2.578±0.339b
$B_{12.5}$	1.201±0.128b	2.573±0.274b	2.573±0.274b	2.573±0.274b
B_{25}	1.224±0.142b	2.624±0.304b	2.624±0.304b	2.624±0.304b
$B_{37.5}$	1.234±0.144b	2.645±0.308b	2.645±0.308b	2.645±0.308b
B_{50}	1.278±0.122a	2.738±0.261a	2.738±0.261a	2.738±0.261a
CV × B				
Aroma 2 × B_0	1.346±0.021	2.886±0.045	2.886±0.045	2.88±0.045
Aroma 2 × $B_{12.5}$	1.317±0.022	2.822±0.048	2.822±0.048	2.822±0.048
Aroma 2 × B_{25}	1.354±0.004	2.901±0.009	2.901±0.009	2.901±0.009
Aroma 2 × $B_{37.5}$	1.365±0.008	2.926±0.016	2.926±0.016	2.926±0.016
Aroma 2 × B_{50}	1.383±0.060	2.964±0.129	2.964±0.129	2.964±0.129
Eleonora × B_0	1.059±0.008	2.269±0.017	2.269±0.017	2.269±0.017
Eleonora × $B_{12.5}$	1.085±0.006	2.325±0.012	2.325±0.012	2.325±0.012
Eleonora × B_{25}	1.095±0.011	2.346±0.024	2.346±0.024	2.346±0.024
Eleonora × $B_{37.5}$	1.103±0.007	2.364±0.015	2.364±0.015	2.364±0.015
Eleonora × B_{50}	1.172±0.003	2.513±0.006	2.513±0.006	2.513±0.006
Significance				
CV	***	***	***	***
B	***	***	***	***
CV × B	n.s.	n.s.	n.s.	n.s.

Data are mean values ± standard deviation, n = 3. Different letters within columns indicate significant mean differences according to Tukey HSD test (p=0.05). CV factor was compared according to t-Test. n.s. and *** denote non-significant or significant effects at $p \leq 0.001$.

The same trend was also observed for Na, which resulted in a NC less than 1%, pointing out that basil is inherently low in Na[57]. Just as observed for macroelements, regardless of cultivar, the intake of biofortified servings of basil would ensure higher EDI-Fe, -Mn, -Cu, and -Se for adults and children (**Table 6** and **Table 7**) compared to the control. Specifically, B_{50} biofortified basil would guarantee higher EDI-Mn, -Fe, and -Cu by 15.80, 3.72, and 19.16% in children and adults, compared to control. Our results show that, for children, consumption of one serving (5 g) of 'Aroma 2' × B_{50} basil would guarantee a nutrient contribution of 7.95% for Mn, an essential cofactor for a number of enzymes involved in neurotransmitter synthesis and metabolism[58]. On the contrary, the consumption of 15 g of 'Eleonora' × B_{50} basil would provide 5% higher NC-Cu for both adults (males and females) and the elderly, considering its essential role in the development and maintenance of the immune system and cardiovascular integrity, lung elasticity, and neuroendocrine functions[8]. Additionally, for Se, the $B_{37.5}$ and B_{50} treatments increased (on avg.) EDI by 19.83%, compared to control, in adults and children (**Table 6** and **Table 7**).

NC-Se related to the consumption of 'Eleonora' × B_{50} was about 4.53% in children and 7.42% in adults and the elderly, exceeding the values obtained by Puccinelli et al.[18] in lettuce biofortified with sodium selenate.

Regarding the primary source of nitrogen in plants, the safety level of nitrate in biofortified hydroponic basil with Zn in children, adults, and the elderly was determined by the hazard quotient (HQ) (**Tables 6** and **Table 7**). Although nitrate is excreted with urine, under specific conditions, it can be reduced to nitrite of recognized carcinogenic effects and responsible for blood diseases such as methemoglobinemia, to which children are much more vulnerable[59]. Regardless of biofortification, nitrate HQ values in children were considered more than safe, with the highest values (0.237) recorded in 'Eleonora'. Significantly, for 'Eleonora', the B_{50} biofortification treatment reduced nitrate HQ by 62.40%, compared to the control ('Eleonora' × B_0), making this cultivar even safer for feeding children. However, the lowest values were obtained from the 'Aroma 2' × $B_{37.5}$. The same trend was also observed for adults, for whom the nitrate HQ were lower due to a higher tolerable intake level of nitrate in the recommended diet (3.7 mg kg^{-1} body $weight^{-1}$).

5. Conclusions

Regardless of social background and economic conditions, about two thirds of the world's population is at risk of mineral deficiency with severe health impacts. Considering that the intake of these elements occurs through food, more and more interest has been shown in crop enrichment, encouraging research to expand studies on biofortification practices. Despite the culinary importance of Genovese basil worldwide, to date, this is the first scientific work to characterize the mineral profile of this multifaceted aromatic herb. The results obtained, in addition to showing a prevalent accumulation of macroelements such as K followed by Ca, P, Mg, and Na and microelements such as Fe followed by Mn, Zn, Cu, and Se, highlight the success of the biofortification program with Zn using an agronomic approach based on increasing Zn in nutrient solution. Zn management in a floating hydroponic system improved the Zn concentration in Aroma 2 and Eleonora basil cultivars but also increased the concentration of the other macro and microelements analyzed. The adaptability of Genovese basil to hydroponic systems (more suitable for biofortification programs) ensure the production of a fresh product biofortified with Zn that would improve, compared to non-biofortified basil, the nutritional status of Zn in consumers and increase the EDI of essential macro and microelements, both in children, adults (males and females) and the elderly.

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Chapter 12

Effect of daily light integral and photoperiod on yield, morpho-physiological characteristics and mineral composition of Genovese basil grown in vertical farming in two successive harvests

Abstract: In recent years, vertical farming is becoming increasingly popular for the production of leafy vegetables and aromatic herbs, such as basil (*Ocimum basilicum* L.). However, research has focused primarily on the effect of light quality levels, quantity, and photoperiod in order to define more energy-efficient basil growing protocols. Although scientists have underlined the positive response of this poliedric aromatic herb to successive harvests, this technique had never been investigated under sole source lighting with crop grown in a vertical farming cultivation. Two experiments were carried out in which the effects of two daily light integrals (DLI, 7.5 and 15 mol m^{-2} d^{-1}) and photoperiod (P, 14/10, 16/8, and 18/4 photoperiod/dark period) were evaluated in relation to two successive harvests on Genovese basil. In the first experiment, at the first cut, a DLI of 15 mol m^{-2} d^{-1} improved yield, yield parameters, and morphophysiological characteristics, but reduced light use efficiency (LUE), compared to a DLI of 7.5 mol m^{-2} d^{-1}. At the second cut, irrespective of the DLI used, plants grown at the first cut with a DLI of 15 mol m^{-2} d^{-1} did not show significant differences in plant fresh biomass. In contrast, for plants grown at the first cut with a DLI of 7.5 mol m^{-2} d^{-1}, the DLI of 15 mol m^{-2} d^{-1} used at the second cut improved yield and photosynthetic performance. Regardless of photoperiod, the cuts increased fresh biomass and DLI by 205.1% (on average), confirming how this pre-harvest factor can be used to achieve desired yield and quality from basil with potentials to enhance LUE and resource savings in the vertical cultivation.

Keywords: DLI; Light use efficiency; LMA; *Ocimum basilicum* L.; RWC; Successive cuts; Vertical agriculture

1. Introduction

The urgent need to feed the world's growing population by preserving natural resources (arable land, clean water, and fuel energy) has driven the development of increasingly sustainable agricultural techniques[1,2]. The prospect of constantly monitoring plant growth by controlling preharvest factors has ensured exponential growth in vertical agriculture[3,4]. Compared to conventional growing systems, vertical farming provides higher yields (about 95%), reduces water waste, the use of pesticides and fertilizers, but, most importantly, avoids the seasonality of production[4-6]. However, this growing system will not replace conventional agriculture (open fields and protected crops), but it will contribute to future of sustainable agriculture[7]. The main strengths that have prompted many developed and developing countries to convert part of their production to a true "Plant Factory" can be attributed to the potential to deal with the problems arising from sudden climate change and uncontrolled growth of the urban population[8,9]. On the other hand, the energy demand required for sole source lighting accounts for 50-55%. Light is one of the crucial factors that can affect plant growth. Although the introduction and subsequent upgrades of LED technology have resulted in significant reductions in energy costs, compared to traditional lighting technology alternatives[10], research efforts persist in investigating methodologies that can optimize the use of light in vertical farms. Specifically, this pertains to light quality, intensity, photoperiod, and delivery of photons to the crop without waste, which, if optimized, can reduce energy costs while improving the quanti-qualitative production performance of crops[5]. Rapid growth, high harvest index, profitability margin (with a high retail price), and versatility have made basil (*Ocimum basilicum* L.) the most studied and cultivated aromatic plant for indoor vertical farming[7]. Unlike lettuce (*Lactuca sativa* L.), due to its tropical and subtropical origin, this aromatic plant is well adapted to extended diurnal irradiation and moderately high photosynthetic photon flux density (PPFD)[8]. As suggested by Sipos, et al.[7], an increase in DLI up to 20 mol^{-1} m^{-2} d^{-1} significantly raised the growth index of basil plants. The right compromise ensuring high yield and nutritional quality with sustainable energy costs was achieved with a DLI of 12.9 mol^{-1} m^{-2} d^{-1} [8]. Although the main goal for a super-intensive commercial production system (such as vertical farming) is yield (as fresh and dry leaf biomass), several studies have focused mainly on increasing secondary metabolites rather than understanding how yield can be improved[11,12]. Specifically, a better understanding of the response of basil to changes in light intensity and photoperiod would, in addition to increasing yield, provide plants with morphometric features better suited to the vertical farming production system (short internodes, high leaf number, and increased carbon partitioning in leaves)[10]. Traditionally, basil cultivation requires at least two successive harvests[13,14] because, as observed in both open field and protected cultivation, this agronomic practice, in addition to lengthening the crop cycle, ensures higher unit yields

in terms of both fresh and dry biomass. Although recent scientific evidence[15-17] highlights the positive effects of cut-induced growth promotion in basil, this agronomic practice has never been studied in vertical farming. The potential to control all production factors and growth conditions (typical aspects of vertical farming) would allow evaluation of various positive effects of cuts on basil growth, development, morphology, and physiology. Therefore, our experiment evaluated the impact of two DLIs (7.5 and 15 mol m^{-2} d^{-1}) and three different photoperiods (14, 16, and 18 hours of light per day with a constant DLI of 15 mol m^{-2} d^{-1}) on the production of basil grown in an indoor vertical farming system with two successive cuts, assessing whether growth promotion could be attributed to changes in light intensity (first experiment) or the effect of photoperiod (second experiment).

2. Materials and Methods

2.1. Growth Conditions and Experimental Set-up

Two different vertical farming experiments were carried out on basil (*Ocimum basilicum* L.) at the Controlled Environment Agriculture Center of the University of Arizona (32° 13′ 18″ N, 110° 55′ 35″ W, 757 m above sea level; Tucson, UA, USA). The two experiments evaluated the productive and physiological response of Genovese basil (Johnny's selected seeds; Winslow, ME, USA) grown under different daily light integrals (DLI) and photoperiods (P) with two successive cuts (first cut-CT_I and second cut-CT_{II}). The first experiment was a completely randomized design with several DLI treatments and was harvested two times. Specifically, during the first experiment, basil was grown under two DLI treatments until the first cut (hereafter CT_I). The two DLI treatments were 7.5 ($DLI_{I\text{-}7.5}$) and 15 ($DLI_{I\text{-}15}$) mol m^{-2} d^{-1}. After the first cut, only half of the $DLI_{I\text{-}7.5}$ plants were exposed to 15 mol m^{-2} d^{-1} ($DLI_{II\text{-}7.5\text{-}15}$) and only half of the $DLI_{I\text{-}15}$ plants were exposed to 7.5 mol m^{-2} d^{-1} ($DLI_{II\text{-}15\text{-}7.5}$). The rest of the $DLI_{I\text{-}7.5}$ plants remained under 7.5 mol m^{-2} d^{-1} until the second cut ($DLI_{II\text{-}7.5\text{-}7.5}$). Similarly, the other half of the $DLI_{I\text{-}15}$ plants remained under 15 mol m^{-2} d^{-1} until the second cut ($DLI_{II\text{-}15\text{-}15}$). Consequently, there were four DLI treatments until the second cut (hereafter CT_2). The four DLI treatments were $DLI_{II\text{-}7.5\text{-}7.5}$, $DLI_{II\text{-}7.5\text{-}15}$, $DLI_{II\text{-}15\text{-}15}$ and $DLI_{II\text{-}15\text{-}7.5}$. The photoperiod was set at 16 hours of light per day. The second experiment was designed as a factorial, completely randomized design, with two main factors photoperiod (P) and cut (CT). Basil was grown under 14, 16, and 18 hours of light per day photoperiods (hereafter P_{14}, P_{16}, and P_{18}) and was harvested two times. The daily light integral (DLI) was constant at 15 mol m^{-2} d^{-1}. Basil seeds were seeded in plastic trays containing 240 Rockwool cubes (Grodan Rockwool B.V., The Netherlands) until the emergence of two true leaves (11 April 2022, 0 days after transplant-DAT), after which morphologically similar seedlings were selected and transplanted at a density of 46 pt m^{-2}. The cultivation cycle lasted 40 days, during which

the plants were cut twice at the first internode. The CTI_I was made at 20 DAT and CTI_{II} at 40 DAT.

The growing chamber (3.68 m wide, 5.94 m long, and 3.8 m high) had two multi-tier cultivation shelves, each holding three deep-water cultivation systems with plants grown on floating rafts (**Figure 1**). The growing area was 2.44 × 1.22 m, and each shelf had a 300 L storage tank containing a continuously circulating nutrient solution (NS). Each shelf had independent 1270 L nutrient tanks and shared a set of peristaltic pumps for acid and nutrients (800-101-3014-17, ANKO, FL, USA). A single CO_2 sensor (GMP222, Vaisala, Vantaa, Finland) and transmitter (GMT220, Vaisala, Vantaa, Finland) measured CO_2 concentrations throughout the growth chamber set at 650 ppm for the duration of the experiment. The light was provided by twelve LED lights (helioSPEC Izar, Heliospectra AB, Sweden) for each level, independently controlled by a networked light controller (Hash Controller, ILUMINAR, CA, USA). The spectrum of these lights was 14% blue, 10% green, 65% red, and 11% far red, as measured by a spectroradiometer (PS-300, Apogee Instruments, UT, USA) before the experiments. The temperature and RH of the air environment for both experiments were set at 25/20 °C photoperiod/dark period and 65%, respectively, and were monitored on one level of each rack with air temperature and RH probes (HMP60, Vaisala, Vantaa, Finland) enclosed in an aspirated and shielded sensor housing. The nutrient solution used consisted of 14 mM $N\text{-}NO_3^-$, 1.75 mM S, 1.5 mM P, 3.0 mM K, 4.5 mM Ca, 1.5 mM Mg, 1.0 mM NH_4^+, 15 μM Fe, 9 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20 μM B and 0.3 μM Mo with a pH of 5.8 and an electrical conductivity (EC) of 2.0 dS m^{-1}. Each rack was also equipped with sensors to monitor the root zone environment and the signals used to control the peristaltic pumps to inject a concentrated nutrient solution to maintain the desired EC and dilute acid solution to control the pH of the nutrient solution. The pH of the nutrient solution (HI 1001, Hanna, RI, USA), EC (HI-EC 3001, Hanna, RI, USA; CDE-100-1 PT100, Omega, UK), and dissolved oxygen (DO1200, Sensorex, CA, USA) were also measured and controlled.

Figure 1: Illustrative figure of the growth chamber used during the experiments.

2.2. Production and Plant Growth Measurements

Before each harvest (20 and 40 DAT), the height and number of nodes of 30 plants per treatment were measured from the top of the Rockwool cube. For each plant, the leaves were separated, counted, and photographed (Nikon D80 camera; Nikon Corporation, Tokyo, Japan) to determine the leaf area by digital image analysis (ImageJ v1.53; National Institutes of Health, Bethesda, MD, USA). The stems and leaves were weighed to determine fresh and dry biomass by drying them in a ventilated oven at 60 °C for 72 hours. The dry leaf-stem ratio was then calculated. The dry matter percentage of the leaves, stems, and the whole plant was calculated using the following formula:

$$DM = \frac{Dry\ biomass}{Fresh\ biomass} \times\ 100$$

2.3. Morphophysiological Analyses

The day before harvest (19 and 39 DAT), the CO_2 net assimilation rate (A_{CO2}), stomatal conductance (gs), and transpiration (E) were measured in 12 fully expanded and unshaded leaves per treatment using a portable gas exchange analyzer (LI-6400; LI-COR, Lincoln, UK) set according to the specific growing conditions. Water use efficiency (WUE) was calculated as A_{CO2}/E.

The same leaves were weighed to determine leaf fresh and dry biomass and then used to determine leaf mass per area (LMA; leaf dry biomass/leaf area). The relative

water content (RWC) expressed as a percentage was determined using the following formula:

$$RWC = \frac{Leaf\ fresh\ biomass - Leaf dry\ biomass}{Saturated\ leaf biomass - Leaf\ dry\ biomass}$$

Saturated leaf biomass was obtained by soaking the leaves in double distilled water for 24 hours.

The light use efficiency (LUE) was calculated as follows:

$$LUE = \frac{Plant\ fresh\ \ biomass \times Plant\ density}{DLI_{400-700\ nm^{-1}}} \times\ days\ of\ cultivation\ (d^{-1})$$

Where,

LUE is expressed as g $mol_{400\text{-}700\ nm}^{-1}$ of fresh plant biomass;

Plant fresh biomass is expressed as g;

Plant density is expressed as plants m^{-2};

DLI (Daily Light Integral) is expressed as mol m^{-2} d^{-1}.

2.4. Mineral Profile Determination

The mineral concentration in basil leaves was determined by ion chromatography (ICS-3000, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) coupled with an electrical conductivity detector according to the method described in detail by Formisano, et al. [18]. Briefly, 250 mg of previously ground dried plant material was extracted in 50 mL of ultrapure water (Arium® Advance EDI pure water system; Sartorius, Goettingen, Lower Saxony, Germany), incubated for 10 min at 80 °C in a shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) and then centrifuged (for 10 min at 6000 rpm; R-10 M, Remi Elektrotechnik Limited, Mumbai, India) and then filtered (using a syringe filter with a pore size of 0.45 µm (What. 45 µm (Whatman International Ltd., Maidstone, Kent, UK)). For the determination of NO_3^-, P, and S, an IonPac AG11-HC 4 × 50 mm guard column and an IonPac AS11-HC 4 × 250 mm analytical column were used, while for the determination of K, Ca and Mg, an IonPac CG12A 4 × 250 mm guard column and an IonPac CS12A 4 × 250 mm analytical column were used. All columns were purchased from Thermo Scientific™ Dionex™ (Sunnyvale, CA, USA). Except for NO_3^- expressed as mg kg^{-1} of fresh biomass, all minerals were expressed as mg g^{-1} of dry biomass.

2.5. Statistical Analysis

For the first experiment, data of each harvest period were subjected separately to analysis of variance (ANOVA) and means comparison according to Tukey-Kramer

multiple range test. For the second experiment, data were subjected to two-way analysis of variance (ANOVA) and means comparison according to Tukey-Kramer multiple range test. Data of both experiments represent mean ± standard error of 3 replicates (n = 3). Statistical analysis was performed using IBM SPSS Statistics software (SPSS Inc., Chicago, IL, USA) version 26.0 for Windows 10.

3. Results

3.1. First Experiment: Productive and Physiological Response of Genovese Basil Under Different Daily Light Integrals (DLI)

3.1.1. First cut

Regarding the first cut (CT_1), $DLI_{1\text{-}15}$ (15 mol m^{-2} d^{-1}) treatment significantly increased plant fresh biomass compared to $DLI_{1\text{-}7.5}$ (7.5 mol m^{-2} d^{-1}) one. The same trend was also recorded for the other biometric parameters shown in **Table 1** and **Table 2**, except for the light use efficiency (LUE; **Figure 2A**) which decreased, and the dry leaf-stem ratio and the number of nodes, which were not significantly affected by DLI treatment.

Chapter 12

Table 1: Effects of different daily light integral (DLI) on plant, leaf, and stem fresh biomass (g plant^{-1}), leaf area (cm^2), leaf and node number, and plant height (cm) at the first and second cuts.

Daily Light Integral (DLI)	Plant fresh biomass	Leaf fresh biomass	Stem fresh biomass	Leaf area	Leaf number	Node number	Plant height
	First Cut (CT_I)						
$DLI_{I\text{-}7.5}$	12.92±0.11	9.34±0.12	3.49±0.08	459.77±14.04	11.81±0.29	3.00±0.00	16.82±0.57
$DLI_{I\text{-}15}$	19.88±0.56	14.18±0.43	5.70±0.12	309.21±11.75	16.42±0.37	3.00±0.00	19.15±0.20
Significance	***	***	***	***	***	n.s.	**
	Second Cut (CT_{II})						
$DLI_{II\text{-}15\text{-}15}$	49.76±2.31ab	31.74±1.78ab	17.17±1.35ab	939.84±51.94	55.33±3.91ab	3.93±0.07	26.97±0.62b
$DLI_{II\text{-}15\text{-}7.5}$	42.51±1.80b	26.64±1.95b	17.21±1.03ab	976.68±64.81	47.40±3.10b	4.00±0.12	35.90±0.40a
$DLI_{II\text{-}7.5\text{-}15}$	58.78±1.93a	37.48±2.58a	22.97±1.50a	1102.80±183.65	67.28±3.03a	4.39±0.31	30.47±2.02b
$DLI_{II\text{-}7.5\text{-}7.5}$	38.64±3.95b	24.54±1.66b	15.34±1.75b	1006.81±98.98	49.94±4.21b	4.39±0.11	32.01±0.95ab
Significance	**	**	*	n.s	*	n.s	**

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

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Table 2: Effects of different daily light integral (DLI) on plant, leaf, and stem dry biomass (g plant^{-1}), plant, leaf and stem dry matter (%), and dry leaf-stem ratio at the first and second cuts.

Daily Light Integral (DLI)	Plant dry biomass	Leaf dry biomass	Stem dry biomass	Plant dry matter	Leaf dry matter	Stem dry matter	Dry leaf-stem ratio
	First Cut (CTI_I)						
$DLI_{I\text{-}7.5}$	1.00±0.01	0.81±0.01	0.18±0.01	7.75±0.11	8.66±0.14	5.25±0.09	4.43±0.12
$DLI_{I\text{-}15}$	1.74±0.06	1.43±0.03	0.32±0.01	8.79±0.31	9.97±0.17	5.72±0.10	4.42±0.06
Significance	***	***	***	**	***	**	n.s
	Second Cut (CTI_{II})						
$DLI_{II\text{-}15\text{-}15}$	3.87±0.34ab	2.61±0.22ab	1.26±0.12	7.96±0.13	8.28±0.18ab	7.38±0.16	2.07±0.05a
$DLI_{II\text{-}15\text{-}7.5}$	3.48±0.36ab	2.13±0.19b	1.35±0.18	7.91±0.34	7.98±0.16ab	7.82±0.60	1.59±0.08b
$DLI_{II\text{-}7.5\text{-}15}$	4.80±0.32a	3.16±0.11a	1.56±0.24	7.94±0.15	8.67±0.23a	6.80±0.13	2.07±0.03a
$DLI_{II\text{-}7.5\text{-}7.5}$	2.96±0.25b	1.91±0.15b	1.05±0.10	7.26±0.12	7.61±0.09b	6.68±0.15	1.83±0.05ab
Significance	*	**	n.s	n.s	*	n.s	***

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

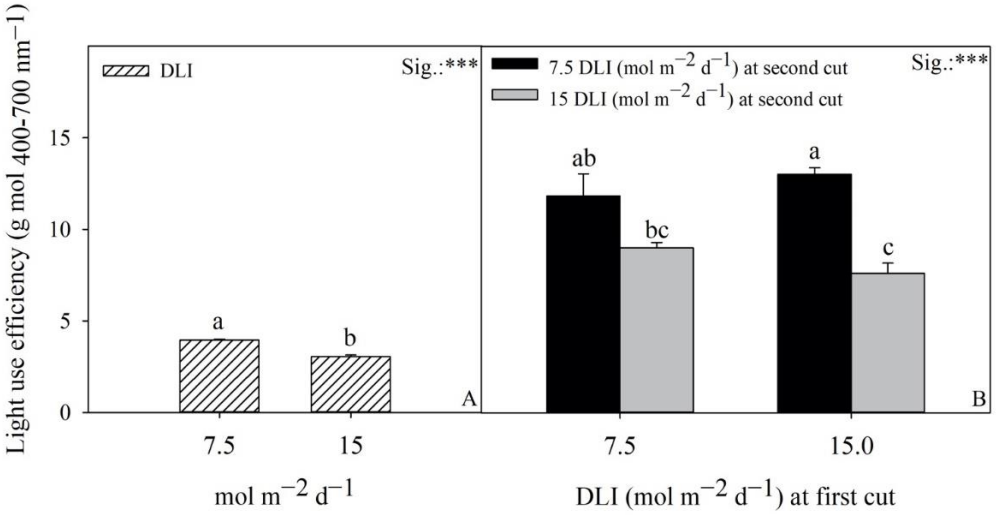

Figure 2: Effects of different daily light integral (DLI) on Light use efficiency at the first (A) and second (B) cuts. Different letters indicate significant mean differences. *** denote significant effects at $p \leq 0.001$.

Regarding photosynthetic performance, A_{CO2}, gs, and E were positively affected by DLI_{I-15} treatment (**Table 3**). A similar trend was observed for LMA, where plants treated with DLI_{I-15} had almost 1.5 times higher values than those treated with $DLI_{I-7.5}$. WUE and RWC were not affected by DLI treatment. Regarding the mineral profile shown in **Table 4**, the $DLI_{I-7.5}$ and DLI_{I-15} treatments did not result in significant differences, except for P and K. Specifically, basil plants treated with $DLI_{I-7.5}$ had higher average concentrations of P and K than plants treated with DLI_{I-15}.

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Table 3: Effects of different daily light integral (DLI) on leaf mass area (LMA: g m^{-2}), relative water content (RWC; %), net CO_2 assimilation rate (A_{CO2}; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}), transpiration (E; mol H_2O m^{-2} s^{-1}), and water use efficiency (WUE; µmol CO_2 mol^{-1} H_2O) at the first and second cuts.

Daily Light Integral (DLI)	LMA	RWC	A_{CO2}	gs	E	WUE
	First Cut (CTI_I)					
$DLI_{I\text{-}7.5}$	19.32±0.86	90.50±0.68	8.59±0.34	0.18±0.00	1.63±0.15	5.51±0.55
$DLI_{I\text{-}15}$	31.18±0.56	89.40±0.39	12.79±0.39	0.23±0.01	2.39±0.17	5.46±0.36
Significance	***	n.s	***	***	**	n.s
	Second Cut (CTI_{II})					
$DLI_{II\text{-}15\text{-}15}$	30.20±3.10a	85.72±0.86b	11.53±0.30ab	0.19±0.01a	2.18±0.09a	5.31±0.22c
$DLI_{II\text{-}15\text{-}7.5}$	17.95±0.17b	87.21±0.48ab	10.73±0.46ab	0.18±0.01a	1.99±0.02a	5.40±0.27c
$DLI_{II\text{-}7.5\text{-}15}$	28.57±0.74a	87.69±0.23ab	12.83±0.81a	0.15±0.00b	1.01±0.02b	12.72±0.98a
$DLI_{II\text{-}7.5\text{-}7.5}$	19.76±0.82b	88.70±0.71a	9.67±0.42b	0.12±0.01b	1.13±0.07b	8.64±0.47b
Significance	**	n.s	*	***	***	***

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

Table 4: Effects of different daily light integral (DLI) on minerals concentration at the first and second cuts. Minerals are expressed as g kg^{-1} of dry biomass, except for nitrate expressed as mg kg^{-1} of fresh biomass.

Daily Light Integral (DLI)	Nitrate	P	K	Mg	Ca
			First Cut (CTI_I)		
$DLI_{I\text{-}7.5}$	3371.11±94.21	9.28±0.33	52.94±1.38	3.12±0.06	13.18±0.64
$DLI_{I\text{-}15}$	3316.26±69.33	10.08±0.09	44.38±1.72	3.12±0.16	14.57±0.30
Significance	n.s	*	**	n.s	n.s
			Second Cut (CTI_{II})		
$DLI_{II\text{-}15\text{-}15}$	3141.40±135.83	9.87±0.61	49.48±0.74bc	4.35±0.10	23.83±0.87
$DLI_{II\text{-}15\text{-}7.5}$	3673.67±62.67	8.14±0.23	55.51±1.21ab	3.95±0.13	20.99±1.63
$DLI_{II\text{-}7.5\text{-}15}$	3379.46±338.81	9.44±0.39	45.06±1.83c	4.14±0.14	23.90±1.40
$DLI_{II\text{-}7.5\text{-}7.5}$	3502.14±167.38	9.45±0.55	57.52±1.72a	4.33±0.10	19.69±1.53
Significance	n.s	n.s	***	n.s	n.s

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

3.1.2. Second cut

For plants grown at CT_I with a DLI of 15 mol m^{-2} d^{-1} (DLI_{I-15}), the use at the second cut (CT_{II}) of a DLI of 7.5 or 15 mol m^{-2} d^{-1} ($DLI_{II-15-7.5}$ and $DLI_{II-15-15}$) did not result in significant differences for plant fresh biomass, leaf fresh biomass, stem fresh biomass, plant dry biomass, leaf dry biomass, stem dry matter, and leaf number (**Table 1** and **Table 2**). On the contrary, for the same plants (DLI_{I-15}), the highest values of the dry leaf-stem ratio were obtained with the $DLI_{II-15-15}$ treatment. The same treatment ($DLI_{II-15-15}$) resulted in the lowest plant height (26.97 cm) and LUE (7.629 g $mol_{400-700\ nm}^{-1}$; **Figure 2B**), compared to $DLI_{II-15-7.5}$ treatment.

Specifically, for plants grown at CT_I under $DLI_{I-7.5}$ treatment, the use of a DLI of 15 mol m^{-2} d^{-1} ($DLI_{II-7.5-15}$) at CT_{II} significantly increased plant fresh biomass, leaf fresh biomass, stem fresh biomass, plant dry biomass, leaf dry biomass, leaf dry matter, and leaf number, compared to $DLI_{II-7.5-7.5}$ treatment (**Table 1** and **Table 2**). On the contrary, for the same plants ($DLI_{I-7.5}$), regardless of the DLI used at CT_{II}, no significant differences were observed for the dry leaf-stem ratio, plant height, leaf area, and LUE (**Table 1**, **Table 2**, and **Figure 2B**).

For plants treated at CT_I with DLI_{I-15} and $DLI_{I-7.5}$, the use at CT_{II} of a DLI of 15 mol m^{-2} d^{-1} ($DLI_{II-7.5-15}$ and $DLI_{II-15-15}$) guaranteed the highest LMA (average 29.4 g m^{-2}) while no significant differences were observed for RWC (**Table 3**).

All photosynthetic parameters and WUE were affected by the modulation of DLI at the CT_{II}. Specifically, for plants grown at CT_I with a DLI of 7.5 mol m^{-2} d^{-1} ($DLI_{I-7.5}$), increasing DLI at CT_{II} ($DLI_{II-7.5-15}$) increased A_{CO2} and WUE by 32.7 and 47.2%, while no significant differences were observed for gs and E (**Table 3**).

Regarding the mineral profile, except for K (the most abundant element), no significant differences were observed (**Table 4**). Specifically, for plants grown at CT_I with 7.5 mol m^{-2} d^{-1} ($DLI_{I-7.5}$), the use at CT_{II} of a DLI of 7.5 mol m^{-2} d^{-1} ($DLI_{II-7.5-7.5}$) significantly increased K concentration, compared to $DLI_{II-7.5-15}$ treatment.

3.2. Second Experiment: Productive and Physiological Response of Genovese Basil Under Different Photoperiods (P)

The cut increased basil yields regardless of photoperiod (P_{14}, P_{16}, and $P_{18;}$ **Figure 3A,B**). Specifically, at CT_{II}, plants grown under P_{14}, P_{16}, and P_{18} treatments increased plant fresh biomass by 204.4, 153, and 258.1%, respectively, compared to CT_{I} (**Figure 4A**).

Figure 3. Illustrative picture of basil plants at first (A) and second (B) cut.

The same trend was recorded for leaf fresh biomass, plant dry biomass, leaf dry biomass, stem dry biomass, and LUE, (**Table 5**, **Table 6**, and **Figure 4B** and **D**).

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Table 5: Effects of different photoperiod (P) on leaf and stem fresh biomass (g plant^{-1}), leaf area (cm^2), leaf and node number at the first and second cuts.

Treatment	Leaf fresh biomass	Stem fresh biomass	Leaf area	Leaf number	Node number
Photoperiod (P)					
P_{14}	26.756±5.515ab	14.056±3.845ab	760.803±140.815	40.178±10.641	3.667±0.304
P_{16}	22.893±4.037b	11.409±2.646b	740.428±127.916	35.208±9.170	3.467±0.211
P_{18}	28.210±6.594a	16.799±5.127a	812.135±177.298	41.628±11.824	3.828±0.372
Cut (CT)					
CT_I	14.194±0.278	5.636±0.134	441.016±10.510	16.076±0.427	3.019±0.019
CT_{II}	37.713±2.046	22.540±1.834	1101.228±32.436	61.933±3.277	4.289±0.138
P×CT					
$P_{14}×CT_I$	14.556±0.497c	5.528±0.104c	447.900±22.656c	16.556±0.611	3.000±0.000
$P_{14}×CT_{II}$	38.956±1.721ab	22.584±1.093ab	1073.705±26.893b	63.800±2.778	4.333±0.133
$P_{16}×CT_I$	14.050±0.316c	5.652±0.129c	457.686±18.923c	15.083±0.403	3.000±0.000
$P_{16}×CT_{II}$	31.737±1.784b	17.167±1.351b	1023.171±38.872b	55.333±3.910	3.933±0.067
$P_{18}×CT_I$	13.976±0.694c	5.729±0.423c	417.462±3.754c	16.589±0.956	3.056±0.056
$P_{18}×CT_{II}$	42.445±3.778a	27.869±2.949a	1206.808±37.307a	66.667±8.434	4.600±0.306
Significance					
P	*	**	n.s	ns	ns
CT	***	***	***	***	***
P×CT	*	**	**	ns	ns

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

Table 6: Effects of different photoperiod (P) on leaf and stem dry biomass (g plant^{-1}), plant, leaf and stem dry matter (%), and dry leaf-stem ratio at the first and second cuts.

Treatment	Leaf dry biomass	Stem dry biomass	Plant dry matter	Leaf dry matter	Stem dry matter	Dry leaf-stem ratio
Photoperiod (P)						
P_{14}	2.539±0.438ab	1.002±0.316ab	8.849±0.265a	9.951±0.528a	6.514±0.454	3.649±0.724a
P_{16}	1.984±0.297b	0.788±0.220b	8.207±0.139b	8.974±0.325b	6.448±0.424	3.209±0.512b
P_{18}	2.654±0.611a	1.143±0.376a	8.505±0.052ab	9.619±0.151ab	6.403±0.273	3.043±0.451b
Cut (CT)						
CT_I	1.433±0.052	0.317±0.010	8.780±0.162	10.186±0.247	5.613±0.069	4.549±0.195
CT_{II}	3.351±0.247	1.639±0.128	8.261±0.111	8.843±0.192	7.297±0.101	2.052±0.033
P×CT						
$P_{14}×CT_I$	1.603±0.077c	0.304±0.007c	9.301±0.291	11.013±0.403a	5.509±0.086c	5.259±0.167a
$P_{14}×CT_{II}$	3.475±0.275ab	1.700±0.105ab	8.398±0.249	8.890±0.328cd	7.520±0.105a	2.039±0.047c
$P_{16}×CT_I$	1.360±0.026c	0.312±0.005c	8.452±0.133	9.665±0.138c	5.516±0.070c	4.351±0.054b
$P_{16}×CT_{II}$	2.608±0.224b	1.264±0.121b	7.962±0.135	8.284±0.175d	7.380±0.155ab	2.068±0.045c
$P_{18}×CT_I$	1.338±0.065c	0.334±0.029c	8.587±0.068	9.882±0.169ab	5.814±0.117c	4.035±0.151b
$P_{18}×CT_{II}$	3.971±0.360a	1.953±0.226a	8.422±0.045	9.357±0.130cd	6.992±0.109b	2.050±0.091c
Significance						
P	*	*	*	**	ns	***
CT	***	***	**	***	***	***
P×CT	*	*	ns	*	**	***

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

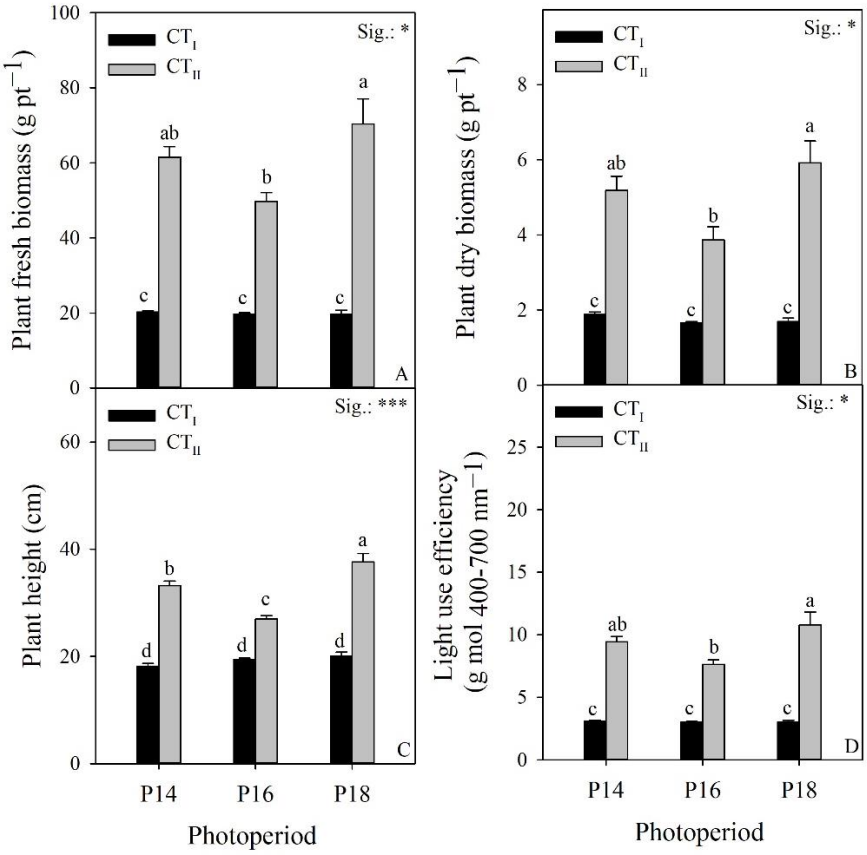

Figure 4: Effects of different photoperiod (P) × Cut (CT) interactions on fresh plant biomass (A), dry plant biomass (B), plant height (C), and light use efficiency (D). Different letters indicate significant mean differences. ns, *, **, and *** denote non-significant or significant effects at $p \leq 0.05$, 0.01, and 0.001, respectively.

Plants grown under the P_{14} treatment had significantly higher plant dry matter and leaf dry matter than those grown under the P_{16} one (**Table 6**). The latter parameter (leaf dry matter), regardless of photoperiod, decreased by 13.2% after the cut; in contrast, stem dry matter increased by 30%. Leaf and node numbers were only significantly affected by cuts (p <0.001), with an increase of 285.3 and 42.1% at CT_{II}, respectively (**Table 5**). Differently, plant height increased at CT_{II} (+69.76%) as photoperiod increased, with the highest values (28.8 cm) obtained from the P_{18} treatment (**Figure 4C**).

Photosynthetic parameters measured by portable photosynthesis system Licor LI-6400 (A_{CO2} and gs) and LMA were not affected by cuts, unlike RWC and WUE, which varied significantly by –2.7 and +15.1%, respectively (**Table 7**).

Except for Mg and Nitrate, the whole mineral profile shown in **Table 8** was not significantly affected by photoperiod. Specifically, the lowest nitrate (2735.4 mg kg^{-1} of fresh biomass) were obtained from plants grown at P_{18}. Regardless of photoperiod, plants harvested at the second cut had higher K, Mg, Ca, and S values, while nitrate and P were unaffected.

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Table 7: Effects of different photoperiod (P) on Leaf mass area (LMA: g m^{-2}), Relative water content (RWC; %), net CO_2 assimilation rate (A_{CO2}; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}), transpiration (E; mol H_2O m^{-2} s^{-1}), and intrinsic water use efficiency (WUE; µmol CO_2 mol^{-1} H_2O) at the first and second cuts.

Treatment	LMA	RWC	A_{CO2}	gs	E	WUE
Photoperiod (P)						
P_{14}	29.039±0.785	88.721±0.855	13.918±0.486a	0.230±0.021a	2.115±0.153ab	6.676±0.285a
P_{16}	30.313±0.429	87.642±0.959	12.595±0.244ab	0.198±0.009ab	2.240±0.073a	5.650±0.203b
P_{18}	30.575±1.026	89.535±0.895	11.959±0.382b	0.172±0.009b	1.789±0.150b	6.910±0.567a
Cut (CT)						
CT_I	30.177±0.727	89.865±0.648	12.789±0.401	0.213±0.016	2.170±0.086	5.963±0.298
CT_{II}	29.775±0.587	87.401±0.611	12.859±0.435	0.187±0.010	1.926±0.139	6.861±0.347
P×CT						
P_{14}×CT_I	28.138±1.084	88.684±1.646	13.983±0.417	0.258±0.029	2.092±0.234	6.804±0.529ab
P_{14}×CT_{II}	29.941±1.046	88.759±0.973	13.852±1.002	0.201±0.023	2.139±0.247	6.549±0.332ab
P_{16}×CT_I	30.431±0.517	89.565±0.398	12.287±0.296	0.201±0.019	2.304±0.121	5.349±0.160b
P_{16}×CT_{II}	30.196±0.799	85.720±0.864	12.904±0.338	0.194±0.007	2.176±0.089	5.952±0.299b
P_{18}×CT_I	31.962±1.096	91.345±0.574	12.096±0.769	0.179±0.006	2.115±0.070	5.735±0.442b
P_{18}×CT_{II}	29.189±1.461	87.725±0.634	11.821±0.344	0.164±0.017	1.463±0.040	8.084±0.188a
Significance						
P	ns	ns	*	*	*	**
CT	ns	**	ns	ns	ns	**
P×CT	ns	ns	ns	ns	ns	**

ns, *, and ** non-significant or significant at $p \leq 0.05$ and 0.01, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

Table 8: Effects of different photoperiod (P) on minerals concentration at the first and second cuts. Minerals are expressed as g kg^{-1} of dry biomass, except for nitrate expressed as mg kg^{-1} of fresh biomass.

Treatment	Nitrate	P	K	Mg	Ca
Photoperiod (P)					
P_{14}	3111.212±87.554a	8.686±0.238	43.050±2.344	3.277±0.139b	17.784±1.52
P_{16}	3181.351±67.155a	9.576±0.336	46.935±1.519	3.739±0.288a	19.200±2.11
P_{18}	2735.387±128.934b	9.136±0.152	46.376±1.280	3.582±0.31ab	17.338±1.622
Cut (CT)					
CT_I	3119.761±81.074	9.188±0.131	43.022±1.299	3.034±0.085	14.528±0.563
CT_{II}	2898.873±109.371	9.078±0.309	47.885±1.226	4.031±0.150	21.686±0.742
P×CT					
$P_{14}×CT_I$	3281.112±74.201	8.978±0.116	39.496±1.659	3.050±0.194b	15.176±1.825
$P_{14}×CT_{II}$	2941.311±62.898	8.395±0.430	46.604±3.476	3.503±0.085b	20.392±1.193
$P_{16}×CT_I$	3221.129±101.559	9.285±0.333	44.385±1.952	3.124±0.164b	14.566±0.149
$P_{16}×CT_{II}$	3141.573±103.209	9.868±0.607	49.485±1.108	4.355±0.100a	23.834±0.871
$P_{18}×CT_I$	2857.041±101.930	9.300±0.223	45.186±2.050	2.928±0.109b	13.842±0.086
$P_{18}×CT_{II}$	2613.734±240.689	8.973±0.196	47.565±1.605	4.236±0.201a	20.833±0.963
Significance					
P	**	n.s	n.s	*	n.s
CT	n.s	n.s	*	***	***
P×CT	n.s	n.s	n.s	*	n.s

ns, *, **, and *** non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$.

4. Discussion

4.1. First Experiment

In indoor growing systems, light direction, duration, quality, and quantity levels play a vital role in plant growth[19-22]. As described in detail in the literature, the different levels of light quantity, *i.e.* the variation of the daily light integral (DLI), have a more significant influence on the morphophysiological traits of higher plants than the instantaneous values of the photosynthetic photon flux density (PPFD) at a given time of day[23-25]. Similarly to the findings of Dou, et al.[8] and Larsen, et al.[10], at CT_I, our results showed a significant increase in plant yield (> plant fresh and dry biomass) under DLI_{I-15} treatment (15 mol m^{-2} d^{-1}; **Table 1** and **2**), underlining the feasibility of using high DLI as a tool to increase plant yield.

However, although Walters and Currey[26] reported three times higher basil yields with a DLI of 15 mol m^{-2} d^{-1}, compared to a DLI of 7.5 mol m^{-2} d^{-1}, our results did not show the same trend, as the yield only increased by 50%. These results are corroborated by statistical data on light use efficiency (LUE), which decreased by 23.04% as the DLI increased (**Figure 1**). The divergence between the reported results highlights the problem of determining optimal DLIs for indoor basil cultivation, first due to genotypic influence and second due to the different environmental conditions adopted. Although almost all scientific contributions concerning the effects of varying the amount of light in plants have described a positive impact of DLI on yield, on some crops, and under particular environmental conditions (temperature, carbon dioxide concentration, and relative humidity), a high DLI can reduce the yield[8] and affect management and production costs[27]. The higher basil yield under DLI_{I-15} treatment was due to increased leaf area, leaf number, and plant height, similar to what Pennisi, et al.[28] reported on basil and lettuce.

Interestingly, productive differences in leaf dry biomass and leaf area in response to different DLIs led to changes in leaf mesophyll structure. As described by Dou, et al.[8], the increase in LMA as the DLI increases suggests a higher leaf thickness, often associated with doubling the cross-sectional area of the mesophyll per unit area. However, as reported by Poorter, et al.[24], the increase in LMA could also be due to an increase in leaf density, often positively correlated with an increase in the concentration of chlorophyll or enzymes directly involved in photosynthesis[29]. In support of the above, we observed a 48.9 and 27.8% increase in net CO_2 assimilation (A_{CO2}) and stomatal conductance (gs) in plants under DLI_{I-15}. The increase in *gs* suggests that basil leaves with the highest DLI had a more open stomata and consequently increased leaf transpiration (**Table 3**). However, the WUE did not show significant differences between treatments ($DLI_{I-7.5}$ and DLI_{I-15}). This result may have been due to the basil leaves of the $DLI_{I-7.5}$ treatment, having fewer leaves, less leaf area, and less thickness (less LMA) than those

of the $DLI_{I\text{-}15}$ treatment, reduced respiratory cost (less E), thus compensating for lower A_{CO2} [30]. In particular, the highest P and K concentrations were found in the $DLI_{I\text{-}7.5}$ treated plants, showing that the uptake of these elements kept pace with the increased growth of the $DLI_{I\text{-}15}$ treated plants[24].

As reported by Larsen, et al.[10], improved photosynthetic performance is positively correlated with increased fresh biomass, as well as increased concentration of sugars and soluble carbohydrates that constitute dry matter. According to Pennisi, et al.[28], increasing DLI at CT_I raised plant, leaf, and stem dry matter **(Table 2**). This result indicates a longer shelf life of basil after harvest, as dry matter correlates with carbohydrate concentration[31].

The above was also partially observed at CT_{II}. Specifically, for plants treated at CT_I with a DLI of 7.5 mol m^{-2} d^{-1} ($DLI_{I\text{-}7.5}$), the use of a DLI of 15 mol m^{-2} d^{-1} at CT_{II} ($DLI_{II\text{-}7.5\text{-}15}$) significantly increased the yield (plant, leaf, and stem fresh biomass) and photosynthetic parameters without affecting LUE, compared to $DLI_{II\text{-}7.5\text{-}7.5}$ treatment. On the contrary, for plants treated at CT_I with a DLI of 15 mol m^{-2} d^{-1} ($DLI_{I\text{-}15}$), the use of a DLI of 7.5 mol m^{-2} d^{-1} at CT_{II} ($DLI_{II\text{-}15\text{-}7.5}$) did not result in significant differences in fresh and dry biomass, compared to the $DLI_{II\text{-}15\text{-}15}$ treatment. Not surprisingly, LUE was higher in plants grown at CT_{II} with the lowest DLI ($DLI_{II\text{-}15\text{-}7.5}$). However, our results cannot be confirmed or refuted due to the lack of similar studies in the literature. All basil studies[7,8,10,28,32] that investigated the response of plants to different DLI and PPFD have not considered the effects of successive cuts. The results observed at CT_{II} suggest that the plants grown at CT_I with a DLI of 15 mol m^{-2} d^{-1} had a well-structured root system that allowed a better regrowth of the non-DLI-dependent root system.

4.2. Second Experiment

Quantitative light management for indoor cultivation often focusses on the total amount of photosynthetically active light received by a plant in a day (DLI), but what is often overlooked is how this light is supplied over time, *i.e.* the photoperiod[33]. The photoperiod is a crucial factor in plants' growth, quality, and circadian rhythm, and optimizing it is vital for indoor cultivation[34,35]. For example, Avgoustaki[34] and Elkins and van Iersel[36] showed that although most indoor basil growers use a 16/8 photoperiod/dark period, the best photosynthetic performance is achieved with a 14/10 photoperiod. This result could be due to increased photosynthetic pigment production, and foliar photosynthesis increases asymptotically with increasing PPFD (within certain limits). However, it is interesting to note that this significant difference did not lead to differences in the same direction regarding production performance. For example, Mao, et al.[37] showed that, despite a reduced activity of RuBisCo (Ribulose-1,5-bisphosphate carboxylase) activity in the leaves of plants grown with a higher photoperiod (18/6), this lighting strategy based on weaker but more prolonged light conditions allowed plants

to improve their light use efficiency, avoiding the negative impact on yield, which was even higher than that obtained by plants grown with the same DLI but with a photoperiod of 16/8.

Interestingly, although numerous studies have evaluated the effect of the photoperiod on the fresh and dry yield of leafy vegetables such as basil, kale (*Brassica oleracea* L. var. acephala D.C.), and others[34,37-40], in our study, this effect was mainly observed at CT_{II}. At CT_{I}, the use of different photoperiods with the same DLI resulted in significant differences in the percentage of leaf dry matter, with the highest values obtained with the treatment characterized by the lowest photoperiod (P_{14} × CT_{I}), although there were no significant differences with the P_{18} × CT_{I} one. Under these conditions, the plants may have allocated drier biomass in the leaves, justifying the higher dry leaf-stem ratio, or they may have enacted an adaptive response to the high PPFD. However, at CT_{II}, an opposite trend was observed. The comparison between the optimal photoperiod (P_{16}) and the lowest photoperiod (P_{14}) showed no significant differences for the main parameters of fresh and dry biomass[34]. Although it is often reported in the literature that reducing the photoperiod with constant DLI significantly reduces biomass, this result must consider the different growth conditions adopted, such as temperature, relative humidity, and carbon dioxide concentration, and the DLI and photoperiod used[35]. Frąszczak, et al.[38] recorded a reduction in basil yield as the photoperiod decreased. However, their experiment compared a 16-hour photoperiod with a 12-hour one. It is important to note that the choice of photoperiod must be carefully chosen and balanced with the crop's needs to achieve positive results. For example, Johnson, et al.[41] found that a 24-hour photoperiod improves the biosynthesis of aromatic molecules in basil but significantly reduces yield and is unsustainable from an energy and economic point of view.

In our study, increasing the photoperiod to 18 hours of photosynthetically active radiation (P_{18}), compared to the commonly used photoperiod (16 hours of light, P_{16}), significantly increased the fresh and dry biomass of plant and leaf, confirming what has been observed in recent studies on lettuce, mizuna (*Brassica rapa* var. niposinica), and Rudbeckia (*Rudbeckia fulgida* var. *sullivantii* 'Goldsturm')[33,37,42,43]. Considering that the better production performance obtained at CT_{II} with P_{18}, compared to P_{16}, did not increase photosynthetic activity, we do not exclude that the use of the P_{18} treatment stimulated root system growth[37], consequently improving the yield at CT_{II}. Although the increase in height and leaf area of plants in the P_{18} treatment, compared to P_{16}, may be attributable to a shade avoidance response, the non-change in LMA and the increase in stem and leaf dry biomass does not support this hypothesis and suggest that they are the result of increased growth. According to numerous studies on Genovese basil and other leafy vegetables[15-17,44,45], regardless of photoperiod, the agronomic practice of successive harvests significantly increased fresh and dry biomass (by 205.14 and

186.23%, respectively). Considering that the days between sowing and the CT_I and those between the CT_I and CT_{II} amounted to 20 days, the economic convenience of adopting this agronomic practice in a vertical cropping system is evident. The only limitation of this technique lies in not cut the primordia of the lateral shoots. For instance, as suggested by Zheljazkov, et al.[46], the increase in production could be due to a well-formed root system that had to reconstitute itself during the second harvest, promoting more rapid regrowth of the epigeal apparatus, probably favored by a preferential allocation of photosynthesis to the green organs to be reconstructed.

Although significant production increases were observed after cut, these were not accompanied by an improvement in photosynthetic performance (**Table 7**), even though there was a significant increase in WUE. This result is not surprising, as a similar study of ours on Genoese basil grown in floating raft systems reported a reduction in photosynthetic parameters after cutting despite the increase in production[15]. However, the increase in cytokinin production in response to cut would justify the more significant number of leaves and greater leaf area and weight at the second cut without affecting LMA (data not shown)[47]. The suppression of apical dominance probably stimulated the emission of lateral buds because of a drastic reduction in auxin fluxes, increasing the fresh and dry weight of the stem. These results would justify the better yields obtained regardless of the photoperiod. Although cut is established to promote the emission of new leaves (> number and weight of leaves), in the present work, the increases in stem weight were more significant than those of leaves; this result led to a decrease in the dry leaf-stem ratio, in line with what has been observed by other authors[13,15,17,48]. The increase in dry matter, node number, and plant height probably pushed the plant to produce more stem components, as this had a supporting function. In addition to production and morphometric changes, similar to what was observed on lettuce and basil[17,49,50], at CT_{II}, the basil plants had a higher concentration of K, Ca, and Mg, minerals that play an essential role in promoting human health (such as stabilizing blood pressure, detoxification, and improving bone structure) and a concentration of nitrate (an antinutritional compound par excellence for leafy vegetables) not significantly different from the CT_I.

5. Conclusion

Growing crops in indoor vertical farming systems require artificial lighting as light is a limiting factor for production. Since the early days, indoor vertical farming-based cultivation of vegetables has been widely researched to achieve high yields. However, studies considering the effects of successive cuts of basil grown in indoor vertical farming systems have been lacking, contrary to those evaluated in traditional open field and protected cultivation. To our knowledge, this research was the first approach to study this “innovative” production technique in indoor vertical farming settings. The

effect of the cut was evaluated with different DLIs and photoperiods. As hypothesized, at CT_I, fresh and dry plant biomass and dry matter content, morphology (plant height and leaf area) and physiology were significantly influenced by the higher DLI. This result was only partially confirmed at CT_{II}. Only for the plants grown at CT_I with a DLI of 7.5 mol m^{-2} d^{-1} was the same trend observed; in contrast, for the plants grown at CT_I with a DLI of 15 mol m^{-2} d^{-1}, the use of a DLI of 7.5 or 15 mol m^{-2} d^{-1} at CT_{II} did not lead to significant differences in yield. The possibility of obtaining the same yield at the second harvest with a halved DLI significantly increased LUE, making cultivation more economically viable. In the second experiment, which evaluated the combined effects of photoperiod and cut, in addition to recording at CT_{II} a significant effect of the photoperiod on the production characteristics of basil, we observed that irrespective of the photoperiod, cutting promoted the growth and production of basil by improving its LUE. These promising results open the door for future research, which in addition to analysing the effects induced by the cut in more detail, should also focus on quality traits.

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Chapter 13

Conclusions

Nutritional quality and production performance of basil are highly influenced by environmental factors, but the genetic variability of the genus *Ocimum* is certainly the main determinant of these aspects. The Genovese type dominates among basil genotypes for the preparation of pesto sauce. Flavor and color are crucial and influential quality indicators for consumer choice. The results presented in **Chapters 4** and **5** showed that for field cultivation, the Aroma 2 cultivar was the best performant with respect to cuttings, high yield, sensory profile, and lowest nitrate concentration. Although all cultivars were also well adapted to growth in a floating raft system, again, 'Aroma 2' stood out in terms of production performance, nitrate concentration, and the highest dry matter percentage. The latter and the aroma profile were not affected by density, while the yield increased with higher density. These results confirmed the positive effects from successive harvests, especially on yield and biosynthesis of key secondary metabolites (**Chapter 6**). The results presented in **Chapter 7** highlighted the productive potential of floating raft systems for basil growth even in the fall season, where the fresh yield (≈3 kg m^{-2}) was higher than yield normally achievable in open field. The Eleonora cultivar, provided high fresh biomass with the lowest electroconductivity (EC), indicating that it exists an interesting genetic variability among basil cv for resource use efficiency. Resource use efficiency in basil can also be improved by using bioproducts with multiple action. In **Chapter 8**, we demonstrated how the application of a plant protein hydrolysate in the nutrient solution (Trainer®) increased all biometric parameters (such as leaf number, fresh and dry yield) and the level of biofunctional molecules such as linalool, and total phenols. The integration of Trainer® into the nutrient solution was correlated by a general improvement of physiological parameters such as net CO_2 assimilation, stomatal conductance, transpiration, and chlorophyll fluorescence (**Chapter 9**). The enrichment of fresh produce with essential minerals can be applied also to minor crops with potential health benefits. In **Chapters 10** and **11**, we evaluated the potential of biofortified Genovese basil production with Zn by supplementing Zn in the nutrient solution (12.5, 25, 37.5, and 50 μM). The Zn management in the FRS system increased the concentration of Zn in the basil cultivars Aroma 2 and Eleonora, while negatively impacting the yield and overall physiology. However, increasing Zn in the nutrient solution significantly increased antioxidant activity, carotenoid and polyphenol concentrations. Consequently, the consumption of Genovese basil biofortified with Zn can increase the intake of Zn by consumers

(children, adults, and the elderly) while providing a product enriched with valuable phytochemicals beneficial to human health a characteristic that customers value. In conclusion, our results not only confirmed, once again, the good adaptability of Genovese basil to the floating raft system, but also highlighted several aspects that could prompt the partner company to become more interested in this cropping system. Higher yields, the ability to deseasonalize production, to integrate the use of biostimulants to reduce dependence on chemical inputs, and to apply biofortification programs represent important upgrades over what is ordinarily achievable in soil-based cultivation. Last but not least, the reduced effect of abiotic and biotic pressures has allowed for a better understanding and evaluation of the components related to the functional, sensory and nutritional quality of this multifaceted leafy vegetable, aspects that could be of greater interest to the company in the near future, as they would add value to the final product. In **Chapter 12**, we evaluated the effects of two daily light integrals (DLI, 7.5 and 15 mol m^{-2} d^{-1}) and photoperiod (P, 14/10, 16/8, and 18/4 photoperiod/dark period) in relation to two successive harvests on Genovese basil. In the first experiment, at the first cut, a DLI of 15 mol m^{-2} d^{-1} improved yield, yield parameters, and morphophysiological characteristics, but reduced light use efficiency (LUE), compared to a DLI of 7.5 mol m^{-2} d^{-1}. At the second cut, irrespective of the DLI used, plants grown at the first cut with a DLI of 15 mol m^{-2} d^{-1} did not show significant differences in plant fresh biomass. In contrast, for plants grown at the first cut with a DLI of 7.5 mol m^{-2} d^{-1}, the DLI of 15 mol m^{-2} d^{-1} used at the second cut improved yield and photosynthetic performance. Regardless of photoperiod, the cuts increased fresh biomass and DLI by 205.1% (on average), confirming how this pre-harvest factor can be used to achieve desired yield and quality from basil with potentials to enhance LUE and resource savings in the vertical cultivation.

Other Publications

1. **Ciriello, M.**, Cirillo, V., Formisano, L., El-Nakhel, C., Pannico, A., De Pascale, S., & Rouphael, Y. Productive, Morpho-Physiological, and Postharvest Performance of Six Basil Types Grown in a Floating Raft System: A Comparative Study. *Plants*, *12*(3), **2023**, 486.

2. **Ciriello, M.**, Formisano, L., Rouphael, Y., & Corrado, G. Volatiles Emitted by Three Genovese Basil Cultivars in Different Growing Systems and Successive Harvests. *Data*, *8*(2), **2023**, 33.

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