# Modulation of light spectrum for controlling plant growth and development of flower species for greenhouse cultivation and leafy vegetables for indoor cultivation


PhD coordinator: Prof. Guido D´Urso
Supervisor: Prof. Roberta Paradiso
Co-supervisor: Prof. Carmen Arena

Ph.D candidate:
Giuseppe Carlo Modarelli



Ph.D program in Agricultural Science and Food Technology
XXXII Cycle
A.Y 2019/2020

DIPARTIMENTO DI AGRARIA

*Illa seges demum votis respondet avari agricolae, bis quae solem, bis frigola sensit; illius emmensae ruperunt horrea messes.*

Publius Vergilius Maro, Georgics, Book I

# Preface

The 21st century is a challenging era for the humanity and the planet's history. The scientific progress in understanding plant behaviour and plant interaction with the surrounding environment is making giant steps; the modern technology together with the multidisciplinary research allows to investigate complex phenomena such as plant adaptation and acclimation to different environmental variables. One of the most important environmental factors for plants is the light.

The aim of this PhD thesis was to investigate physiological and morphological effects of light modulation on ornamental and vegetables species, to contribute to the development of new lighting protocols for the cultivation of vegetable and ornamental crops.As ornamental species for these studies, we selected an emerging species namely *Ranunculus asiaticus* L., a quantitative long day geophyte originally from the Mediterranean basin and Asia minor, cultivated for cutted stems and potted plants production. In this species, we aimed to investigate plant physiological and morphological behaviour in response to light radiation, considering both light intensity and quality, to define lighting protocol able to anticipate flowering response.

The lack of information on physiology of *Ranunculus asiaticus* L. was the reason to carry out the first experiment (Chapter 1). In this experiment, the photosynthetic process was characterized in plants grown in pots in a chamber under fluorescent white light (Photosynthetic Photon Flux Density, PPFD 200 µmol $m^{-2}$ $s^{-1}$) and controlled conditions: photoperiod 16 hours, temperature 18°C, relative humidity 60-70%). Two *Ranunculus asiaticus* L. hybrids (MBO and MDR), characterized by different earliness in flowering, and two tuberous roots preparation procedures, rehydration (C) and rehydration followed by vernalization (V), were compared. The results obtained in the first experiment indicated an hybrid-specific response to the tuberous roots preparation procedure and highlighted that flowering response in ranunculus is a complex process, in which the temperature and the photoperiod play a crucial role. On the basis of these results, we then focused our attention

on the influence of vernalization and photoperiodic light quality on plant physiology and flowering earliness. In a second experiment (Chapter 2) we further investigated on the same hybrids (MBO and MDR), the effect of vernalization on the photosynthetic process and flowering earliness in potted plants, grown in a cold greenhouse obtained by three different tuberous roots preparation procedures: rehydration (C), rehydration followed by two and four weeks of vernalization at 3.5°C (V1 and V2, respectively).

In Chapter 3, the attention has been concentrated on the effect of photoperiodic lighting spectral composition to promote flowering in our model of ornamental species *R. asiaticus* L.. It is well known that, in some long day species, photoperiodic lighting with Red and Far Red, creating at plant level an intermediate phytochrome photo-equilibrium (PPE), anticipates flowering compared to conditions of high or low PPE. In particular, we evaluated the influence of four different lighting treatments on photosynthesis, metabolic profiling and flowering earliness to identify the best spectral composition to anticipate flowering. The lighting treatments utilized in this study were: natural day length (NL, short day), NL plus white fluorescent photoperiodic lighting (estimated PPE 0.84, a typical high value), NL plus Light Emitting Diodes (LEDs) photoperiodic lighting with R: FR ratio of 3:1 (PPE 0.84) and NL plus LEDs photoperiodic lighting with R:FR ratio of 1:3 (estimated PPE 0.63, an intermediate value).

In Chapter 4 the attention was moved on leafy vegetables (*Lactuca sativa* L. cv. 'Blackhawk' and *Chicorium endivia* L. cv. 'Lindo') for their production in plant factories under artificial light, and especially on the influence of light quality on plant growth, photosynthesis and polyphenols synthesis. In a first experiment, we compared the effect of lighting treatments with different percentages of blue (B) and red (R) wavelengths, compared to white LEDs light as control, on plant growth, photosynthesis and polyphenols content. From this experiment, the most performing B:R ratio was used as control in a second experiment on the same species over different Far-Red (FR) intensities added to the B:R spectrum to determine the FR effects on polyphenol synthesis, plant growth and photosynthesis.

The possibility to optimize the plant growth by modulating the light spectrum is an attractive perspective to obtain specific morphological and physiological traits for different plant types. Moreover, the modulation of light quality could represent a valid alternative to the

more expensive cultivation practices. In the framework of global climate change and overconsumption of resources the possibility to obtain healthier products improving biomass production by light modulation in indoor cultivation, especially for crops, will allow to preserve also the soil and other precious natural resources.

# Index

# General Introduction

The term “light” derives from the latin *lux* and it is defined as the electromagnetic radiation within the portion of the electromagnetic spectrum, comprised between 400-700 nm, that can be perceived by human eyes, between the infrared and ultraviolet radiation (Ouzounis et al., 2015). Light has a dual nature known as the wave-particle duality. As radiation, it propagates like a wave with a determined frequency. Particles of light are called photons. Photons of different wavelengths have different energy according to the Planck relation (Pattison et al., 2018). In fact, blue photons have higher energy than red photons for instance, this means that red photons cost less energetically than blue photons. On the other hand, Far Red radiation (FR) is known to be less energetically active than ultraviolet (UV) radiation.

From the beginning, light has always been associated with the life on our planet. Light is the main driver for photosynthetic organisms. The photoautotrophs, especially plants, developed and shaped their entire evolution to optimize light harvesting, to produce food, colonizing the whole planet from equatorial to polar latitudes, adapting to different environments, and surviving to extreme conditions.

Depending on the latitude and climate, plants have developed different types of photosynthesis, namely C3, C4 and CAM, to optimize their growth and biomass accumulation in a wide range of environments (Taiz and Zeiger, 2002). The light harvesting in photosynthesis is due to photosynthetic pigments, including chlorophyll a, chlorophyll b and carotenoids. Chlorophyll *a* absorbs at 430 and 665 nm of visible spectrum, while chlorophyll *b* absorbs mainly at 453 and 642 nm. Also carotenoids and anthocyanin are able to harvest light working in conjunction with chlorophylls to transfer light to the photosystems and convert it into primary metabolites (Sager and McFarlane, 1997; Ouzounis et al., 2015). Light composition or light quality affects the rate of photosynthesis. In fact, chlorophylls absorb mainly in the red and blue regions of the spectrum while carotenoids mainly in the blue, indicating that photosynthesis can be mainly enhanced by these wavelengths (McCree, 1971). Red and blue radiation does not penetrate deep through the leaf while green or far red radiation penetrate deeper in the leaf tissue, contributing to the carbon fixation in the inner layers of the canopy or more in depth in the plant cell (Pattison et al., 2018; Eilers et al., 2019).

**Light and photoreceptors role**

Changes in light intensity, light quality and light duration are perceived by plants even at low irradiances, thanks to specific photoreceptors able 1) to distinguish the different wavelengths of the light and 2) to regulate various functions such as plant growth, shape and life. Photoreceptors can be divided into five families: Phytochromes, Phototropins, Cryptochromes, Zeitlupe/Flavin-Binding, Kelch repeat, F-Box 1/Light Oxigen Voltage Kelch protein2 (ZTL/FKF1/LKP2) and ultraviolet receptor 8 (UVR8) (Fukuda, 2019; Paik and Huq, 2019).

In the next sections of this paragraph a general overview of the main functions regulated by the different photoreceptors in response to light will be presented.

***Phytochromes***

Phytochromes are the first photoreceptor family discovered in late 1959 by the biophysicist Warren Butler and the biochemist Harold Siegelman, in *Arabidopsis.* Five types of phytochromes have been characterised: namely phytochrome A, B, C, D and E (Kami et al., 2010). Phytochromes consist of a protein covalently linked to a light-sensing chromophore. They absorb mainly red (R, 600-700 nm) and far red (FR, 700-800) light, and are present in all plant tissues, in two forms: Phytochrome red (Pr), with an absorption peak at 660 nm, and Phytochrome far red (Pfr), with a absorption peak at 730 nm (Sager et al., 1988). R light converts the Pr to the Pfr form while FR light has an opposite effect, and the ratio between these two forms (Pfr/Ptot) determines the phytochrome photoequilibria (PPE) (Sager et al., 1988). Phytochromes regulate many functions including seed germination, seedlings de-etiolation, stem elongation and leaf expansion (known as shade avoidance responses), tuberization, bud dormancy, day-length perception and hence circadian rhythm, transition from vegetative to flowering stage (Demotes-Mainard et al., 2016, and references therein). The active form is the Pfr form that interacts directly with transcription factors (Chen et al., 2010; Jang et al., 2010; Demotes-Mainard et al., 2016). Generally, phytochromes can perceive variation of the light even at low intensity, for example red and far red wavelenghts are perceived even at very low light intensity.

***Cryptochromes***

Cryptochromes perceive mainly blue and UV-A light and are divided into two sub families CRY 1 and CRY 2. They have two chromophores, pterin (with a peak of absorption at 380 nm) and flavin (with a peak of absorption at 450 nm), present in plants as well as in mammals

and insects (Meng et al., 2013; Fukuda, 2019). They negatively regulate costitutive photomorphogenesis 1 gene (COP1) and they can work toghether with phytochromes in a synergistic and antagonistic way (Liu et al., 2011b; Casal, 2013). In fact from a photo-morphological point of view cryptochromes regulate seedling de-etiolation, reduce internode length and leaf senescence and induce a more compact size in plants (Ouzounis et al., 2015; Fukuda, 2019). Cryptochromes, together with phytochromes, regulate the circadian rhythm and flowering (Devlin et al., 2007), stomata opening and closure, and are involved in the biosynthesis of gibberellic acid (Fukuda et al., 2016).

***Phototropins***

Phototropins and flavinoproteins are photoreceptors able to perceive blue light and UV A. To date little is known on their roles and functions. Two forms of phototropins have been characterized till now in *Arabidopsis*: PHOT 1, PHOT 2. The main function of these compounds is to control a wide range of responses in optimizing photosynthetic efficiency, preventing photo-damage in plants, regulating leaf positioning (phototropism) and expansion. Moreover they also affect chloroplasts distribution and stomata opening (Kami et al., 2010; Fukuda, 2019).

***ZTL/FKF1/LKP2***

ZTL/FKF1/LKP2 are a complex of proteins formed by Zeitlupe, Flavin binding, Repeat F-box 1, and LOV Kelch Protein 2. These proteins share three characteristics: Light Oxygen Voltage (LOV) domain, like phototropins, for UV-A and Blue light detection, an F-box to elicit degradation of protein targets and a C-terminal Kelch domain to mediate protein-protein interactions. Their role as photoreceptors is not completely understood, however as far we know, their main role is to regulate the circadian response and flowering (Zoltowski and Imaizumi, 2014; Huché-Thélier et al., 2016).

***UVR8***

UVR8 is a receptor responsible for detecting and absorbing ultraviolet-B radiation (in the range of 280-315 nm). It is involved in response to stress inhibiting plant growth mediating auxin signals and promoting the synthesis of photoprotective compounds such as polyphenols (Jenkins, 2009; Casal, 2013; Yang et al., 2019).

**The day-length response of flowering in plants**

Photoreceptors like phytochromes and cryptochromes allow plants to regulate their circadian rhythm and to perceive daily or seasonal variations in day and night duration, to synchronize biological processes, allocate resources and induce phenological modification during plant growth cycle, like flowering. Flowering response involves the transcription, activation and regulation of several genes (Putterill et al., 2004). Plants can be classified based on their day-length response of flowering, into: day neutral (DN), plants that are able to flower without differences in day length; long day plant (LD) able to flower when the day length is above a threshold amount; short day plant (SD) able to flower when the day length is shorter than a threshold time.

Both LD and SD plants can be further divided into quantitative or facultative and qualitative or obligate; a quantitative or facultative response means that LD or SD plants are also able to flower under SD or LD conditions respectively, while a qualitative or obligate response does not allow plants to flower in unfavorable conditions (Thomas and Vince-Prue, 1997).

Ornamentals and flower stem production schedule has always been of interest to enter the market earlier, control yields and distribute them throughout the year.

The forcing technique used in the ornamental sector to schedule productions are usually vernalization and photoperiodic lighting (De Hertogh, 1996).

Vernalization, the induction of a plant's flowering process by exposure to the prolonged cold exposure, induces the end of dormancy, especially in bulbous species, by decreasing abscissic acid content, increasing gibberellic acid, and indolacetic acid biosynthesis and availability (Liu et al., 2011a), reducing starch into soluble sugars and activating the respiration process inside the bulbs. The raise in availability of soluble sugars and gibberellic acid induces in plant first the growth and hence the flowering in the long term (De Hertogh, 1996; Wang et al., 2018).

In the past, the traditional light source used for photoperiodic lighting was the incandescent lamp, today abandoned for its high energy cost; later fluorescent lamp used as a substitute, was less effective due its spectral composition (Runkle et al., 2012). A mixture of R and FR is needed to promote flowering in several ornamentals, and, at present, LED technology is the most promising solution at this purpose with less energy consumption (Nelson and Bugbee, 2014).

Photoperiodic lighting to extend the day length can be applied as day extension, supplied at the end of the day (EOD) or at the beginning of the day, or as a night interruption (NI). Photoperiodic lighting can be subdivided into 3 main types of irradiation: continuous, intermittent and cyclic (Blanchard and Runkle, 2009).

Red and far red light are perceived even at very low light intensity. Photoperiodic lighting with R and FR proportion creating an intermediate PPE (0.63-0.80) has been proven to be more effective in promoting flowering in some LD species (*Antirrhinum majus*, *Fuchsia x hybrida*, *Petunia x hybrida*, *Rudbeckia hirta*) compared to R and FR creating an high PPE (above 0.80) (Craig and Runkle, 2016). However, the light requirement in terms of intensity and quality can vary among species and are still unknown for many crops.

**Light affects phenolic compounds and nutrients within plant tissues**

Environmental changes in terms of light intensity or quality or low nutrients may stress plants through a resource imbalance. As adaptation response to these conditions, plants synthetize photo-protective phytochemicals such as phenolic compounds (Dudt and Shure, 1994). Phenolic compounds can be classified in phenols, phenolic acids, hydroxynnamic acid derivatives and flavonoids (Holopainen et al., 2018). From a nutritional point of view, phenolic compounds presents in plants have beneficial effect on human health with a marked antioxidant activity and anti-inflammatory effects, acting against the risk of diabetes or cancer (Taulavuori et al., 2013 and reference therein).

Light quality actively influences the biosynthesis and the content of phenolic compounds in many species. In fact, B light had positive effect to increase phenolic compounds concentration in parsley, basil, tomatoes and lettuce (Taulavuori et al., 2013, 2018; Hyöky et al., 2016). Moreover, in baby leaf lettuce, additional UV-A, B, G, R or FR light to a white fluorescent lamp spectrum influenced ascorbic acid, anthocyanin and phenolics (Li and Kubota, 2009). Light modulation in term of B:R ratios influenced nutrient uptake and the aromatic profile in basil (Pennisi et al., 2019). On the other hand, FR light induce modification at chloroplast level decreasing chlorophyll content (Kerckhoffs et al., 1992; Yanovsky et al., 1998; Demotes-Mainard et al., 2016), and it was effective, in lettuce, to promote chlorogenic and caffeic acids content (Lee et al., 2016).

In the light of these observations, the experimental work at the basis of this PhD thesis was to assess the influence of light quality, especially the role played by FR and the relative PPE,

on flowering and secondary metabolite production. More specifically we indagated 1) how light quality in photoperiodic treatments affect flowering, photosynthesis and metabolic profile in the ornamental crop *Ranunculus asiaticus* L., used as model, to develop *ad hoc* photoperiodic lighting recipes for greenhouse cut stem production in Mediterranean latitudes, and 2) how BR ratio and the addition of FR may influence secondary metabolites in leafy vegetables (i.e *Chicorium endivia* L. and *Lactuca sativa* L. ) to develop vertical farm lighting strategies.

# Chapter 1

**ORIGINAL RESEARCH ARTICLE**

Front. Plant Sci., 12 March 2019 | https://doi.org/10.3389/fpls.2019.00241

## Photosynthesis in *Ranunculus asiaticus* L.: The Influence of the Hybrid and the Preparation Procedure of Tuberous Roots

Petronia Carillo[1], Carmen Arena[2], Giuseppe Carlo Modarelli[3], Stefania De Pascale[3] and Roberta Paradiso[3*]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta, Italy
[2]Department of Biology, University of Naples Federico II, Naples, Italy
[3]Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy

Modarelli, G.C., C. Arena, C., De Pascale, S., Paradiso, R. (2018). Photosynthesis in *Ranunculus asiaticus* L.: characterization in two Mediterranean hybrids under fluorescent white light. *Acta Hort*: III International Symposium on Innovation and New Technologies in Protected Cultivation, International Horticultural Congress 2018 (IHC 2018) Organized by the International Society for Horticultural Sciences (ISHS). Istanbul (Turkey), in press.

Modarelli G.C., Arena C. De Pascale S., Paradiso R. (2018). Studi sulla fotosintesi di *Ranunculus asiaticus* L.. XII Giornate Scientifiche SOI, Innovazione e meccanismo per favorire la sua adozione e applicazione in ortoflorofrutticoltura. Organized by the Società di Ortoflorofrutticoltura Italiana (SOI). *Alma Mater Studiorum* – University of Bologna, Bologna,

# Photosynthesis in *Ranunculus asiaticus* L.: The influence of the hybrid and the preparation procedure of tuberous roots

Petronia Carillo[1], Carmen Arena[2], Giuseppe Carlo Modarelli[3], Stefania De Pascale[3] and Roberta Paradiso[3*]

[1] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta, Italy

[2] Department of Biology, University of Naples Federico II, Naples, Italy

[3] Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy

***Abstract***

*Ranunculus asiaticus* L. is a quantitative long-day geophyte, grown in a cold greenhouse for cut flowers and potted plants. Flowering in ranunculus is a complex process, strongly steered by temperature and photoperiodism. Vernalization of rehydrated tuberous roots anticipate sprouting and leaf rosette formation and flowering. It is known that the time for flowering and the sensitivity to cold treatment, in terms of flowering anticipation, varies in numerous hybrids, while no information seems to be available on the influence of hybrids and on the vernalization on the photosynthetic process and primary metabolite profiling. We investigated the influence of two ranunculus hybrids, MDR and MBO, and two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V), on the photosynthesis and photochemistry of plants grown in a climatic chamber, under a controlled environment. In addition, in MBO plants, in which the vernalization showed the main effects, carbohydrate, amino acid and protein levels were also investigated. In control plants, the response of leaf photosynthesis, to increasing white light, revealed higher photosynthetic activity in MDR than in MBO. The quantum yield of PSII (ΦPSII), electron transport rate (ETR) and nonphotochemical quenching (NPQ) did not differ between the two hybrids. The maximal photochemical efficiency (Fv/Fm) was higher in MBO than in MDR and showed a decrease in both hybrids after vernalization. The preparation treatment of propagation material affected the light response of photosynthesis

in the two hybrids differently, which increased in plants from vernalized tuberous roots, compared to those from only rehydrated in MBO and decreased in MDR, in accordance to the effects of vernalization observed in leaf photosynthetic pigments. In MBO vernalized tuberous roots, starch was rapidly degraded, and the carbon skeletons used to synthesize amino acids. Control plants of MBO, developed more leaves than those of MDR and a consequent larger plant leaf area. Compared to only rehydration, vernalization of rehydrated tuberous roots increased the plant leaf area in both the hybrids. Compared to the control, vernalized tuberous roots of MBO showed higher concentrations of sucrose and free amino acids, which could act as a long-distance signal promoting floral transition in young leaf primordia.

**Introduction**

*Ranunculus asiaticus* L. is a perennial geophyte, from the family Ranunculaceae native to the Mediterranean basin and Asia Minor, grown as annual crop for cut flowers and potted plants (De Hertogh, 1996). In the wild Mediterranean environment, tuberous roots of *R. asiaticus* sprout in Autumn, when the first rain rehydrates the dried tissue, and develop a rosette of longpetiole leaves (Horovitz, 1985). Floral induction occurs when six to eight leaves are formed, and flowering lasts from February to May. Plants then begin the dormancy process, as the roots dry and the aerial part wilts and disappears during the summer (Meynet, 1993).

For production of propagation material, the harvested tuberous roots are dehydrated to less than 15% moisture (Meynet, 1993).

*Ranunculus asiaticus* exhibits a low temperature requirement (night/day regime 5–10/12–25°C, optimum day 16°C) and has a medium to high light intensity requirement. It is also considered a quantitative long day plant (Horovitz, 1985). The duration of the vegetative period and the number of flowers vary in the genotypes and are affected by the size of the tuberous root: floral induction is earlier, and the flowers are more numerous in plants from greater tuberous roots compared to those from smaller tuberous roots (Meynet, 1993).

Flowering in ranunculus is a complex process, in which both the thermal history of tuberous roots and the photoperiod play a crucial role. Cold treatments of tuberous roots (vernalization) anticipate sprouting, leaf rosette formation, and flowering (Meynet, 1993;

Beruto et al., 2009). This has been ascribed to the need for a cold period to break the summer vegetative dormancy in tuberous roots, as in the natural growth conditions (Kamenetsky et al., 2005).

Vernalization and a short day (SD) stimulate meristematic activity in tuberous roots, increasing the number of buds sprouting, as well as the number of leaves and flowers (Meynet, 1993). Tuberization and flowering show a direct antagonism. In fact, a long day (LD) increases the growth of tuberous roots and anticipates flowering compared to SD, however it reduces the number of flowers as it promotes the growth and development of already formed buds (Ohkawa, 1986; Meynet, 1993). Temperature interacts with the photoperiod: a LD promotes flowering at low temperatures, while being ineffective at high temperatures (Farina et al., 1985).

Vernalization is commonly used to break dormancy in many flower geophytes, as it promotes the starch degradation, increasing the sucrose content (Wang et al., 2018). In addition, dormancy in bulbs is regulated by the balance of abscisic acid (ABA) and gibberellic acid (GA) (Liu et al., 2011). Vernalization activates several metabolic pathways involved in the down regulation of ABA and primes the biosynthesis of active GA forms, releasing dormancy (Wang et al., 2018). However, while in other "bulb" species (e.g., Iris, Lilium and Tulipa), application of GA3 can partially replace the effect of vernalization (Moe and Berland, 1986). The effects of exogenous GA3 in *R. asiaticus* is controversial, as it anticipated flowering and improved the yield of flowers in some experiments (Hassan et al., 1985), while it did not affect flowering time and reduced yield in others (De Pascale and Scognamiglio, 1998; Mayoli et al., 2009).

In the south of Italy, ranunculus is cultivated in a cold greenhouse from rehydrated tuberous roots that are planted from the end of August to the beginning of September and harvested from the end of November until the beginning of April.

Over the past several years, cultivation of this crop has increased all over the world, also by means of breeding and the development of new hybrids (Hamrick, 2003). Contrary to other wild ranunculus species (Madsen, 1993; Madsen and Brik, 1997), little is known in the literature about the plant physiology of *Ranunculus asiaticus*, and no information seems to be available on its photosynthesis and on the influence vernalization has on the photosynthetic process and on the partitioning of sugars and amino acids within the different plant organs.

The aim of this experiment is to investigate the influence of two hybrids, MDR and MBO, and two preparation treatments of tuberous roots, only rehydration (Control, C) and rehydration, followed by vernalization (V), on the photosynthesis, photochemical efficiency and the photosynthetic pigment contents in plants of *R. asiaticus* L., grown in pots, in a controlled environment. In addition, the MBO hybrid, in which vernalization exerted the strongest effects on pigment contents, leaf development and leaf area, profiling of sugars and amino acids in tuberous roots at three plant phenological stages (planting, leaf rosette, and beginning of flowering), in response to the two preparation procedures, was carried out. Our study is the first to complete an analysis of carbohydrates and free amino acids performed on different tissues of *R. asiaticus.*

## Materials and Methods

### *Plant Material, Growth Conditions and Experimental Design*

The experiment was carried out at the Department of Biology of the University of Naples Federico II (Naples, Italy).

Plants of two hybrids of *Ranunculus asiaticus* L., MDR (medium earliness) and MBO (early flowering) (Biancheri Creations, Italy), were obtained from dry tuberous roots subjected to two preparation procedures, only rehydration (exposure to 11°C for 24 h in a humid chamber, Control, C) and rehydration followed by vernalization (exposure to 3.5 °C for 10 days, V). Tuberous roots of the most common size for each cultivar were used (4–5 cm for MDR and 3–4 cm for MBO). Four treatments derived by the factorial combination of the two hybrids and the two preparation procedures were compared: MBO-C, MDR-C, MBO-V, and MDR-V.

Plants were grown in pot, on a mixture of perlite and peat (70:30 in vol.), in a climatic chamber under a controlled environment (temperature 20/18°C day/night, relative humidity 60–80%, ambient air $CO_2$ concentration 370–400 ppm). The mean values of air temperature and relative humidity (day/night) recorded at the end of the experimental period (70 days, from the beginning of September to the middle of November) were 20.1 ± 0.9/17.7 ± 1.1°C and 60.9 ± 9.8/86.7 ± 7.0%, respectively (Mean Value ± Standard Deviation).

Light was provided by white fluorescent tubes (Sylvania luxline plus -T8, F36W/840, Cool white deluxe, Germany) at a Photosynthetic Photon Flux Density (PPFD) of 200 μmol $m^{-2}$ $s^{-1}$ at the canopy level (16 h photoperiod).

Irrigation was alternated with fertigation (three pulses per week in total). In the nutrient solution, pH and electrical conductivity were kept at 6, 0, and 1.5 dS/m, respectively, and the concentration of the nutrient elements was: (in mM) 12.6 N, 2.9 K, 0.9 P, 4.0 Ca, 1.0 Mg, 1.0 S; (in μM) 38.80 Fe, 45.00 B, 0.60 Cu, 3.80 Zn, 9.10 Mn, 1.00 Mo.

***Sampling and Measurements Net Photosynthesis, Chlorophyll a Fluorescence Measurements and Leaf Photosynthetic Pigments***

Light response curves and instantaneous values of leaf net photosynthesis (NP) were determined using an Infra-Red Gas Analyzer WALZ HCM-1000 (Walz, Effeltrich, Germany) on fully expanded leaves, in plants at the vegetative stage (leaf rosette, 4th week after planting). Measurements of response curves of NP were carried out after a 10 min acclimation to darkness, increasing PPFD by 0, 50, 100, 250, 500, 1000, 1500, and 2000 μmol $m^{-2}$ $s^{-1}$ using a built-in white halogen lamp, under constant conditions in the leaf chamber (20°C, RH 60%, 400 ppm ambient $CO_2$ concentration, air flow rate 600 ml $min^{-1}$). Contextually to the measurements of NP, chlorophyll *a* fluorescence emission measurements were performed on the same leaves using a portable fluorometer FP 100-MAX-LM equipped with a light sensor (Photon System Instruments, Czechia). Maximal PSII photochemical efficiency was calculated as the ratio of variable to maximal fluorescence (Fv/Fm): Fv/Fm = (Fm-Fo)/Fm, where Fv is the difference between maximal and minimal fluorescence (Fm-Fo). The measurements in the light were carried out under climatic chamber conditions at PPFD of 200 μmol photons $m^{-2}$ $s^{-1}$ at canopy level. The quantum yield of PSII electron transport (φPSII) was determined according to Genty et al. (1989). The linear electron transport rate (ETR) was derived by the Krall and Edwards (1992). Non-photochemical quenching (NPQ) was calculated as described by Bilger and Björkman (1990). Measurements of photosynthesis and photochemistry were performed on one leaf per plant, in four plants per combination *Hybrid x Preparation treatment*.

Total chlorophyll (a + b) and total carotenoids (x + c, xanthophylls and carotenes) were analyzed in three samples per plant, on frozen leaf disks grounded in 2 mL of ice-cold 100% acetone (three plants per combination *Hybrid x Preparation treatment*). Samples were

centifuged at 3000 rpm for 5 min, and surnatant was read spectrophotometrically (Cary 100 UV-VIS, Agilent Technologies, Santa Clara, CA, United States) at 470, 645, and 662 nm, according to Sorrentino et al. (2018).

Tuberous root tissues of *R. asiaticus* hybrid MBO, subjected to two preparation procedures, only rehydration (Control, C) and rehydration followed by vernalization (V), were sampled at three different plant phenological stages: presprouted tuberous roots, at planting; leaf rosette, plants at vegetative stage with at least five fully developed leaves (3 weeks after planting); beginning of flowering, plants with primordia of the first flower stem visible (10 weeks after planting).

***Starch, Soluble Sugars and Amino Acids in Tuberous Roots***

Starch and soluble sugars were extracted according to Ferchichi et al. (2018) with some modifications. Frozen powdered samples (50 mg) were suspended in 300 µL of ethanol (98%, v/v), incubated for 20 min at 80 °C in a water bath and centrifuged at 14,000 g for 10 min at 4°C. The clear supernatants were separated from the pellets and stored in 1 mL tubes at 4°C. The pellets were then submitted to two subsequent extractions with 150 µL of 80% ethanol (v/v) and 150 µL of 50% ethanol (v/v). Each extraction was followed by an incubation for 20 min at 80°C in a water bath and a centrifugation at 14,000 g, for 10 min at 4°C. The supernatants of the first and the two following extractions were pooled and stored at -20°C until analysis. For starch determination, the pellets of the ethanol extraction were heated at 90°C for 2 h in 250 µl of 0.1 M KOH. After cooling the samples in ice, they were acidified to pH 4.5 with 70 µl of 1 M acetic acid. Hundred µL of 50 mM sodium acetate pH 4.8 containing 0.2 U α-amylase and 2 U amyloglucosidase were added to an aliquot of 100 µL of acidified samples and the starch was hydrolizedhydrolyzed at 37°C for 18 h. The samples were vortexed and then centrifuged at 13,000 g for 10 min at 4°C and the supernatant containing the glucose derived from hydrolyzed starch was used for measurement. Soluble glucose as well as glucose originating from starch hydrolysis were analyzed enzymatically by a FLX-Xenius spectrophotometer (SAFAS, Monaco) according to Geigenberger et al. (1996). Amino acids were extracted according to Carillo et al. (2012) and measured according to Woodrow et al. (2017).

***Plant growth***

Plant growth was determined as the number of leaves and the leaf area. The number of leaves was monitored weekly. Plant leaf area was estimated by non-destructive analysis of digital images of leaves with ImageJ software 1,50i version (Wayne Rasband National Institute of Health, United States), on five leaves per plant (five plants per combination *Hybrid x Preparation treatment*) and expressed in $cm^2$ per plant.

***Statistical Analysis***

The experiment was conducted on 10 plants per combination *Hybrid x Preparation treatment*.

All experimental data were analyzed by two-way analysis of variance (ANOVA) using the SPSS 13 software package1. To compare the means of the treatments for each parameter measured, a Tukey post hoc test was performed at a significance level of $P \leq 0.05$ and $P \leq 0.01$.

For sugars and amino acids, the principal component analysis (PCA) was conducted using Minitab 16.2.1 statistical software, aimed to extract trends when multiple qualitative variables were used, by formulating new variables correlated to the original ones. The PCA outputs included treatment component scores as well as variable loadings to each selected component (Ciarmiello et al., 2015; Carillo et al., 2019).

The heat map was generated using the https://biit.cs.ut. ee/clustvis/ online program package with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage

**Results**

In Control plants of *Ranunculus asiaticus* L., obtained through only rehydrated tuberous roots, the rate of leaf net photosynthesis (NP) increased with the level of white light until a saturating intensity of about 500 µmol $m^{-2}$ $s^{-1}$ in the hybrid MBO and 1000 µmol $m^{-2}$ $s^{-1}$ in MDR was reached (Figure 1). Leaf NP was higher in MDR compared to MBO plants at all the light intensities above 250 µmol $m^{-2}$ $s^{-1}$, with the value of 4.47 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 1000 µmol $m^{-2}$ $s^{-1}$ PPDF vs. 2.92 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 500 µmol $m^{-2}$ $s^{-1}$ PPDF, respectively (Figure 1).

Vernalization of rehydrated tuberous roots affected the response of NP to light intensity differently in the two hybrids of Ranunculus asiaticus L. Indeed, this preparation procedure enhanced NP in plants of MBO, while it decreased NP in plants of MDR, at a PPDF above 250 µmol $m^{-2}$ $s^{-1}$, compared to only rehydration (Figure 1).

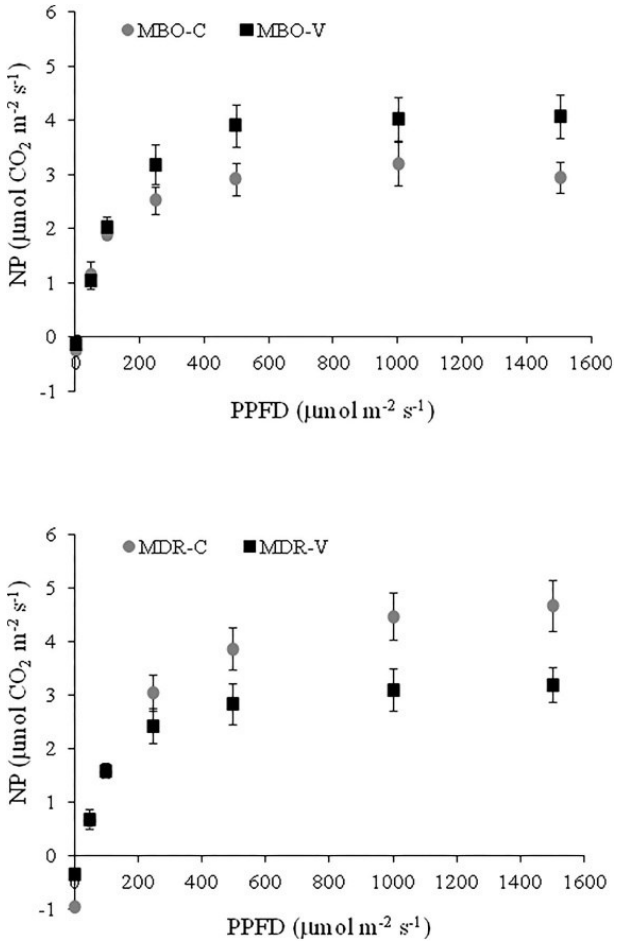



Figure 1. Response curve of leaf net photosynthesis (NP) to increasing white light intensity in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration plus vernalization (V). Plants at vegetative stage (4th week after planting), grown in a climatic chamber under a controlled environment. Measurement conditions in the leaf chamber: 20°C, RH 60%, 400 ppm ambient $CO_2$ concentration. Mean values ± standard errors; n = 4.

The PSII maximal photochemical efficiency (Fv/Fm) was higher in MBO than in MDR (0.814 in MBO-C vs. 0.801 in MDR-C) and showed a significant decrease ($P < 0.05$) in both hybrids after vernalization (Table 1). In control plants from only rehydrated tuberous roots,

a chlorophyll a fluorescence emission analysis evidenced no significant difference in the quantum yield of PSII electron transport (ΦPSII), the linear ETR and the NPQ between the two hybrids, including those with or without vernalization treatment.

Table 1. Maximal PSII photochemical efficiency (Fv/Fm), quantum yield of PSII electron transport ($\Phi_{PSII}$), linear electron transport rate (ETR) and non-photochemical quenching (NPQ) in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration plus vernalization (V). Plants at vegetative stage (4$^{th}$ week after planting), grown in climatic chamber under controlled environment. Mean values ± standard errors; n=4; ns and * indicate non-significant or significant at P <0.05, respectively.

| | | Fv/Fm | ΦPSII | ETR | NPQ |
|---|---|---|---|---|---|
| MDR | C | 0.801±0.004 | 0.756±0.003 | 38.120±0.171 | 0.267±0.042 |
| | V | 0.789±0.003 | 0.749±0.008 | 37.765±0.416 | 0.165±0.017 |
| *mean* | | *0.796* | *0.753* | *37.943* | *0.217* |
| MBO | C | 0.814±0.003 | 0.764±0.008 | 38.545±0.432 | 0.336±0.048 |
| | V | 0.804±0.003 | 0.767±0.006 | 38.680±0.338 | 0.293±0.066 |
| *mean* | | *0.810* | *0.766* | *38.613* | *0.315* |
| Significance | | | | | |
| Hybrids (H) | | * | ns | ns | ns |
| Preparation treatment (P) | | * | ns | ns | ns |
| H × P | | ns | ns | ns | ns |

In the fully developed leaves of control plants, photosynthetic pigment content was higher in MDR than in MBO hybrids, for both total chlorophylls (75.08 µg/cm$^2$ vs. 40.42 µg/cm$^2$) and total carotenoids (16.80 vs. 8.52 µg/cm$^2$) (Figure 2). The preparation procedure of tuberous roots affected the synthesis of photosynthetic pigments in the two hybrids differently (Figure 3). More specifically, the pigment amount did not change in the two preparation procedures in MDR, while it was significantly increased by vernalization in MBO (+70% in total chlorophyll and +26% in total carotenoids in MBO-V compared to MBO-C) (Figure 2).

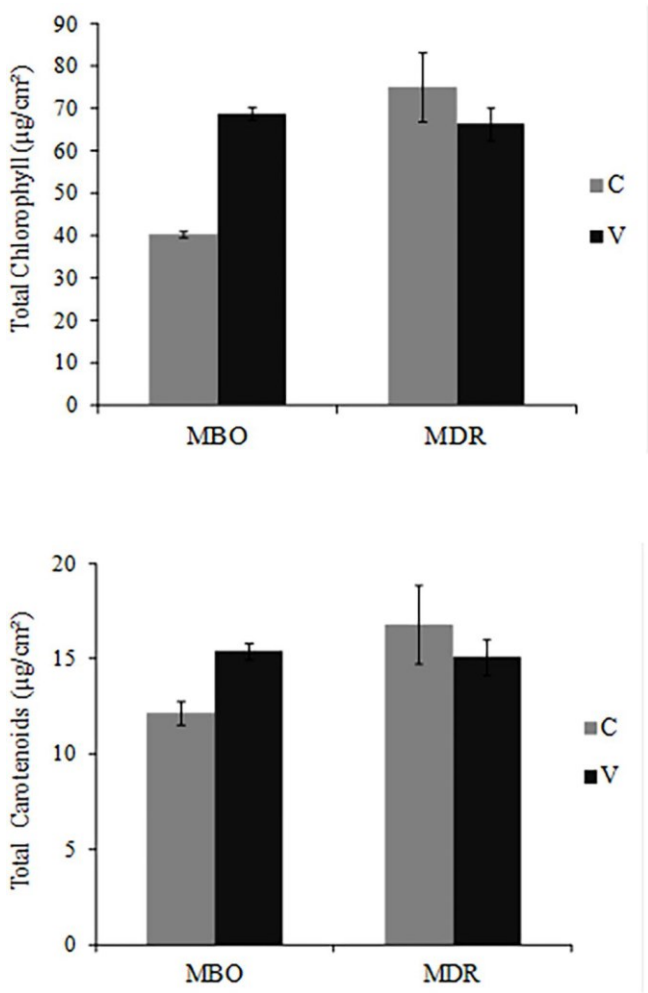



Figure 2. Total chlorophyll (a + b) and total carotenoid (x + c) content in fully expanded leaves of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V). Plants at vegetative stage (4th week after planting), grown in a climatic chamber under a controlled environment. Mean values ± standard errors; n = 3.

At the complete growth of the aerial part (10th week after planting), in Control plants from only rehydrated tuberous roots, the number of leaves was higher in the MBO compared to the MDR hybrid (27.0 in MBO-C vs 17.2 leaves per plant in MDR-C). Accordingly, the leaf area was higher in MBO-C than in MDR-C (414.8 vs. 294.0 $cm^2$ per plant in MDR-C) (Figure 3). Plants of the two hybrids showed a different response to the preparation procedure of propagation material in terms of plant growth. In fact, while in MBO vernalization increased both the number of leaves and the leaf area (+7 leaves and +18.5% leaf area per plant, respectively in MBO-V compared to MBO-C) significantly, in MDR it promoted only the leaf expansion (+30% leaf area per plant) (Figure 3).

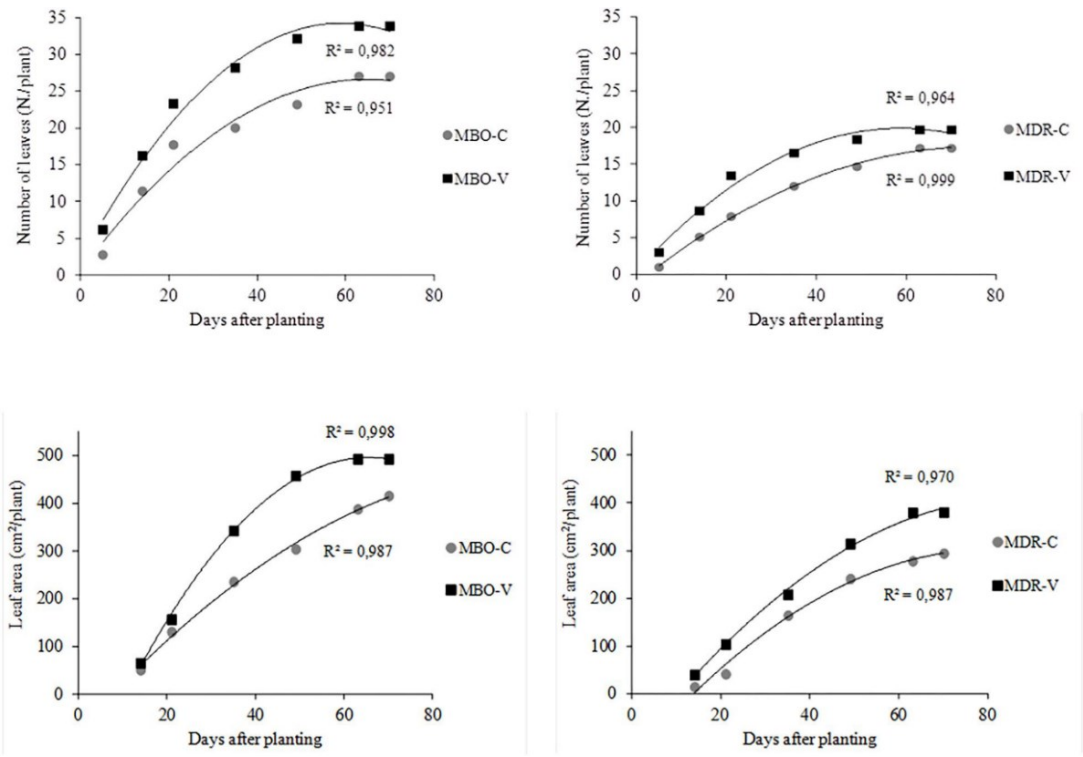



Figure 3. Time evolution of the number of leaves and plant leaf area in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V). Mean values; n = 5.

In MBO plants, in which vernalization exerted a positive effect on photosynthesis and pigment content, sugars and amino acids were also determined in tuberous roots at three phenological stages (planting, leaf rosette, and beginning of flowering) of *Ranunculus asiaticus* L., in response to the two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V) (Supplementary Tables S1, S2; see Appendix 1). A comprehensive view of the results of sugars and amino acids in these plant tissues were obtained through the PCA (Figure 4). The first two principal components (PCs) were associated with Eigen values higher than one and explained 75.7% of the cumulative variance, with PC1 and PC2 accounting for 54.3% and 21.4% (Figure 4). PC1 was positively correlated to fructose, glucose, proline, minor amino acids, and was included in branched chain amino acids (BCAAs), serine and alanine. PC1 was negatively correlated to ornithine and threonine, asparagine and arginine. Moreover, PC2 was positively

correlated to glutamate, total amino acids, starch, glutamine and aspartate. In the current experiment, the score plot of the PCA superimposed on the above matrix of variables in both tissue x treatments revealed a strong clustering of the tuberous root tissues at planting along the PC1 in the first quadrant, concentrating the sucrose, fructose, part of the minor amino acids, alanine and proline, and the tissues of tuberous roots at initial flowering, in the second quadrant, concentrating the threonine asparagine and total amino acids. The PCA also revealed a strong clustering of MBO-C and MBO-V tuberous roots at the rosette stage, along the negative side of PC2 in the third and fourth quadrants, respectively (Figure 4).

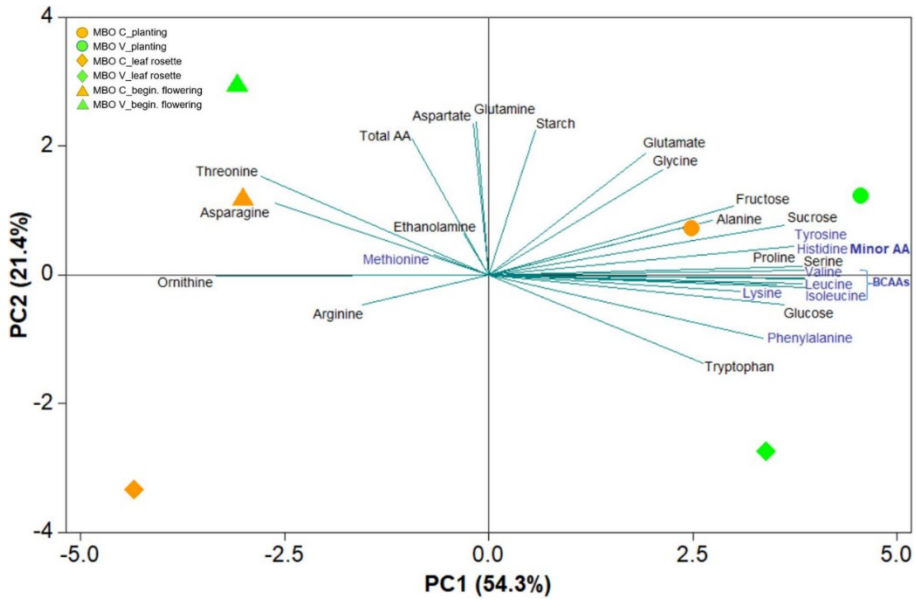



Figure 4. Principal component loading plot and scores of principal component analysis (PCA) of sugars and free amino acids content in tuberous roots at three plant phenological stages (planting, leaf rosette, and beginning of flowering) of *Ranunculus asiaticus* L. hybrid MBO, obtained by two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V).

The heat map in Figure 5 provides an integrated view of the responses of sugars and amino acids in MBO tuberous roots, at the three plant phenological stages, to control or vernalization treatment. The heat-map identified two main clusters: I and II (Figure 4). Tuberous roots of MBO-C and MBO-V at planting, and roots of MBO-V at rosette stage, were clustered together in the cluster I and clearly separated from the other three samples present in cluster II. The main clustering factor was the concentration of hexoses (glucose

plus fructose), minor amino acids and BCAAs, which were, on average, 3.4, 2.3, and 3.8-fold higher in the samples of cluster I than in those of cluster II, respectively. Glutamate highly contributed to the determination of a sub-cluster in cluster I, since the concentration of glutamate in the two tuberous roots was on average, 2.7-fold higher than in all other samples of tuberous roots. In cluster II, grouping MBO-C at rosette stage and MBO-C and MBO-V at beginning of flowering, minor amino acids included BCAAs, and sucrose drastically decreased, while total amino acids, and in particular asparagine, aspartate and glutamine in tissues at beginning of flowering, increased. In MBO-V at beginning of flowering, there was a 50% and 69% increase in starch and ethanolamine concentration, respectively, compared to MBO-C.

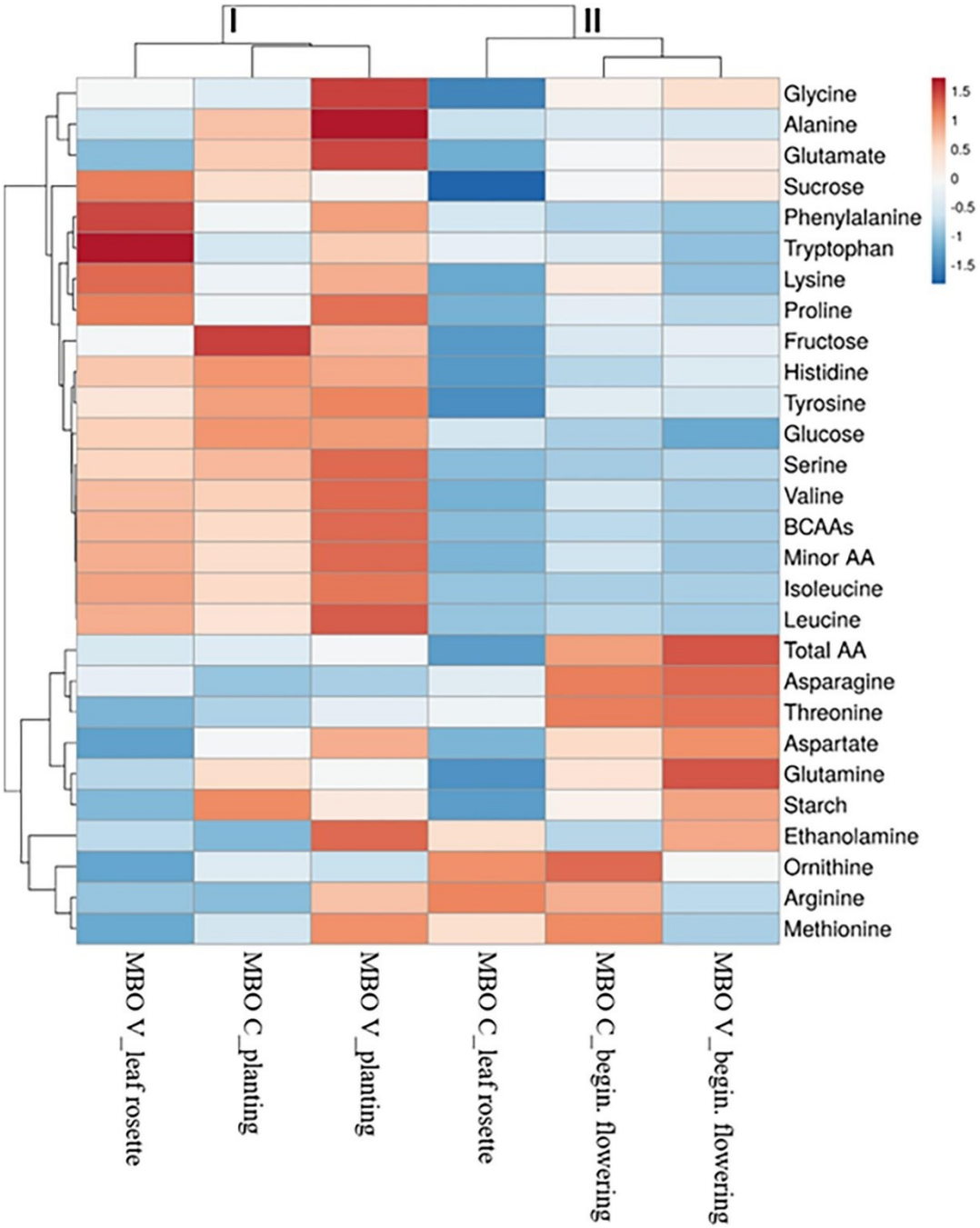



Figure 5. Cluster heat map analysis summarizing sugars and free amino acid changes in tuberous roots at three plant phenological stages (planting, leaf rosette and beginning of flowering) of *Ranunculus asiaticus* L. hybrid MBO, in response to two preparation procedures of tuberous roots, only rehydration (Control, C) and rehydration followed by vernalization (V).

## Discussion

The response curves of leaf net photosynthesis to increasing white light, obtained in plants of *Ranunculus asiaticus* L. at vegetative stage, showed some intrinsic differences between the hybrids, indicating a higher photosynthetic capacity in MDR compared to MBO. In fact, in control plants obtained by only rehydrated tuberous roots, net photosynthesis was higher in MDR compared to MBO plants, at all the light intensities above 250 μmol $m^{-2}$ $s^{-1}$. No data seems to be available in the literature for photosynthesis of *Ranunculus asiaticus* L. In general, the rate of photosynthesis measured in our experiment seems to be lower than that reported for other geophytes (e.g., *Lilium*), grown in a greenhouse for flower production (Barrera-Aguilar et al., 2013). This result could depend on genetic reasons, but it could also be due to the adaptation of ranunculus plants to the relatively low PPFD applied in the experiment, as well as to the lower photosynthetic capacity known for several species in the young developmental stage (i.e., $4^{th}$ week after planting) (Kitajima et al., 2002; Paradiso et al., 2018). However, the values recorded for both the hybrids of ranunculus, grown under artificial light in a controlled environment, are in the order of magnitude of those measured in wild plants of the spring tuberous roots species *Anemone raddeana* Regel (Family *Ranunculaceae*; Yoshie and Yoshida, 1987).

The different photosynthesis of the two hybrids seems to be due to a diverse amount of photosynthetic pigments rather than differences in photochemistry. In fact, the fluorescence emission analysis indicates a good performance of the photosynthetic apparatus in a light reaction of photosynthesis, in both hybrids. In our opinion, the intrinsic difference in the photosynthetic pigment content may in part explain the differences recorded in photosynthesis, and in particular, the opposite response to vernalization in the two hybrids for both pigment content and net photosynthesis.

The lower content of photosynthetic pigments in MDR-V compared to MDR-C could also explain the occurrence of differences in maximal PSII photochemical efficiency (Fv/Fm) and net photosynthesis found between the hybrids. In fact, no difference was found between the hybrids in the quantum yield of PSII electron transport (ΦPSII) and in the linear ETR. Conversely, Fv/Fm was lower in MDR-C than in MBO-C, indicating a better condition for the photosystem II and a higher potential capability to convert light energy to reaction centers in the MBO-C hybrid. It is noteworthy that Fv/Fm can be considered as a good

indicator of the plant health status; in absence of physiological stress the threshold value is around 0.83 (Björkman and Demmig, 1987). Lower values can provide an indication of unfavorable conditions for photosynthetic apparatus, highlighting a possible photoinhibition under stressful events (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004). In our case, even if the Fv/Fm was lower in MDR-C compared to MBO-C we can exclude phenomena linked to photosystem photoinhibition, since the Fv/Fm values for both hybrids were close to the optimal value. In both the hybrids, vernalization seems to determine an apparent reduction of NPQ, even if the differences are not statistically significant. It may be hypothesized that this treatment reduces the need for the photosynthetic apparatus to dissipate as heat the light energy was not utilized in photochemistry (Johnson et al., 1993; Horton et al., 1996; Maxwell and Johnson, 2000). It can be supposed that vernalization may likely help photosynthetic apparatus of MBO to withstand the low temperatures. However, further studies on *R. asiaticus* physiology may clarify these aspects.

In our experiment, the increase of pigment content and thus of photosynthesis, observed in MBO-V compared to MBO-C, agrees with studies on herbaceous crops chilled at the pre-sowing seed stage, such as *Foeniculum vulgare* Mill (Rashad and ElSoud, 2009), *Beta vulgaris* L. (Sakr et al., 2013) and *Solanum lycopersicum* L. (Haroun et al., 2011). Our results are also consistent with studies on *Cicer arietinum* L. and *Luffa aegyptiaca* Mill (Singh, 1984) and on *Lolium perenne* L. (Stapleton and Jones, 1987) plants subjected to cold treatment during vegetative development. Conversely, the decrease observed for MDR-V compared to MDR-C is in line with a study on *Glicine max* (L.) Merr seedlings (Yadegari et al., 2008), *Ocimum basilicum* L. plants (Borowski and Blamowski, 2009) and on Coffea arabica L. plants (Partelli et al., 2009) exposed to cold treatment. The effect of different hybrid x tuberous roots preparation procedure interaction on pigment content and photosynthesis carried out in our study is consistent with some findings in which it has been hypothesized the existence of a different genotype x abiotic stress response in various species (Bączek-Kwinta et al., 2007; Bekhradi et al., 2015; Kalisz et al., 2016; Tuteja and Gill, 2016). On the other hand, the different behavior observed in *R. asiaticus* hybrids may also be due to a less pronounced effect of vernalization in MDR, likely suggesting the occurrence of a diverse cold requirement in these hybrids. In addition, the sensitivity to the cold treatment may have been influenced by the different sizes of tuberous roots in the two hybrids: bigger tuberous sizes may likely require a longer vernalization period to obtain the

same effect. However, these aspects in *R. asiaticus* remain in part unsolved and further studies are required to confirm these hypotheses.

In plants from rehydrated tuberous roots, the growth in terms of number of leaves and total leaf area per plant differed between the hybrids. Specifically, in the control preparation procedure, MBO plants produced more leaves and reached a greater leaf area compared to MDR, despite the smaller size of the tuberous roots. This result confirms the existence of different vegetative types in *R. asiaticus* and suggests that larger tuberous root sizes are advised for less vigorous genotypes (Meynet, 1974; Beruto et al., 2018).

Compared to the control treatment (only rehydration), vernalization of rehydrated tuberous roots significantly increased the plant leaf area in both the hybrids, in accordance with the promoting effect of low temperature on the meristematic activity known in *R. asiaticus* (Meynet, 1993) and in other bulbous species (Wang et al., 2018).

Sugars and amino acids were analyzed in the MBO hybrid, in which vernalization exerted the strongest effects on photosynthesis and pigment content. These metabolites provide energy and precursors for plant growth and sustain the development of flowers, serving as chemical precursors and energy for floral primary and secondary metabolism (Muhlemann et al., 2014; Borghi and Fernie, 2017).

Vernalization-related cold stress can compromise membrane fluidity and increase oxidation, due to the uncoupling of the electron transport chain (Taïbi et al., 2018). As a consequence, high levels of reactive oxygen species (ROS) are formed, which, in turn, further affect the integrity and functionality of the same membranes and subcellular structures (Thakur and Nayyar, 2013).

In MBO-V tuberous roots, a faster hydrolysis of starch compared to MBO-C, and a strong accumulation of glutamate, alanine, glycine, proline and minor amino acids in addition to glucose and fructose (hexoses), but not sucrose, occurred.

The hexoses, that accumulate in MBO-V tuberous roots after vernalization, can function as osmoprotectants and ROS (hydroxyl radicals) scavengers, maintaining the integrity and stability of membranes and macromolecules (Taïbi et al., 2018). Moreover, the accumulation of minor amino acids can be closely related to the high levels of hexoses, which particularly increase under stress (Fritz et al., 2006). Minor amino acids included BCAAs, which can

serve both as compatible compounds, antioxidants and as alternative electron donors for the mitochondrial electron transport chain (Woodrow et al., 2017 and references therein).

It is also likely that the increase of the other amino acids in MBO-V is due to the stressing effects of vernalization. Glutamate, in fact, can serve as precursor of proline, that over and above its supposed role as an osmolyte, can scavenge ROS and buffer cellular redox potential, stabilizing membranes and proteins, and lead to the expression of stress responsive genes containing proline response elements (Carillo, 2018). In addition, glutamate can be converted to GABA, through a decarboxylation catalyzed by glutamate decarboxylase which consumes protons, buffering stress-induced cytosolic acidosis (Carillo, 2018). Subsequently, GABA undergoes a series of reactions, known as GABA shunt, which use alanine as a precursor and supply NADH and/or succinate to the mitochondrial electron-transport chain, under conditions in which respiration and the TCA cycle are impaired and ROS is increased (Bouché et al., 2003). Additionally, glycine, that significantly increases in cold-sensitive seedlings, can be related to the synthesis of glutathione, of which it is a constituent (Taïbi et al., 2018). Glutathione functions not only as a ROS scavenger but also as a cofactor for several enzymes involved in the oxidative stress response (Woodrow et al., 2017).

Furthermore, the increase in ethanolamine in MBO-V tuberous roots, an amino acid derivative obtained by decarboxylation of serine, is useful for the synthesis and/or regeneration of phospholipids, and for changes in the membrane phospholipid profile under cold stress (Kogan et al., 2000).

Glutamate concentration in tuberous roots strongly decreased at the stages of leaf rosette and at the beginning of flowering, in contrast with previous reports (Lea and Forde, 2007; Ishizaki et al., 2010). The decrease of glutamate could depend on its use as a nitrogen donor in biosynthetic transamination in the production of other amino acids. In fact, in MBO-V leaf rosettes, phenylalanine and tryptophan increased while proline was maintained at the same level as in the MBO-V tuberous roots. Phenylalanine and tryptophan are not only essential for protein synthesis in plants, but they are used as precursors for a variety of plant hormones, such as auxin and salicylate, and aromatic secondary metabolites with various biological functions, such as volatile organic compounds (Tzin and Galili, 2010; Muhlemann et al., 2012). In particular, phenylalanine synthesis and accumulation are essential to provide

the building blocks for floral enzymes and structural proteins, even in plants containing low amounts of volatile organic compounds (Borghi and Fernie, 2017).

Furthermore, in MBO-C there was a strong increase of two amino acids, arginine and ornithine. The arginine biosynthetic pathway in plants consists of nine enzymatic steps from glutamate, also involving the production of ornithine as an intermediate. These two amino acids can function as precursors of polyamines, which are involved in many pivotal biological processes such as flowering, fruit ripening and osmotic stress protection (Kalamaki et al., 2009). The active role of single types of polyamines in floral development has been reported in several crops, and in particular in long-day plants. The inhibition of the polyamine biosynthetic pathway, preventing flowering in *Spirodela punctata* (G.F.W. Mey.) Thompson and Polianthes tuberosa, can be resumed when spermidine, one of the most abundant polyamines, is applied exogenously (Liu et al., 2006 and references therein). However, spermidine has been proven as necessary but not sufficient for promoting flower induction in *Spirodela punctata* (Bendeck de Cantú and Kandeler, 1989). Therefore, the accumulation of arginine and ornithine, precursors of polyamines, can represent a plant strategy to be ready to produce polyamines, which is one of the signals that play a direct role in the promotion of flowering (Kalamaki et al., 2009). The presence of high levels of these two amino acids in tuberous roots of MBO-C, but not in MBO-V, at leaf rosette and beginning of flowering may indicate that in the absence of vernalization the synthesis of metabolites involved in the induction of flowering is delayed.

The signal responsible in MBO-V rosettes, for the use of arginine and ornithine to synthetize polyamines and promote flowering, could be the accumulation of sucrose. It is well known that exogenous sucrose can promote flowering in several species (Bernier et al., 1993). In the juvenile phase, the expression of the florigen gene (FT) is inhibited by miR172-targeted AP2- like transcription factors. miR172 is a conserved miRNA, that is a short, single-stranded non-coding RNA regulating the FT gene expression at a post-transcriptional level. Sucrose is able to inactivate the inhibition of miR172 and promote flowering. In addition, high levels of sucrose induce an increase of trehalose 6 phosphate T6P, which contributes to the production of FT in leaves through a distinct genetic pathway, allowing plant flowering at the correct time when sucrose levels are suitable (Wahl et al., 2013; Yadav et al., 2014).

Finally, at the stage of beginning of flowering, tuberous roots of MBO-V show a strong accumulation of primary amino acids, in particular amides and aspartate, starch and again ethanolamine. The amides asparagine and glutamine, with a high N:C ratio (2:4), can efficiently serve as a carrier and storage metabolite for nitrogen. Increasing the concentration of these two amino acids and aspartate, in addition to starch and ethanolamine can be an effective strategy to store reserves or precursors that can be used during ovule maturation and embryo growth (Borghi and Fernie, 2017).

**Conclusion**

In control plants obtained by only rehydrated tuberous roots, the hybrid MDR showed a lower plant leaf area but better efficiency in light interception, and higher net photosynthesis and photosynthetic pigment content compared to MBO. However, the lower photosynthesis in MBO plants was not due to the impairment of photosystems as the Fv/Fm values were comparable in the two hybrids.

Compared to only rehydration, rehydration followed by vernalization had a positive effect in the hybrid MBO, increasing the photosynthetic pigments and the net photosynthesis as well as the plant leaf area. The reasons why vernalization had different effects in the hybrids may be linked to a genotype specific response as well as the different sizes of the tuberous roots, confirming that the size of propagation material should be considered in the selection of both the hybrid and the preparation procedure. Our findings also suggest to perform physiological screenings, in order to define the optimal combination hybrid/vernalization treatment. Moreover, the MBO hybrid, in response to vernalization showed a fine tuning of selected sugars and amino acids in tuberous roots, which prepared plants for flowering. In particular, the concerted action of protectant metabolites in tuberous roots helped the plant to cope with the vernalization related-cold stress, and subsequently in rosette tissues to divert metabolism toward the utilization or accumulation of particular amino acids and sucrose functions to promote flowering.

**Author Contributions**

GCM performed plant cultivation and contributed to measurements. RP, CA, PC performed measurements and statistical analysis, and wrote the manuscript. SDP provided scientific oversight in experimental design.

# Chapter 2

Modarelli, G.C., Arena, C., De Pascale, S., Paradiso, R. (2019). The influence of the hybrid and the preparation procedure of tuberous roots on plant growth and flowering in *Ranunculus asiaticus* L. *Acta Hort*. GreenSys 2019 International Symposium on Advanced Technologies and Management for Innovative Greenhouses. Organized by the International Society for Horticultural Sciences (ISHS). Angers (France), in press.

# The influence of the hybrid and the preparation procedure of tuberous roots on photosynthesis and flowering in *Ranunculus asiaticus* L.

Modarelli G.C.[1], Arena C.[2], De Pascale S.[1], Paradiso R.[1 *]

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100 - 80055, Portici, Naples (Italy)

[2] Department of Biology, University of Naples Federico II, Via Cinthia 4 - 80126, Naples (Italy)

*Corresponcence: rparadis@unina.it

***Abstract***

In *Ranunculus asiaticus* L., we investigated the influence of two hybrids, MBO and MDR, and three tuberous roots preparation procedures, only rehydration (Control, C), rehydration plus vernalization at 3.5 °C for 2 weeks (V1), and rehydration plus vernalization at 3.5°C for 4 weeks (V2), on plant growth, leaf photosynthesis and flowering, in plants grown in pot, in a cold greenhouse.

A significant interaction was found between the genotype and the preparation treatment on net photosynthesis: compared to Control, it did not change in V1 and increased in V2 in MBO while it was unaffected by vernalization in MDR.

Quantum yield of PSII electron transport (ΦPSII), linear electron transport rate (ETR) and non-photochemical quenching (NPQ) did not differ in the two hybrids, whereas maximal PSII photochemical efficiency (Fv/Fm) was higher in MBO than in MDR. Fluorescence indexes were unaffected by the preparation procedure, with the exception of ETR, which varied only in MDR, decreasing in V1 compared to C and V2.

Genotype and preparation procedure significantly interacted also on total plant leaf area, which was reduced in V2 procedure only in MBO plants, while decreased in both the vernalization procedures in MDR plants.

In Control plants, flowering started in 65 days in MBO and 69 days in MDR. Compared to controls, both the vernalization procedures resulted in earlier flowering in MDR, while they were detrimental or only slightly efficient in MBO. Vernalization always reduced the quality of flower stems in both the hybrids.

In conclusion, the two hybrids showed a different response to the root preparation procedure in terms of photosynthesis, plant growth and flowering earliness. These results highlight that specific response of the hybrids should be considered in the choice of the preparation procedure.

**Keywords:** geophytes, cold treatment, photosynthesis, photosynthetic pigments, flowering

## Introduction

*Ranunculus asiaticus* L. is an ornamental geophyte cultivated for flower stems and potted plants production, mainly propagated by tuberous roots. *R. asiaticus* is characterized by low temperature requirement (night/day regime 5-10/12-25 °C, optimum day 16°C) and is classified as a quantitative long day plant (Horovitz, 1985). Floral induction in the rosette occurs when 6 to 8 leaves are formed and flowering lasts from February to May, then the plants enter in dormancy as the roots and the aerial part wilt and disappear during summer (Meynet, 1993).

In the wild Mediterranean environment, the dry and dormant tuberous roots sprout in Autumn, when the first rain rehydrated the dried tissue, and develop a rosette of long petioled leaves (Horovitz, 1985). For production of propagation material, the tuberous roots are harvested and dehydrated to less than 15% moisture content (Meynet, 1993). The genotype and the size of tuberous roots affect the duration of vegetative period and the number of flowers, in fact plants from bigger roots show an earlier floral induction and produce more flowers compared to those from smaller roots (Meynet, 1993).

Flowering in ranunculus is a complex process influenced by the thermal history of tuberous roots and the photoperiod, and vernalization of tuberous roots is widely used to anticipate sprouting, leaf rosette formation and flowering (Meynet, 1993; Beruto et al., 2009). Indeed, cold temperature causes starch degradation, increases the sucrose content (Wang et al., 2018), and influences the balance of abscisic acid (ABA) and gibberellin acid (GA) (Liu et

al., 2011), which regulates the dormancy in bulbs by determining the down regulation of ABA and promoting the biosynthesis of active GA forms, releasing dormancy (Wang et al., 2018).

Several studies on *R. asiaticus* focused on the interaction between thermo and photoperiod. It is known that vernalization and short day (SD) stimulate meristematic activity increasing the percentage of sprouting buds as well as the number of leaves and flowers (Meynet, 1993), while flowering is in antagonism with the tuberization process. In fact, compared to SD, long day (LD) anticipates flowering, promoting the growth of already formed buds with negative effect on flowers yields and quality, while increases the size of tuberous roots (Meynet, 1993; Ohkawa, 1986). Temperature interacts with photoperiod: LD promotes flowering at low temperature, while it is ineffective at high temperature (Farina et al., 1985).

In the South of Italy, ranunculus is cultivated in cold greenhouse from rehydrated tuberous roots, with planting from the end of August to the beginning of September and harvesting from the end of November until the beginning of April.

Cultivation of *Ranunculus asiaticus* has been rising during the last years all over the world, also thanks to the breeding and the development of new hybrids (Beruto et al., 2018). However, conversely to other wild ranunculus species (Madsen, 1993; Madsen and Brik, 1997), little is known about the plant physiology in this species and, apart from one recent study (Carillo et al., 2019a), no information seems to be available on photosynthesis and photochemistry and on the influence of vernalization on plant physiology in commercial hybrids.

We investigated the influence of two hybrids of *R. asiaticus* L. and three preparation procedures of tuberous roots on photosynthesis, photochemical efficiency and flowering earliness of potted plants grown in cold glasshouse.

**Materials and Methods**

The experiment was carried out in an unheated greenhouse, at the Department of Agriculture of the University of Naples (Portici, Italy - 40°49′ N, 14°20′ E), from September 2018 to February 2019.

Dry tuberous roots of two hybrids of *Ranunculus asiaticus* L., MDR (medium earliness) and MBO (early flowering) (Biancheri Creazioni, Italy), were subjected to three preparation procedures:

- only rehydration: exposure to 12 °C for 24 h in humid chamber (Control, C);
- rehydration followed by vernalization at 3.5 °C for 2 weeks (V1);
- rehydration followed by vernalization at 3.5 °C for 4 weeks (V2).

Tuberous roots of the most common size for each hybrid were used (3-4 cm for MBO and 4-5 cm for MDR). Plants were grown in pot, on a mixture of perlite and peat (70:30 in vol.). Six treatments, derived by the factorial combination of the two hybrids and the three preparation procedures, were compared: MBO-C, MDR-C, MBO-V1, MDR-V1, MBO-V2, MDR-V2.

Irrigation was alternated with fertigation (4 pulses per week in total). In the nutrient solution (recipe Hoagland full strength), pH and electrical conductivity were kept at 5.5 and 1.7 dS/m, respectively.

The number of leaves was monitored weekly. Plant leaf area was estimated by non-destructive analysis of digital images of leaves with ImageJ software 1,50i version (Wayne Rasband National Institute of Health, USA), on 3 leaves per plant (on 5 plants per combination *Hybrid x Preparation treatment*). The results obtained in fully grown plants (11 weeks after planting) are reported. The beginning of flowering was calculated as the 10% of plants with visible flower buds.

Physiological measurements were performed on fully expanded leaves in plants at vegetative stage (leaf rosette, 7 weeks after planting), on 1 leaf per plants, in 4 plants per combination *Hybrid x Preparation treatment*. Net photosynthesis (NP) was determined by an Infra-Red Gas Analyzer Walz HCM-1000 (Walz, Effeltrich, Germany). On the same leaves, chlorophyll *a* fluorescence emission measurements were conducted using a portable fluorimeter MINI PAM (Walz, Effeltrich, Germany). The maximal PSII photochemical efficiency was calculated as the ratio of variable to maximal fluorescence (Fv/Fm): Fv/Fm = (Fm−Fo)/Fm where Fv is the difference between maximal and ground fluorescence (Fm-Fo). The measurements in the light were carried out under natural light, which was on average 436±107 μmol $m^{-2}$ $s^{-1}$ at canopy level (Mean value ± Standard deviation, n = 25). The quantum yield of PSII electron transport ($\Phi_{PSII}$) was determined according to Genty et al. (1989). The linear electron transport rate (ETR) was derived by the Krall and Edwards equations (1992). Non-photochemical quenching (NPQ) was calculated as described by Bilger and Björkman (1990).

The experiment was conducted on 25 plants per combination *Hybrid x Preparation*

*treatment*. Data were analysed by ANOVA using SPSS 25 software package (www.ibm.com/software/analytics/spss) and means were compared by Duncan *post-hoc* ($P \leq 0.05$).

**Results**

The mean values of air temperature and relative humidity (day/night) recorded during the plant vegetative phase (7 weeks from planting) were 18.2±4.4/9.4±1.4 °C and 79.3±18.1/98.6±2.5 %, respectively (Mean Value ± Standard Deviation).

In plants from rehydrated tuberous roots (Control), leaf net photosynthesis of *Ranunculus asiaticus* L. was higher in the hybrid MDR than in the hybrid MBO (Figure 1). A significant interaction ($P<0.05$) was found between the genotype and the preparation procedure on net photosynthesis: compared to Control, it did not change in V1 and increased in V2 in MBO, while control and vernalisation showed no significant differences in MDR (Figure 1).

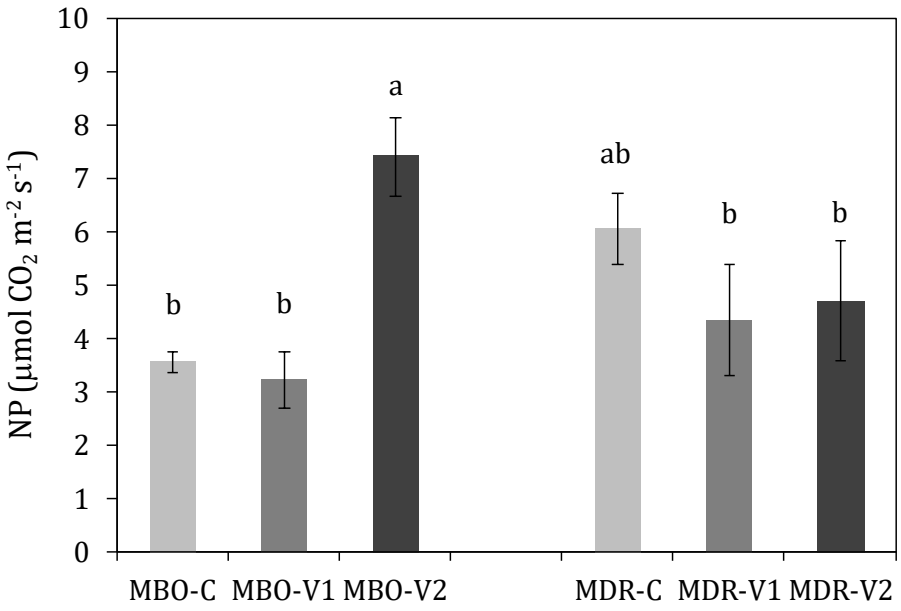



Figure 1. Photosynthesis in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by three preparation procedures of tuberous roots, only rehydration (Control, C), rehydration plus vernalization for 2 weeks (V1), rehydration plus vernalization for 4 weeks (V2). Mean values ± Standard errors; n=5. Different letters indicate significant difference according to Duncan's test at $P \leq 0.05$.

The fluorescence emission measurements evidenced no significant effect of the hybrid for quantum yield of PSII electron transport ($\Phi_{PSII}$), linear electron transport rate (ETR), and non-photochemical quenching (NPQ) (Table 1). Conversely, Fv/Fm ratio showed higher values ($P<0.05$) in MBO than in MDR (Table 1).

A significant interaction was found between the genotype and the preparation procedure in the electron transport rate. ETR did not change with vernalization in MBO, while it significantly decreased ($P<0.05$) compared to the other procedures in MDR subjected to V1 treatment (Table 1).

Table 1. Maximal PSII photochemical efficiency (Fv/Fm), quantum yield of PSII electron transport ($\Phi_{PSII}$), linear electron transport rate (ETR) and non-photochemical quenching (NPQ) in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, obtained by three preparation procedures of tuberous roots, only rehydration (Control, C), rehydration plus vernalization for 2 weeks (V1), rehydration plus vernalization for 4 weeks (V2). Mean values ± Standard errors; n=5; ns and * indicate non-significant or significant difference at P =0.05.

| | | Fv/Fm | $\Phi_{PSII}$ | ETR | NPQ |
|---|---|---|---|---|---|
| MBO | C | 0.789±0.006 | 0.497±0,035 | 86.654±13.677 | 1.572±0.193 |
| | V1 | 0.807±0.009 | 0.497±0.020 | 91.141±4.889 | 1.521±0.253 |
| | V2 | 0.802±0.006 | 0.484±0.026 | 85.687±8.517 | 1.273±0.101 |
| *mean* | | *0.799* | *0.492* | *87.827* | *1.455* |
| MDR | C | 0.790±0.006 | 0.419±0.080 | 72.379±6.449 | 1.619±0.389 |
| | V1 | 0.785±0.009 | 0.566±0.034 | 54.118±5.153 | 1.130±0.179 |
| | V2 | 0.787±0.004 | 0.514±0.023 | 95.845±9.064 | 1.293±0.228 |
| *mean* | | *0.787* | *0.499* | *74.114* | *1.347* |
| Significance | | | | | |
| Hybrids (H) | | * | ns | ns | ns |
| Preparation procedure (P) | | ns | ns | ns | ns |
| H × P | | ns | ns | * | ns |

In Control plants, the total leaf area was significantly higher in MDR compared to MBO (883.6 vs 646.5 cm², respectively).

Genotype and preparation treatment significantly interacted also on total plant leaf area, which was reduced significantly only in V2 procedure in MBO plants (-82% compared to Control), while decreased in both the vernalization procedures in MDR (-34% in V1 and -72% in V2).

In plants from only rehydrated tuberous roots (C), flowering started earlier in the hybrid MBO (65 vs. 69 days after planting in MDR). Compared to only rehydration, the procedure V1 delayed flowering in MBO (+30 days than in MBO-C) and promoted it in MDR (-41 days than in MDR-C), while the procedure V2 anticipated flowering only slightly in MBO (-7 days than in MBO-C) and strongly in MDR (-59 days than in MDR-C).

The quality of flower stems differed between the hybrids and was influenced by the preparation procedure. Flower stems were significantly taller in MBO compared to MDR (57.1 cm vs. 49.9 cm, respectively), however the flower fresh weight did not vary in the two hybrids (24.52 g/flower on average).

Compared to Control procedure, V1 and V2 treatments did not influence the stem height in the MBO plants, while they both reduced it in MDR (-24% and -38%, respectively). Despite the different genotype response to vernalization in terms of stem height, both the vernalization procedures determined lighter stems in both the hybrids compared to Control (-41% in V1 and -66% in V2 in MBO, and -58% and -71% respectively in MDR).

**Discussion and conclusions**

Different studies show the influence of the thermo-photoperiod on growth and flowering time of *R. asiaticus* (Karlsson, 2003; Meynet, 1993). In our experiment, plants of two hybrids characterized by different earliness, obtained by different roots preparation procedures were grown from September till March, under short day condition in an unheated greenhouse.

Measurements of leaf net photosynthesis in plants of *Ranunculus asiaticus* grown in greenhouse confirmed the occurrence of intrinsic differences between MBO and MDR, we already observed in the same hybrids grown in growth chamber (Carillo et al., 2019a). Accordingly, the two hybrids also showed different response of photosynthesis to the thermal preparation treatment of tuberous roots, as highlighted in the previous study (Carillo et al., 2019a).

The absence of significant difference in $\Phi_{PSII}$ and NPQ in both the hybrids, indicated that the preparation procedures utilized in this study did not affect the plant capability to convert the light energy in photochemistry as well as the thermal dissipation mechanisms. Also, this result is in agreement with our research on the same hybrids, when treated with only two preparation procedures of tuberous roots (rehydration and rehydration plus vernalization for 2 weeks) (Carillo et al., 2019a). In addition, the statistically higher value in MBO than in MDR for the Fv/Fm ratio confirmed a different intrinsic physiological potential between the hybrids, indicating a better healthy status in MBO plants (Carillo et al., 2019a). Specifically, the Fv/Fm ratio closer to 0.83 in MBO indicates a lighter stress condition for photosynthetic apparatus in this hybrid compared to the other one (Björkman and Demmig, 1987; Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004), suggesting that this hybrid is less sensitive to vernalization treatment.

In plants from only rehydrated tuberous roots flowering was earlier in MBO compared to MDR, as expected. Cold treatment of rehydrated tuberous roots has been proved to anticipate flowering in different ranunculus hybrids (Beruto et al., 2009), however in our experiment it was effective only in the hybrid MDR and ineffective or detrimental in MBO. This confirms that the efficacy of vernalization depends on hybrid-specific cold requirements (Beruto et al., 2009). Indeed, our results are in agreement with the findings of Ohkawa (1986) who studied the influence of different vernalization procedures (5°C for 2 weeks and 5°C for 4 weeks compared to only rehydration) on flower initiation of *R. asiaticus* and revealed a close relationship between flowering anticipation and the duration of cold treatment, as we observed in MDR. The same Author also reported a negative influence of the 4 weeks thermal treatment on flowering, in line with our finding in the hybrid MBO.

In our experiment, flower stem characteristics were comparable in the two hybrids in plants obtained with only rehydration of tuberous roots. Vernalization reduced the stem height in MDR and the fresh weight in both the hybrids, with stronger effect after 4 weeks of treatment, confirming negative effects on flower quality (Ohkawa, 1986).

In conclusion, the two hybrids had a different response to the root preparation procedure in terms of photosynthesis, plant growth and flowering earliness. These results highlight that specific response of the hybrids should be considered in the choice of the preparation procedure at large scale.

# Chapter 3

## Unravelling the role of light quality of photoperiodic lighting on photosynthesis, flowering and metabolic profiling in two *Ranunculus asiaticus* L. hybrids

Giuseppe Carlo Modarelli[1], Carmen Arena[2], Giuseppe Pesce[3], Emilia Dell´Aversana[4], Giovanna Marta Fusco[4], Petronia Carillo[4], Stefania De Pascale[1], Roberta Paradiso[1]

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100 - 80055, Portici, Naples (Italy)

[2] Department of Biology, University of Naples Federico II, Via Cinthia 4 - 80126, Naples (Italy)

[3] Department of Physics, University of Naples Federico II, Via Cinthia 4 - 80126, Naples (Italy)

[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta (Italy)

**submitted to:**

Physiologia Plantarum

# Unravelling the role of light quality of photoperiodic lighting on photosynthesis, flowering and metabolic profiling in two *Ranunculus asiaticus* L. hybrids

Giuseppe Carlo Modarelli[1], Carmen Arena[2], Giuseppe Pesce[3], Emilia Dell´Aversana[4], Giovanna Marta Fusco[4], Petronia Carillo[4], Stefania De Pascale[1], Roberta Paradiso[1*]

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100 - 80055, Portici, Naples (Italy)

[2] Department of Biology, University of Naples Federico II, Via Cinthia 4 - 80126, Naples (Italy)

[3] Department of Physics, University of Naples Federico II, Via Cinthia 4 - 80126, Naples (Italy)

[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta (Italy)

*Correspondence: rparadis@unina.it

*Keywords:* light spectrum, geophytes, photosynthesis, PSII photochemical efficiency, sugars, amino acids

***Abstract***

Light quality of photoperiodic lighting affects flowering of long day plants, by influencing the phytochrome photoequilibria (PPE) at plant level, however the most effective light spectrum to promote flowering is still unknown for most the flower crops.

We evaluated the influence of light spectrum of three light sources for photoperiodic lighting, with different PPE induced at plant level, on photosynthesis, metabolic profiling and plant growth and flowering in two hybrids of *Ranunculus asiaticus* L., MBO and MDR. Three photoperiodic treatments were compared to natural day length (NL): fluorescence

light (FL, estimated PPE 0.84), LEDs red:far red light at 3:1 ratio (R:FR 3:1, estimated PPE, 0.84), and LEDs red:far red light at 1:3 ratio (R:FR 1:3, estimated PPE 0.63).

In plants grown under natural light, net photosynthesis was higher in MDR than in MBO, while no difference in Chlorophyll a fluorescence emission was observed between the hybrids. Compared to NL, photoperiodic treatments did not affect the rate of net photosynthesis, while they promoted the quantum yield of PSII (ΦPSII) and reduced the non-photochemical quenching (NPQ).

Under NL, plant growth was greater in MBO, while flowering started earlier in MDR and flower stems characteristics were similar in the hybrids. Photoperiodic treatments influenced plant growth and reduced the time for flowering only in MBO. Metabolic profiling enabled the identification of phenotypic variation associated with plants genotype and/or lighting conditions. In particular, MDR plants were all characterized by higher soluble sugars, polyphenols, photosynthetic pigments and proteins, while MBO plants by higher starch and amino acid content. These latter showed also higher number of leaves and leaf area, and root and leaf fresh and dry weight than MDR. Photoperiodic treatment R:FR 3:1 stimulated higher photosynthetic pigment concentration in both hybrids.

**Abbreviations**

ABA, abscisic acid; Chl, chlorophyll; EOD, end of day; FL, fluorescence lamp; FR, far red light; Fv/fm, maximal PSII photochemical efficiency; GA, gibberellic acid; GABA, γ aminobutyric acid; GAE, gallic acid equivalents; LD, long day; LEDs, light emitting diodes; MEA, ethanolamine; NL, natural day length; NP, net photosynthesis; NPQ, non-photochemical quenching; PPE, phytochrome photoequilibria; PCs, principal components; PHYB, phytochrome B; Pfr, phytochrome far red; Pr, phytochrome red; PPFD, Photosynthetic Photon Flux Density; R, red light; ROS, reactive oxygen species; ΦPSII, quantum yield of PSII electron transport.

**Introduction**

The influence of light quality on plant growth and photosynthesis and photomorphogenetic responses, including flowering has been well documented (Devlin et al., 2007; Dueck et al., 2016). Plants perceive changes in the light environment thanks to photoreceptors, specific for the detection of the different wavelengths, which dictate specific signals which regulate plant development and shaping and metabolism (Craig and Runkle, 2016).

Plant photoreceptors are classified in five families: phytochromes, phototropins, cryptochromes, ZTL/FKF1/LKP2 and UVR8 (Fukuda, 2019). Phytochromes, appointed for the absorption of red (R, 600-700 nm) and far red (FR, 700-800) light, are present in all plant tissues, in two forms: Phytochrome red (Pr), with a peak of absorption at 660 nm, and Phytochrome far red (Pfr), with a peak of absorption of 730 nm (Sager et al., 1988). R light converts the Pr to the Pfr form while FR light has an opposite effect, and the ratio between these two forms (Pfr/Ptot) determine the phytochrome photoequilibria (PPE) (Sager et al., 1988). Phytochrome ratio is involved in several morphological and physiological functions such as seed germination, flowering, tuberization, bud dormancy and shade-avoidance responses, ultimately influencing plant shaping (Fukuda, 2019).

Red and Far red light are perceived even at very low light intensity. In photoperiodic lighting, the threshold irradiance of R:FR lighting effective to regulate flower induction in some herbaceous species is between 0.05 and 0.40 μmol $m^{-2}$ $s^{-1}$, and the saturation irradiance varies between 0.2 and 1.0 μmol $m^{-2}$ $s^{-1}$ (Whitman et al., 1998). Accordingly, a light intensity between 1 to 3 μmol $m^{-2}$ $s^{-1}$ is usually suggested to growers for photoperiodic lighting (Zhang and Runkle, 2019).

The importance of the PPE value induced at plant level by R and FR light in the regulation of the flowering process of long day (LD) plants has been recently investigated, thanks to the diffusion of light emitting diodes (LEDs) in agricultural (Craig and Runkle, 2016; Dueck et al., 2016). Photoperiodic lighting with R and FR proportion creating an intermediate PPE (0.63-0.80) has been proved to be more effective to promote flowering in some LD species (*Antirrhinum majus*, *Fuchsia x hybrida*, *Petunia x hybrida*, *Rudbeckia hirta*) compared to a R and FR lighting creating an high PPE (above 0.80) (Craig and Runkle, 2016). However, light requirement in terms of intensity and quality can vary among the species and are not known for many crops.

*R. asiaticus* (family Ranunculaceae) is a perennial geophyte, native of the Mediterranean basin and Asia Minor, grown as annual crop for cut flowers and flowering potted plants (De Hertogh, 1996), whose cultivation increased exponentially in the last decades in Europe. In the wild Mediterranean environment, tuberous roots of *R. asiaticus* sprout in Autumn, when the first rain rehydrates the dried tissue, and develop a rosette of long petiole leaves (Horovitz, 1985). Floral induction occurs when six to eight leaves are formed, and flowering lasts from February to May, when roots dry and the aerial part wilts and the dormancy begins (Meynet, 1993). For production of propagation material, the harvested tuberous roots are dehydrated to less than 15% moisture (Meynet, 1993).

*R. asiaticus* exhibits a low temperature requirement (night/day regime 5-10/12-25°C) and a medium to high light intensity requirement. It is considered a quantitative LD plant (Horovitz, 1985). The earliness of flowering and the number of flowers vary in the genotypes and are affected by the size of the tuberous root: floral induction is earlier and flowers are more numerous in plants from greater compared to those from smaller tuberous roots, due to the greater quantity of reserve starch available for flower stem development (Meynet, 1993).

Flowering in *R. asiaticus* is a complex process, in which the thermal history of tuberous roots and the photoperiod play a crucial role. Cold treatments of tuberous roots (vernalization) anticipate sprouting, leaf rosette formation, and flowering (Meynet, 1993, Beruto et al., 2009). Consequently, the use of vernalized propagation material is a common practice in Italy for ranunculus, as well as for many flower geophytes, to anticipate flowering. In general, exposure to cold temperature allows the breaking of dormancy in bulbs by promoting the starch degradation and increasing the sucrose content (Wang et al., 2018). In addition, since dormancy in bulbs is regulated by the balance of abscisic acid (ABA) and gibberellic acid (GA) (Liu et al., 2011), vernalization releases dormancy by activating metabolic pathways involved in the down regulation of ABA and primes biosynthesis of active GA forms (Wang et al., 2018). However, a recent work from Carillo and coworkers (2019a) showed that vernalization is not only able to induce a faster hydrolysis of starch reserves in *R. asiaticus* L. tuberous roots, but it promotes a strong accumulation of hexoses and proline acting as compatible osmolytes and reactive oxygen species (ROS) scavengers. Moreover, the minor amino acids, whose accumulation is correlated to that of hexoses under stress (Fritz et al., 2006), can act as antioxidant and/or an

alternative electron donor for the mitochondrial electron transport chain accelerating plant metabolism and flowering (Woodrow et al., 2017; Carillo et al., 2018).

Earlier studies aimed to evaluate the response of *Ranunculus asiaticus* L. to photoperiod demonstrated that flowering was anticipated by long day, compared to short day, however, photoperiod interacted with temperature and the promoting effect of LD was null or detrimental when plants were grown at high temperatures (Farina, 1985; Ohkawa, 1986; De Pascale and Scogliamiglio, 1998).

To the best of our knowledge, no study is available in literature on the influence of light quality of photoperiodic lighting in *Ranunculus asiaticus* L. The objective of this study was to evaluate the influence of light spectrum of three light sources for photoperiodic lighting, with different PPE induced at plant level, on photosynthesis and PSII photochemical efficiency, metabolic profiling and plant growth and flowering in two hybrids with different flowering earliness.

**Materials and methods**

The experiment was carried out in Portici (Naples, Italy; 40°49' N latitude).

**Plant material and culture**

Plants of the hybrids MBO (early flowering) and MDR (medium earliness) (Biancheri Creazioni, Italy) were obtained from dry tuberous roots subjected to rehydration, followed by vernalization (exposure to 3.5 °C for 2 weeks), then pre-spouted at 12 °C for 2 weeks. Tuberous roots of the most common size for each cultivar were used (3-4 cm for MBO and 4-5 cm for MDR).

Plants were grown in pot, on a mixture of perlite and peat (70:30 in vol.), in a cold greenhouse from November 16, 2018 till March 21, 2019. The mean values of air temperature and relative humidity (day/night) recorded during the experimental period (125 days) were 21.0±3.0/9.8±1.9 °C and 59.9±10.7/71.4±21.7%, respectively (hourly measurements; Mean Value ± Standard Deviation).

Irrigation was alternated with fertigation (4 pulses per week in total). In the nutrient solution (recipe Hoagland full strength), pH and electrical conductivity were kept at 5.5 and 1.7 dS/m, respectively.

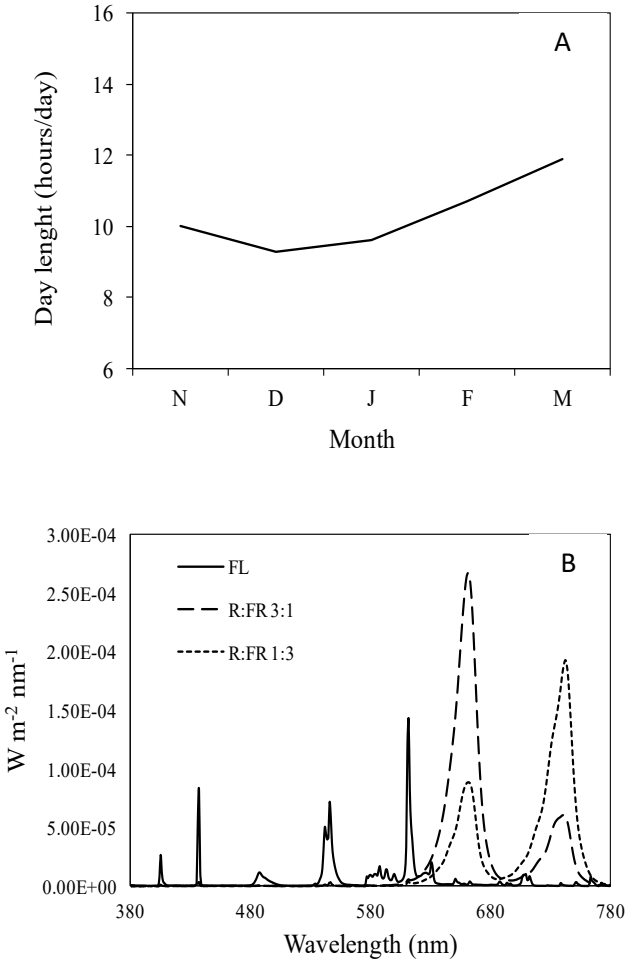
**Light sources and photoperiodic treatments**

Photoperiodic lighting was applied as end of day (EOD) lighting treatment to extend the day length to 14 hours per day (critical photoperiod for *R. asiaticus* L.), from December 10 (24 days after planting), when all the tuberous roots were fully sprouted, until the end of the experiment. The duration of lighting treatment to reach 14 hours day length was calculated on a weekly basis, starting from the sunset (Figure 1). Four photoperiodic treatments were compared:

- Natural day length (NL), ranging from 9:16 hours (2nd week of December) to 11:54 hours (3rd week of March; Figure 1);
- FL: NL+EOD lighting with a compact white fluorescence lamp (Phillips Master 13W, colour temperature 2700 °K), estimated PPE 0.84;
- R:FR 3:1: NL+EOD lighting with monochromatic R and FR LEDs at 3:1 ratio (emission peak at 662 nm and 743 nm, respectively), estimated PPE 0.84;
- R:FR 1:3: NL+EOD lighting with monochromatic R and FR LEDs at 3:1 ratio (emission peak at 662 nm and 743 nm, respectively), estimated PPE 0.63.

LEDs used for R:FR lighting treatments were FD-39R-Y 3W 740 nm and FD-33R-Y 3W 660 nm (Shenzhen Fedy Technology Co., LTD Guanlan Shenzhen 518110, China). The light emission spectra of the light sources were determined by an integrating sphere (1 cm diameter opening) coupled with a spectroradiometer OL770 (Optronic Lab. Inc., FL, United States) (Figure 1). In all photoperiodic lighting treatments, Photosynthetic Photon Flux Density (PPFD) at canopy level was 3-4 μmol $m^{-2}$ $s^{-1}$. The estimated PPE for each lighting treatment was calculated according to Sager et al. (1988).

Figure 1 - Natural day length at 40°49' N latitude used to calculate the duration of photoperiodic lighting to reach the photoperiod of 14 hours (A) and light emission spectra of light sources (B): FL: compact white fluorescence lamp (Phillips Master 13W, colour temperature 2700 °K); R:FR 3:1: monochromatic red and far red LEDs at ratio 3:1; R:FR 1:3: monochromatic red and far red LEDs at ratio 1:3.



**Net photosynthesis, Chlorophyll a fluorescence and leaf photosynthetic pigments**

Measurements of net photosynthesis (NP) and chlorophyll *a* fluorescence emission were performed on fully expanded leaves of plants at the 12$^{th}$ week after planting (phase of flowering), on 1 leaf per plants, in 4 plants per combination *hybrid x lighting treatment*.

NP was determined through an Infra-Red Gas Analyzer Walz HCM-1000 (Walz, Effeltrich, Germany). On the same leaves, chlorophyll *a* fluorescence emission was measured by and a portable pulse amplitude modulated fluorometer (MINI-PAM, Walz, Effeltrich, Germany). The measurement in the dark were conducted on 45 minutes dark-adapted leaves. The maximal PSII photochemical efficiency was calculated as the ratio of variable to maximal fluorescence Fv/Fm = (Fm−Fo)/Fm where Fv is the difference between the maximal and the minimal fluorescence (Fm-Fo). The measurements in the light were carried out under natural light on the same leaves. During NP and Chl a fluorescence measurements in the light, temperature was 26.8±2.8 °C, and Photosynthetic Photon Flux Density (PPFD) was on average 343.3±86.7 µmol $m^{-2}$ $s^{-1}$ at canopy level (Mean value ± Standard deviation, n = 64). The quantum yield of PSII electron transport ($\Phi_{PSII}$) was determined according to Genty et al. (1989). Non-photochemical quenching (NPQ) was calculated according to Bilger and Björkman (1990).

Photosyntetic pigments were extracted by homogenization of 10 mg lyophilized leaf tissues in 1 ml methanol according to Annunziata et al. (2013). The resulting extracts were centrifuged at 4.800 g for 15 min, and chlorophylls a and b and total carotenoids were estimated by measuring the absorbance of the supernatants at 470, 652 and 665 nm in polypropylene microplates by a microplate reader (Synergy HT, BioTEK Instruments, Bad Friedrichshall, Germany) according to Woodrow et al. (2017).

**Starch, soluble sugars, soluble proteins, amino acids and polyphenols in leaves**

Three fully expanded leaves per plant per 3 plants per combination *hybrid x lighting treatment* were sampled during the 15$^{th}$ week after planting (flowering phase). Leaves were immediately frozen in liquid nitrogen prior storage at –80°C and, before the analysis, leaves samples were frozen dried at –50°C for three days and powdered in a cooled mortar.

Starch and soluble sugars were extracted according to Ferchichi et al. (2018) with some modifications. Leaves frozen dry powdered samples (50 mg) were suspended in 300 mL of ethanol (98%, v $v^{-1}$), incubated for 20 min at 80 °C in a water bath and centrifuged at 14,000

g for 10 min at 4 °C. The clear supernatants were separated from the pellets and stored in 1 mL tubes at 4 °C. The pellets were then submitted to two subsequent extractions with 150 mL of 80% ethanol (v $v^{-1}$) and 150 mL of 50% ethanol (v $v^{-1}$). Each extraction was followed by an incubation for 20 min at 80 °C in a water bath and a centrifugation at 14,000 g, for 10 min at 4 °C. The supernatants of the first and the two following extractions were pooled and stored at -20 °C until analysis. For starch determination, the pellets of the ethanol extraction were heated at 90 °C for 2 h in 250 ml of 0.1 M KOH. After cooling the samples in ice, they were acidified to pH 4.5 with 70 ml of 1 M acetic acid. Hundred mL of 50 mM sodium acetate pH 4.8 containing 0.2 U a-amylase and 2 U amyloglucosidase were added to an aliquot of 100 mL of acidified samples and the starch was hydrolized at 37 °C for 18 h. The samples were vortexed and then centrifuged at 13,000 g for 10 min at 4 °C and the supernatant containing the glucose derived from hydrolyzed starch was used for measurement. Soluble glucose as well as glucose originating from starch hydrolysis were analyzed enzymatically by a FLX-Xenius spectrophotometer (SAFAS, Monaco) according to Woodrow et al. (2017). Amino acids were extracted according to Carillo et al. (2012) and measured according to Woodrow et al. (2017).

Soluble proteins were extracted by mixing 20 mg of lyophilized plant material with 0.1M Tris-HCl 200 mM pH 7.5 containing 500 mM $MgCl_2$ at 4°C for 24 h, the samples were vortexed and then centrifuged at 16,000g, for 10 min at 4 °C, and the clear supernatants separated from the pellets. Triplicate aliquots of 20 µL of extracts, as well as protein standards were dispensed into wells of a polypropylene microplate. The wells contained also 180 µL of Bio-Rad protein assay dye reagent diluted 1:5 with bidistilled water Woodrow et al. (2017). The solutions were mixed and then absorbance at 595 nm was recorded on a microplate reader (Synergy HT, BioTEK Instruments, Bad Friedrichshall, Germany). The absorbance of samples was referred to the calibration curve of protein standards used, and the concentration was calculated accounting for dilution factor.

The total polyphenols content was determined by the Folin-Ciocalteu method according to Singleton et al. (1999) with few modifications. Lyophilized tissues (30 mg) were extracted in 700 µl of 60% methanol (v $v^{-1}$); 35 µl of extract was mixed with 125 µl of the Folin-Ciocalteu reagent diluted with distilled water (1:4 v $v^{-1}$) and, after mixing for 6 minutes, 650 µl of 3% (v $v^{-1}$) sodium carbonate was added. After 90 min at room temperature, the absorbance at 760 nm was determined in a microplate reader (Synergy HT, BioTEK

Instruments, Bad Friedrichshall, Germany). The polyphenols concentration was expressed as gallic acid equivalents (GAE) as described in Carillo et al. (2019b). The analyses were carried out in three independent measurements and the results were expressed as mean values ± standard deviation.

**Plant growth and flowering**

Plant growth was measured during the 7$^{th}$ week after planting (vegetative phase) on 3 plants per combination *Hybrid x Lighting treatment*, as total number of leaves and total leaf area, estimated by image analysis of the all plant leaves with ImageJ software 1,50i version (Wayne Rasband National Institute of Health, USA).

The flowering process was studied as beginning of flowering (appearance of the first visible flower buds) and duration of flowering (100% of plants with flower buds), on 25 plants per combination *Hybrid x Lighting treatment.*

Flower stems were collected at the anthesis, and the date of appearance and the harvest were recorded. Flower stem length was measured with a ruler; flower stem diameter was measured twice with an electronic caliper and calculated as average of the two cross measurements.

Fresh weight and dry weight (after oven-drying for 48 hours at 70 °C) of roots and leaves and flower stems were measured with an electronic balance.

**Statistical analysis**

The experiment was conducted on 25 plants per combination *Hybrid x Lighting treatment.* Data were analysed by ANOVA using SPSS 25 software package (www.ibm.com/software/analytics/spss) and means were compared by Duncan *post-hoc* test at $P \leq 0.05$.

For all the analysed parameters, the principal component analysis (PCA) was conducted using Minitab 16.2.1 statistical software, aimed to extract trends when multiple qualitative variables were used, by formulating new variables correlated to the original ones (Ciarmiello et al., 2015). The PCA outputs included treatment component scores as well as variable loadings (Ferchichi et al., 2018).

The heat map was generated using the https://biit.cs.ut. ee/clustvis/ online program package with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.

## Results

### Net photosynthesis, Chlorophyll a fluorescence and leaf photosynthetic pigments

In plants of *R. asiaticus* grown under natural light, net photosynthesis was higher in the hybrid MDR compared to the hybrid MBO (Table 1).

The lighting conditions (day-length and light spectrum of photoperiodic treatment) did not affect the rate of net photosynthesis (Table 1).

The Chl *a* fluorescence measurements showed no significant difference between the two hybrids MBO and MDR as concerns the maximal PSII photochemical efficiency (Fv/Fm), the quantum yield of PSII electron transport ($\Phi_{PSII}$), and the non-photochemical quenching (NPQ). Conversely, the diverse lighting treatments determined statistically significant difference in ΦPSII and NPQ; more specifically NL treatment promoted NPQ and reduced ΦPSII in both hybrids; on the contrary photoperiodic lighting treatments reduced NPQ and increased ϕPSII compared to NL. In particular, the treatment R:FR 3:1 determined the strongest reduction of NPQ and the strong increase of ΦPSII (Table 1). No effect of different lighting treatments was found in Fv/Fm.

Interactions *Hybrid x Lighting treatment* (H x L) were found in $\Phi_{PSII}$ and NPQ. More specifically R:FR 1:3 reduced NPQ in MDR compared to MBO, whereas FL and NL promoted $\Phi_{PSII}$ in MBO compared to MDR (Table 1).

Table 1. Net photosynthesis (NP), Maximal PSII photochemical efficiency (Fv/Fm), quantum yield of PSII electron transport ($\Phi_{PSII}$), and non-photochemical quenching (NPQ) in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting treatments, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). Mean values ± Standard errors; n=4; Mean values ± Standard errors; n=3; ns and * indicate non-significant or significant difference at P <0.05. Week 12, phase of flowering.

| | NP | Fv/Fm | $\Phi_{PSII}$ | NPQ |
|---|---|---|---|---|
| MBO | | | | |
| NL | 4.42 ± 1.14 | 0.770 ± 0.019 | 0.546 ± 0.045 | 1.086 ± 0.137 |
| FL | 5.01 ± 0.62 | 0.804 ± 0.012 | 0.642 ± 0.018 | 0.476 ± 0.125 |
| R:FR 3:1 | 4.73 ± 1.01 | 0.770 ± 0.017 | 0.680 ± 0.008 | 0.254 ± 0.021 |
| R:FR 1:3 | 4.74 ± 0.44 | 0.796 ± 0.011 | 0.586 ± 0.048 | 0.951 ± 0.258 |
| *mean* | *4.72* | *0.785* | *0.613* | *0.692* |
| MDR | | | | |
| NL | 6.50 ± 1.35 | 0.786 ± 0.017 | 0.410 ± 0.033 | 1.323 ± 0.220 |
| FL | 6.59 ± 1.11 | 0.813 ± 0.010 | 0.559 ± 0.033 | 0.586 ± 0.102 |
| R:FR 3:1 | 7.04 ± 0.85 | 0.801 ± 0.005 | 0.676 ± 0.011 | 0.419 ± 0.103 |
| R:FR 1:3 | 6.67 ± 0.94 | 0.782 ± 0.006 | 0.594 ± 0.030 | 0.685 ± 0.146 |
| *mean* | *6.70* | *0.796* | *0.559* | *0.753* |
| Significance | | | | |
| Hybrid (H) | * | ns | ns | ns |
| Lighting treatment (L) | ns | ns | * | * |
| H x L | ns | ns | * | * |

**Starch, soluble sugars and amino acids in leaves**

MBO plants showed a higher starch (on avg. 19.0 and 14.0 mg $g^{-1}$ DW, respectively), and free amino acids (on avg. 12.4 and 6.7 μmol $g^{-1}$ DW, respectively) content than MDR. Total chlorophylls, carotenoids, polyphenols and soluble proteins were, on average, 2.1-, 2-, 1.2- and 1.4-fold higher in MDR than in MBO plants, respectively (Table 2). The hybrid did not significantly affect the content of soluble sugars ($P<0.05$), while the different light treatment induced glucose, fructose and sucrose from MDR plants to peak in NL, R:FR 3:1 and FL treated leaves, respectively.

.

Table 2. Chlorophyll *a*, *b*, total, Carotenoids, Soluble proteins, Starch, Glucose, Fructose, Sucrose and Polyphenols (in mg/g D.W.) in leaves of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). Mean values ± Standard errors; n=3. ns and * indicate non-significant or significant difference at P <0.05. Week 15, phase of flowering.

| | MBO | | | | | MDR | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NL | FL | R:FR 1:3 | R:FR 3:1 | *mean* | NL | FL | R:FR 1:3 | R:FR 3:1 | *mean* | Hybrid (H) | Lighting treatment (L) | H xL |
| Chlorophyll *a* | 0.060±0.007 | 0.049±0.006 | 0.041±0.002 | 0.061±0.007 | *0.053* | 0.108±0.003 | 0.121±0.006 | 0.065±0.006 | 0.149±0.015 | *0.110* | * | * | * |
| Chlorophyll *b* | 0.018±0.002 | 0.017±0.001 | 0.013±0.001 | 0.019±0.002 | *0.017* | 0.033±0.004 | 0.036±0.002 | 0.021±0.001 | 0.046±0.005 | *0.034* | * | * | * |
| Chlorophyll Total (*a*+*b*) | 0.078±0.009 | 0.066±0.006 | 0.053±0.003 | 0.080±0.009 | *0.069* | 0.141±0.006 | 0.157±0.007 | 0.085±0.007 | 0.195±0.020 | *0.145* | * | * | * |
| Carotenoids | 0.017±0.002 | 0.015±0.002 | 0.013±0.001 | 0.017±0.002 | *0.016* | 0.032±0.001 | 0.036±0.002 | 0.020±0.002 | 0.040±0.004 | *0.032* | * | * | * |
| Soluble proteins | 18.861±0.352 | 15.573±1.328 | 18.809±0.485 | 19.819±0.674 | *18.266* | 30.393±0.294 | 25.905±0.835 | 19.626±0.357 | 24.481±1.117 | *25.101* | * | * | * |
| Starch | 17.939±0.319 | 18.159±1.689 | 23.635±1.496 | 16.303±0.874 | *19.009* | 11.238±1.186 | 15.833±0.335 | 15.115±0.446 | 13.758±0.850 | *13.986* | * | * | * |
| Glucose | 33.391±0.489 | 33.210±1.009 | 34.659±0.073 | 25.826±0.535 | *31.771* | 36.764±0.605 | 34.790±0.356 | 33.578±2.606 | 26.281±0.358 | *32.853* | ns | * | * |
| Fructose | 22.186±0.296 | 23.499±0.548 | 25.952±0.180 | 25.070±0.571 | *24.177* | 24.417±0.412 | 26.413±0.182 | 27.183±0.899 | 27.614±0.435 | *26.407* | * | * | * |
| Sucrose | 5.775±0.090 | 6.137±0.048 | 6.942±0.082 | 6.792±0.188 | *6.412* | 6.453±0.152 | 7.681±0.071 | 6.550±0.218 | 8.084±0.023 | *7.192* | * | * | * |
| Polyphenols | 9.968±0.074 | 9.752±0.247 | 10.238±0.165 | 9.561±0.140 | *9.880* | 11.997±0.326 | 11.431±0.013 | 13.670±0.699 | 10.898±0.110 | *11.999* | * | * | * |

The free amino acids profile was affected by the hybrid and the lighting treatment (Table 3). In particular, in MBO plants some amino acids strongly increased compared to MDR under the following treatments: i) FL treatment, asparagine, α aminobutyric acid (GABA), glutamine, glycine, serine and threonine (+12.6-, 2.8-, 4.5-, 7.7-, 4.8- and 8.6-fold, respectively); ii) NL treatment, glycine and threonine (+3.3- and 9.2-fold, respectively); iii) R:FR 1:3 treatment, GABA, glycine, histidine, ornithine, threonine and tryptophan (+3.5-, 11.2-, 6.7-, 3.7-, 5.2- and 6.7-fold, respectively) and iv) R:FR 3:1 treatment, asparagine, GABA, histidine, serine and tryptophan (+8.7-, 3.4-, 7.0-, 5.7- and 6.0-fold, respectively). Minor amino acids (the sum of arginine, isoleucine, histidine, leucine, lysine, methionine, phenylalanine, tyrosine, tryptophan and valine) accounted, on average, for 7.4 and 9.2 % of total amino acids in MBO and MDR plants, respectively; however, they got the 9,2% also in MBO plants under R:FR 3:1 treatment.

Table 3. Free amino acid content (µmol/g D.W.) in leaves of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). Mean values ± Standard errors; n=3. ns and * indicate non-significant or significant difference at P <0.05. Week 15, phase of flowering.

| | MBO | | | | | MDR | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NL | FL | R:FR 1:3 | R:FR 3:1 | *mean* | NL | FL | R:FR 1:3 | R:FR 3:1 | *mean* | Hybrid (H) | Lighting treatment | H x L |
| Alanine | 4.143±0.508 | 5.622±0.599 | 7.499±0.448 | 8.404±0.396 | *6.417* | 4.644±0.295 | 2.892±0.439 | 4.015±0.690 | 4.499±0.365 | *4.012* | * | * | * |
| Arginine | 2.096±0.327 | 3.331±0.347 | 3.373±0.305 | 6.455±0.608 | *3.814* | 2.533±0.179 | 1.695±0.236 | 1.833±0.231 | 2.349±0.265 | *2.102* | * | * | * |
| Asparagine | 2.137±0.308 | 17.732±2.130 | 5.093±0.336 | 20.953±3.369 | *11.479* | 3.955±0.273 | 1.506±0.238 | 2.184±0.150 | 2.477±0.427 | *2.531* | * | * | * |
| Aspartate | 5.662±0.796 | 4.216±0.387 | 5.553±0.301 | 3.896±0.591 | *4.832* | 4.270±0.203 | 4.266±0.603 | 3.475±0.606 | 4.785±0.618 | *4.199* | ns | ns | ns |
| GABA | 3.572±0.593 | 3.060±0.533 | 7.085±0.797 | 3.306±0.245 | *4.256* | 3.377±0.286 | 1.117±0.065 | 2.118±0.183 | 1.017±0.136 | *1.907* | * | * | * |
| Glutamate | 13.468±2.061 | 10.243±1.066 | 16.220±0.701 | 10.839±1.622 | *12.692* | 13.572±0.940 | 16.077±2.297 | 13.366±2.381 | 13.208±2.328 | *14.056* | ns | ns | ns |
| Glutamine | 16.592±2.252 | 29.344±3.138 | 33.462±1.487 | 37.423±5.511 | *29.205* | 20.666±1.405 | 6.881±0.975 | 9.180±1.601 | 14.898±1.858 | *12.906* | * | * | * |
| Glycine | 0.609±0.078 | 2.058±0.131 | 1.573±0.045 | 0.700±0.064 | *1.235* | 0.188±0.017 | 0.270±0.015 | 0.148±0.021 | 0.430±0.060 | *0.259* | * | * | * |
| Histidine | 0.478±0.067 | 0.461±0.043 | 0.792±0.055 | 0.869±0.112 | *0.650* | 0.385±0.029 | 0.375±0.053 | 0.121±0.019 | 0.127±0.017 | *0.252* | * | ns | * |
| Isoleucine | 0.356±0.063 | 0.656±0.062 | 0.456±0.017 | 1.205±0.366 | *0.668* | 0.476±0.033 | 0.322±0.042 | 0.323±0.053 | 0.522±0.058 | *0.411* | * | * | * |
| Leucine | 0.642±0.092 | 0.897±0.082 | 0.946±0.046 | 1.133±0.151 | *0.904* | 0.542±0.035 | 0.405±0.057 | 0.536±0.089 | 0.444±0.050 | *0.482* | * | ns | * |
| Lysine | 0.744±0.070 | 0.598±0.061 | 0.869±0.078 | 0.981±0.073 | *0.798* | 0.364±0.026 | 0.331±0.058 | 0.353±0.030 | 0.453±0.054 | *0.375* | * | * | * |
| MEA | 2.269±0.553 | 1.867±0.279 | 3.797±0.293 | 3.597±0.271 | *2.882* | 2.724±0.236 | 2.940±0.178 | 2.809±0.245 | 3.948±0.547 | *3.105* | ns | * | * |
| Methionine | 0.152±0.022 | 0.214±0.020 | 0.275±0.012 | 0.354±0.049 | *0.249* | 0.165±0.011 | 0.103±0.015 | 0.173±0.031 | 0.130±0.015 | *0.143* | * | * | * |

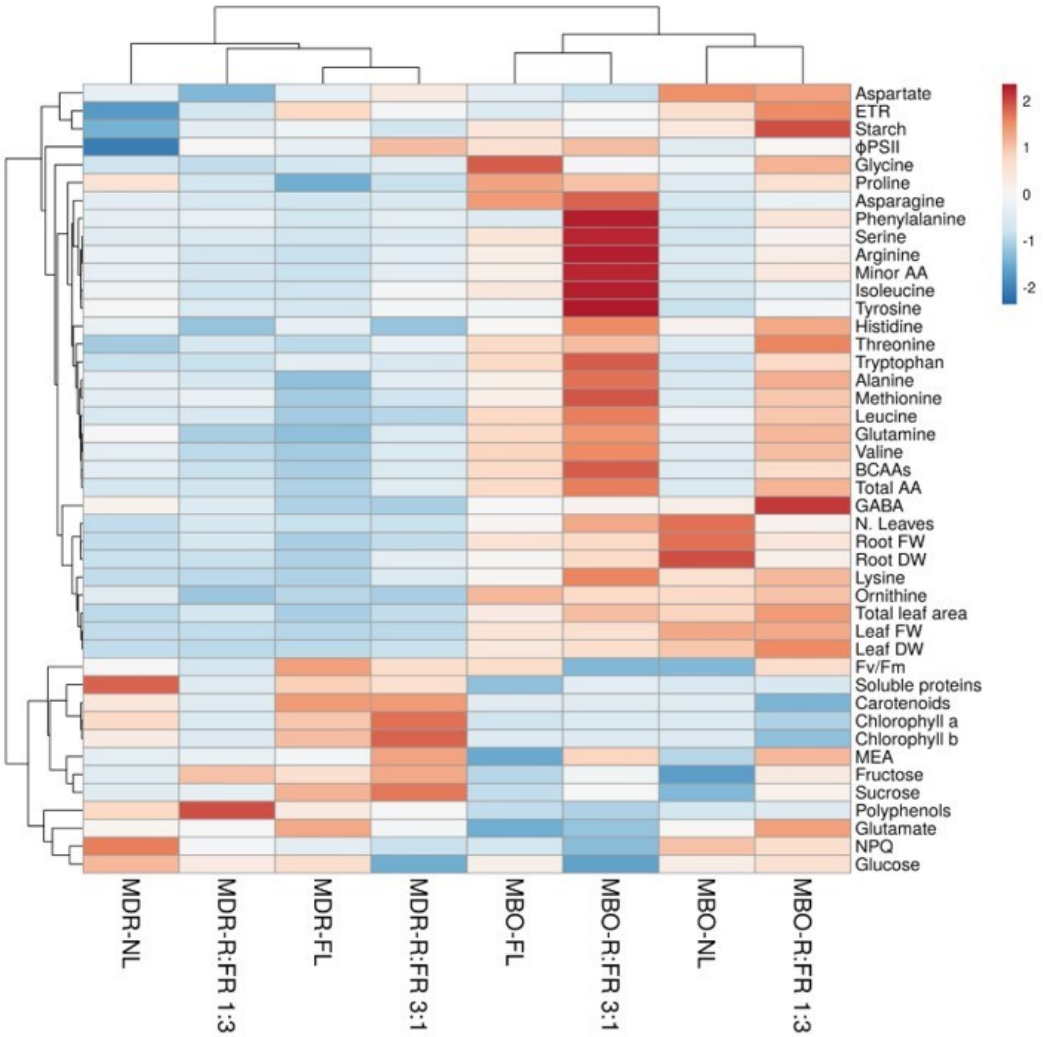
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ornithine | 0.308±0.050 | 0.346±0.021 | 0.344±0.007 | 0.311±0.049 | *0.327* | 0.177±0.016 | 0.118±0.016 | 0.096±0.013 | 0.112±0.011 | *0.126* | * | ns | * |
| Phenylalanine | 0.283±0.061 | 0.303±0.030 | 0.419±0.040 | 0.634±0.099 | *0.410* | 0.319±0.022 | 0.282±0.035 | 0.331±0.058 | 0.319±0.057 | *0.313* | * | * | * |
| Proline | 3.391±0.113 | 5.739±0.163 | 4.776±0.156 | 5.371±0.062 | *4.819* | 4.707±0.063 | 1.980±0.060 | 3.063±0.123 | 2.907±0.061 | *3.164* | * | ns | * |
| Serine | 5.090±0.728 | 20.823±3.080 | 15.510±1.436 | 43.276±3.103 | *21.175* | 8.733±0.634 | 4.806±0.833 | 7.515±0.613 | 8.124±1.370 | *7.295* | * | * | * |
| Threonine | 21.693±3.802 | 56.415±6.445 | 81.489±3.777 | 65.519±10.707 | *56.279* | 2.628±0.422 | 7.230±1.256 | 15.827±1.282 | 26.797±3.890 | *13.120* | * | * | * |
| Tryptophan | 0.077±0.010 | 0.458±0.034 | 0.448±0.018 | 0.738±0.106 | *0.430* | 0.069±0.004 | 0.189±0.022 | 0.068±0.005 | 0.126±0.012 | *0.113* | * | * | * |
| Tyrosine | 0.868±0.238 | 2.034±0.282 | 2.122±0.037 | 6.200±0.834 | *2.806* | 2.176±0.160 | 1.067±0.150 | 1.372±0.108 | 2.087±0.246 | *1.676* | * | * | * |
| Valine | 1.011±0.136 | 1.706±0.156 | 1.818±0.073 | 2.089±0.276 | *1.656* | 1.077±0.074 | 0.655±0.093 | 0.755±0.122 | 0.959±0.104 | *0.862* | * | * | * |
| BCAAs | 2.009±0.284 | 3.259±0.300 | 3.220±0.132 | 4.427±0.790 | *3.229* | 2.095±0.142 | 1.382±0.191 | 1.614±0.264 | 1.925±0.213 | *1.754* | * | * | * |
| Minor AA | 6.708±1.045 | 10.659±1.111 | 11.519±0.423 | 20.657±1.946 | *12.386* | 8.104±0.573 | 5.424±0.757 | 5.867±0.738 | 7.516±0.848 | *6.728* | * | * | * |
| Total AA | 85.64±12.48 | 168.12±18.67 | 193.92±8.53 | 224.25±27.28 | *167.98* | 77.74±5.24 | 55.51±7.61 | 69.66±8.36 | 90.72±11.33 | *73.41* | * | * | * |

**Principal Component Analysis and Heat map of the analysed leaf parameters**

To obtain an in-depth overview of the morpho-physiological and biochemical profile of the two hybrids under the different treatments, a heat map and a principal component analysis (PCA) were conducted for all the above-mentioned measured parameters.

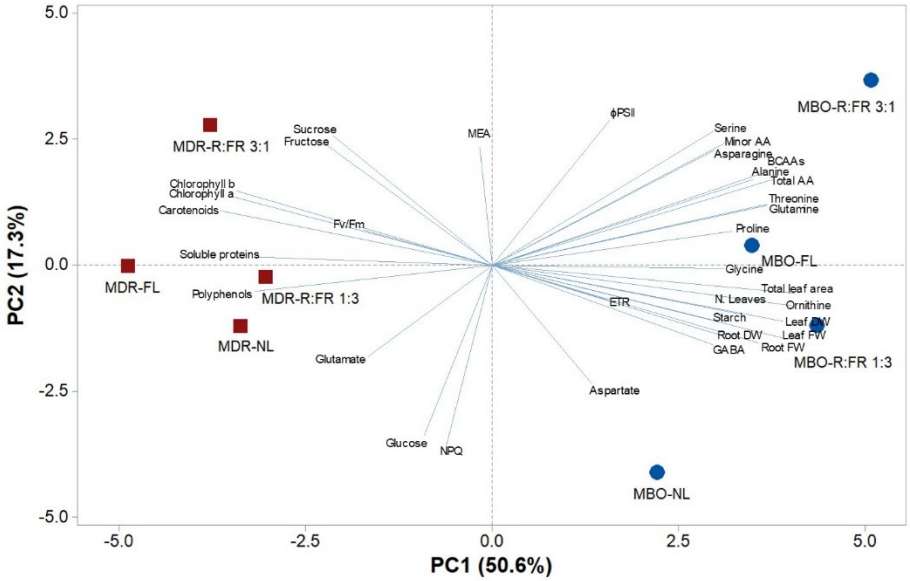
The aggregated data heat-map analysis identified two main clusters corresponding to the hybrids MBO and MDR, respectively (Figure 2). Separated sub-clusters could be defined under the first and second cluster, which fundamentally depended on the light treatments. In the cluster on the right, MBO-NL and MBO-R:FR 1:3 clustered separately from MBO-FL and MBO- R:FR 3:1. While the cluster on the left related to MDR samples, showed a first sub-cluster including only MDR-NL, and then a second sub-cluster which was further sub-clustered with MDR-R:FR 1:3 on the left, close to MDR-NL, and the other two treatments on the right. This indicated that the hybrid was the main clustering factor, while the light treatment was the second one. The main cluster on the left, related to MDR samples, was separated by the cluster on the right because of the lower fresh and dry weight of roots and leaves, lower leaf area, number of leaves, starch and amino acid content (except for glutamate), but higher soluble sugars, polyphenols, chlorophylls, carotenoids and proteins content. In particular, in the MDR cluster, the condition R:FR 3:1 showed the highest sucrose, fructose, chlorophylls, and MEA content, FL the highest Fv/Fm, R:FR 1:3 the highest polyphenols and NL the highest NPQ. While in the main MBO cluster on the right, the treatments R:FR 1:3 and R:FR 3:1 were particularly evident because the former accumulated the highest starch and GABA content, while the latter the highest total amino acids content, and in particular minor amino acids including BCAAs, showing also the highest leaf fresh and dry weight and leaf area. The R:FR 3:1 treatment in both hybrids induced higher rates of photosynthesis.

Figure 2 - Cluster heat map analysis summarizing all the results obtained by the analysis of data on *Ranunculus asiaticus* L. hybrids MBO and MDR, , grown under four photoperiodic lighting treatments, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). It was generated using the https://biit.cs.ut.ee/clustvis/ online program package with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.



The PCA of all analyzed parameters highlighted that the first three principal components (PCs) were related with eigen values higher than 1 and explained 79.6% of the total variance, with PC1, PC2 and PC3 accounting for 50.6%, 17.3% and 11.7%, respectively (data not shown). The type of hybrid contributed to the clear separation on PC1, while the light treatment contributed to the separation on PC2 (Figure 3). MDR treatments were concentrated in negative side of PC1 in the upper and lower left quadrant, closed to the x axis, while MBO treatments were in the positive side of PC1. MBO-NL, MBO-R:FR 1:3 were in the lower right quadrant, with in particular the former in the negative side of PC2. While, and MBO-FL and MBO-R:FR 3:1 in the positive side of PC2 in the upper right quadrant (Figure 3). PC1 was positively correlated to total amino acids, glutamine, minor amino acids, BCAAs, leaf area, leaf fresh and dry weight. PC1 was also negatively correlated with carotenoids, chlorophylls, polyphenols and soluble proteins. PC2 was positively correlated with $\Phi_{PSII}$, sucrose, fructose, serine and MEA. While it was negatively correlated to NPQ, glucose, aspartate, glutamate and GABA. In addition, the MBO-R:FR 3:1 treatment showed the highest minor amino acids content, in particular BCAAs, and MBO-NL treatment had the highest fresh and dry root weight and number of leaves. While MDR-R:FR 3:1 treatment had the highest photosynthetic pigments and polyphenols content (Figure 3).

Figure 3 - Principal component loading plot and scores of principal component analysis (PCA) of all the parameters analyzed in samples of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting treatments, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3).



**Plant growth and flowering**

In plants grown under natural light conditions, the number of leaf rosettes at the end of the vegetative phase was higher in the hybrid MBO compared to the hybrid MDR (5 vs 2 rosettes per plant, data not shown). Accordingly, the number of leaves, the total leaf area, and the leaf dry matter accumulation were higher in MBO compared to MDR (Table 4). Compared to natural light conditions, EOD photoperiodic treatments influenced plant growth differently in the two hybrids (Table 2). Indeed, a significant interaction *Hybrid x Lighting treatment* was found in the number of leaves, which was reduced significantly under fluorescent light and R:FR 1:3 light compared to NL control in MBO plants, while it was unaffected by lighting tratments in MDR plants (Table 4).

Table 4. Plant growth in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). Mean values ± Standard errors; n=3; ns and * indicate non-significant or significant difference at P <0.05. Week 7, full grown plants at the end of vegetative phase.

| | Number of leaves (N./plant) | Total leaf area ($cm^2$/plant) | Leaves fresh weight (g/plant) | Leaves dry weight (g/plant) | Root fresh weight (g/plant) | Root dry weight (g/plant) |
|---|---|---|---|---|---|---|
| MBO | | | | | | |
| NL | 36.3 ± 4.9 | 274.1 ± 25.7 | 15.31 ± 1.79 | 1.52 ± 0.07 | 5.72 ± 0.80 | 0.41 ± 0.031 |
| FL | 21.0 ± 1.5 | 218.4 ± 14.0 | 11.74 ± 1.76 | 1.24 ± 0.11 | 4.07 ± 0.60 | 0.24 ± 0.045 |
| R:FR 3:1 | 32.0 ± 3.5 | 300.4 ± 46.3 | 12.15 ± 1.30 | 1.36 ± 0.19 | 4.32 ± 0.54 | 0.30 ± 0.047 |
| R:FR 1:3 | 21.7 ± 3.4 | 335.6 ± 51.6 | 15.21 ± 0.17 | 1.81 ± 0.22 | 3.96 ± 0.45 | 0.25 ± 0.035 |
| *mean* | *27.8* | *282.1* | *13.60* | *1.48* | *4.52* | *0.299* |
| MDR | | | | | | |
| NL | 12.3 ± 0.7 | 86.1 ± 8.8 | 5.34 ± 0.60 | 0.61 ± 0.10 | 2.15 ± 0.19 | 0.16 ± 0.021 |
| FL | 13.0 ± 1.5 | 69.9 ± 5.6 | 4.86 ± 0.54 | 0.59 ± 0.08 | 1.90 ± 0.24 | 0.14 ± 0.012 |
| R:FR 3:1 | 12.7 ± 3.8 | 93.0 ± 15.7 | 5.06 ± 1.07 | 0.62 ± 0.16 | 2.14 ± 0.53 | 0.20 ± 0.050 |
| R:FR 1:3 | 14.7 ± 1.8 | 113.4 ± 20.2 | 5.22 ± 1.03 | 0.57 ± 0.11 | 2.44 ± 0.51 | 0.16 ± 0.030 |
| *mean* | *13.2* | *90.6* | *5.12* | *0.60* | *2.16* | *0.16* |
| Significance | | | | | | |
| Hybrid (H) | * | * | * | * | * | * |
| Lighting treatment (L) | ns | * | ns | ns | ns | ns |
| H x L | * | * | * | * | * | * |

Under NL, flowering started earlier in MDR than in MBO (47 vs 60 days after planting), while the time to complete flowering (100% of plants with visible flower buds) was shorter in MBO (34 vs 76 days in MDR). Flower stems characteristics were similar in the two hybrids (Table 5).

Compared to NL, in MBO all photoperiodic treatments anticipated flowering (-5 days on average in FL and R:FR 1:3 and -8 days in R:FR 3:1), and both R:FR ratios also decreased the time to complete it (-13 days on average); in MDR photoperiodic lighting had no significant effect on earliness but always reduced the time to complete flowering (-29 days on average). R:FR 3:1 increased stem diameter and dry weight in MBO (Table 5).

Table 5. Flower stem biometrics in plants of *Ranunculus asiaticus* L. hybrids MBO and MDR, grown under four photoperiodic lighting treatments, Natural day length (NL), Natural day length + photoperiodic lighting with fluorescence light (FL), Natural day length + photoperiodic lighting with LEDs R:FR 3:1 (R:FR 3:1), Natural day length + photoperiodic lighting with LEDs R:FR 1:3 (R:FR 1:3). Mean values ± Standard errors; n=10; ns and * indicate non-significant or significant difference at P <0.05.

| | Stem length (cm) | Stem diameter (mm) | Flower diameter (mm) | Stem fresh weight (g/ stem) | Stem dry weight (g/ stem) |
|---|---|---|---|---|---|
| MBO | | | | | |
| NL | 51.15±1.40 | 3.34±0.18 | 69.04±2.37 | 7.12±0.66 | 0.94±0.06 |
| FL | 54.03±2.08 | 3.78±0.24 | 65.80±4.00 | 6.97±0.58 | 0.91±0.08 |
| R:FR 3:1 | 55.09±1.99 | 4.19±0.24 | 68.54±2.72 | 8.78±1.10 | 1.16±0.14 |
| R:FR 1:3 | 56.29±1.35 | 3.59±0.24 | 67.50±2.99 | 7.16±1.06 | 0.76±0.12 |
| *mean* | *54.14* | *3.72* | *67.72* | *7.51* | *0.94* |
| MDR | | | | | |
| NL | 57.83±1.80 | 3.17±0.20 | 60.44±5.35 | 8.06±1.00 | 1.09±0.14 |
| FL | 57.69±1.56 | 3.86±0.29 | 67.89±1.31 | 8.41±0.48 | 1.24±0.04 |
| R:FR 3:1 | 62.42±3.73 | 3.35±0.25 | 62.32±1.51 | 8.13±0.89 | 1.09±0.10 |
| R:FR 1:3 | 64.21±2.35 | 3.55±0.23 | 62.40±4.43 | 8.60±1.13 | 1.24±0.17 |
| *mean* | *60.54* | *3.48* | *63.26* | *8.30* | *1.17* |
| Significance | | | | | |
| Hybrid (H) | * | ns | ns | ns | ns |
| Lighting treatment (L) | * | * | ns | ns | ns |
| H x L | * | * | ns | ns | * |

**Discussion and conclusions**

Measurements of leaf net photosynthesis in plants of *Ranunculus asiaticus* grown in greenhouse under natural light confirmed a higher photosynthetic capacity in MDR compared to MBO, we already observed in the same hybrids grown in growth chamber under fluorescence light, in controlled environment (Carillo et al., 2019a). However, photosynthesis measured in this experiment in plants at the flowering phase (week 12), grown in greenhouse under natural light, was higher compared to those of young plants (week 4) of the same hybrids cultivated in growth chamber under white fluorescent light (200 mmol $m^{-2}$ $s^{-1}$ at the canopy level). This result is presumably due to the plant adaptation to the different light environments, with lower photosynthetic rate under lower radiation intensity, as well as to the lower assimilation capacity known for several species in the young developmental stage (Paradiso et al., 2018).

The lighting conditions (day length and light spectrum of photoperiodic treatment) did not affect the rate of net photosynthesis, even though the high intrinsic variability within each treatment could have masked some differences.

Chlorophyll *a* fluorescence measurements indicate a good health status of photosynthetic apparatus in *R. asiaticus* plants grown under the different lighting regimens; in fact, the Fv/Fm ratio was always close to 0.83, the typical value for plants without any physiological stress (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004).

In the present experiment no significant difference in photochemistry was found between the two hybrids, conversely to our previous experiment in a growth chamber under fluorescence white light, where PSII photochemical efficiency was higher in MBO compared to MDR (Carillo et al., 2019a). This result could depend on genetic reasons and it has been ascribed to the higher potential capability of MBO to convert light energy to reaction centers. As the health status of plants was comparable under the four lighting treatments, it is reasonable to deduce that the day length and the light spectrum of photoperiodic lighting did not affect photosystem capability to utilise the harvested light.

Our data demonstrate that natural light conditions (NL) promoted thermal dissipation processes within photosynthetic apparatus and reduced photochemistry in both hybrids, compared to photoperiodic lighting. Conversely, the EOD photoperiodic treatments enhanced the photochemical processes compared NL, regardless of the emission spectrum

of the light source, thus reducing the need for thermal dissipation at photosystem level (Johnson et al., 1993; Maxwell and Johnson, 2000). The greater thermal dissipation in NL plants represents a safety mean to avoid the occurrence of photoinhibitory damages risks to photosystems, ensuring their integrity and functionality. The strong increase of thermal dissipation in NL plants can be interpreted as a compensation mechanism for the reduced PSII photochemical efficiency and may represent a safety mean to prevent photoinhibitory damages risks, ensuring photosystem integrity and functionality. The absence of stress conditions is confirmed by the Fv/Fm values that in NL and all photoperiodic treatments are comparable and near the to the threshold for unstressed plants (De Micco et al., 2011). Among the three photoperiodic treatments, R:FR 3:1 seems to be the most efficient in guaranteeing the best photochemical efficiency and then the highest yield. This suggests that the spectral composition rather than the PPE affects plant photosynthetic efficiency, as shown by the best fluorescence indexes under R:FR 3:1 compared to FL, although the PPE induced at plant level was the same in the two treatments. It is known that the high R:FR light present in open spaces, where leaves do not shade each other, enhances stomata density and amphistomy, facilitating the transpiration and the diffusion of $CO_2$, the photosynthetic efficiency and leaf cooling (Mott et al., 1982; Boccalandro et al., 2009). Accordingly, photoperiodic treatment R:FR 3:1 significantly increased the quantum yield of PSII in both *R. asiaticus* hybrids while reducing the amount of excitation energy not used for photochemistry and dissipated as heat, as proved by the lower NPQ.

NL and, even more, R:FR 3:1 light increased the content of chlorophylls in both hybrids, in particular in MDR plants. Moreover, photoperiodic treatment R:FR 3:1 light condition increased also fructose and sucrose content, in addition to photosynthetic pigments and ethanolamine (MEA) in MDR plants. The higher concentration of chlorophylls and recent photosynthates well matched with the increase of photosynthetic rate previously discussed in MDR plants under the same light conditions. In addition, the increase in MEA, precursor of phosphatidylcholine and phosphatidylethanolamine, which are the most abundant glycerophospholipids in plants with a fundamental role in membranes structure and function, represents a proof of an ongoing chloroplast ultrastructure regeneration. Luo et al. (2013) demonstrated that an active reconstruction of the chloroplast ultrastructure can delay leaf senescence, enhancing photosynthetic capacity. These data are in agreement with those

reviewed by Demotes-Mainard et al. (2016), showing that high R:FR or increased expression of *PHYB* increase foliar chlorophyll content per unit area and leaf duration in many species because of the reduction of the progressive loss of chlorophyll and photosynthetic proteins. However, Demotes-Mainard et al. (2016) reported also that contrasting responses related to photosynthetic pigments may depend on variability in growing conditions and between species and/or genotypes, as revealed by the differences between MDR and MBO plants under the different light treatments. This metabolic profile completely changed under low R:FR light treatment in MDR plants, which were characterized only by a very high polyphenol content. While, MBO plants showed an opposite metabolic profile in comparison with MDR ones, with a strong increase of total amino acids content independently of light treatment. In particular, the photoperiodic treatment R:FR 3:1 increased minor amino acids included BCAAs, which may actively function as antioxidants and alternative electron donors for the mitochondrial electron transport chain (Woodrow et al., 2017 and references therein), limiting the occurrence of photo-oxidative damages. However, their increase appeared unrelated to the levels of hexoses, differently from Fritz et al. (2006) and Carillo et al. (2019a). In EOD R:FR 1:3 light treatment, on the contrary, the increase of total amino acids was due to primary amino acids and GABA. Probably the increase of GABA may be related to the lower efficiency of PSII and the higher NPQ. The excitation energy not channelled towards photochemistry and only in part dissipated as heat can be responsible for the production of ROS (Boccalandro et al., 2009 and references therein). In this scenario, GABA can be rapidly synthesized for buffering pH, scavenging ROS and stabilizing membranes and macromolecules. Moreover, this metabolite can be broken down soon after the relief of stress to supply energy, carbon and nitrogen to the damaged tissues (Carillo, 2018). These plants showed also the highest starch content, proving that notwithstanding the photosynthesis was working quite efficiently and well protected by GABA, they did not break down it to produce new proteins to invest in new leaves. Since low R:FR is perceived by plants as a signal of shade conditions, presumably due to high plant density environments as those of forests, prairies or orchard communities, plants enact a ubiquitous mechanism known as “shade-avoidance syndrome” characterized by an enlargement of leaves already present, and an acceleration of flowering (Martínez-García et al., 2014).

In this experiment, significant changes in morphological and physiological traits of *R. asiaticus* plants were related to both genotype and light quality of photoperiodic treatment.

Plant growth, in terms of number of leaves, plant leaf area, leaves and roots fresh and dry weight at the end of vegetative phase was higher in MBO than in MDR, confirming the occurrence of intrinsic growth difference between the two genotypes, in agreement with the results of our previous study on the same hybrids (Carillo et al., 2019a).

Photoperiodic lighting affected plant growth and flowering earliness only in MBO plants, with different effects on leaf formation and expansion and flowering anticipation depending on the lighting source spectrum. In fact, plants under FL and R:FR 1:3 light developed a fewer number of leaves, albeit with similar total leaf area compared to NL: this may be linked to the inhibitory effect of blue radiation in the emission spectrum of FL lamps, and has been reported as effect of the addition of FR to light spectrum in lettuce (Meng and Runkle, 2019a). Compared to white FL, R:FR 1:3 determined larger total leaf area per plant, with similar number of leaves. Indeed, the addition of FR to the natural light spectrum (reducing the PPE value at plant level) increases the plants leaf area by increasing the leaf expansion (Li and Kubota, 2009; Park and Runkle, 2017, 2018; Fukuda, 2019; Meng and Runkle, 2019). Accordingly, Annunziata et al. (2017) found that higher R:FR ratios induces an increased expression of phytochrome B (*PHYB*), reducing phytochrome signalling and phytochrome-dependent processes such as leaf development and circadian clock. Generally, as the photoperiod and FR intensity increase, linearly plant height and stem length increase (Zhang and Runkle, 2019). A low R:FR ratio can trigger shade-avoidance responses promoting apical dominance and so stem elongation by increasing auxin and gibberellic acid accumulation, this response is mediated through phytocrome B in antagonism with phytocrome A (Franklin, 2008a); in our experiment flower stems of both the hybrids were significantly elongated as the FR in the photoperiodic light spectrum increased.

In our experiment we observed intrinsic differences in flowering time between the two *R. asiaticus* hybrids, regardless the lighting conditions, in accordance to the technical information provided by the Breeder (https://www.bianchericreazioni.it/), and also a genotype specific response to EOD photoperiodic lighting. Indeed, photoperiodic treatments were effective to anticipate flowering only in MBO plants, even though flowering occurred also in plants under the natural short-day condition, confirming *R. asiaticus* as a quantitative

long-day plant able to flower in short-day (Horovitz, 1985; Ohkawa, 1986). The spectral emission of the light source influenced the time of appearance of the first visible flower bud, with stronger advance under R:FR 3:1 light (estimated PPE at plant level 0.84). This results does not confirm for *R. asiaticus* the evidence obtained in several other LD species (such as snapdragon and *Petunia*), in which flower induction was better promoted by PPE lower than 0.80 (Craig and Runkle, 2016). However, it has been hypothesized that different mechanisms involving the amount of the Pfr phytocrome form and possibly threshold values for flower induction or inhibition, can exist in the different plant species (Thomas and Vince-Prue, 1997)

The heat map and PCA carried out in this work facilitated a broad view of morpho-physiological and biochemical traits and enabled the identification of phenotypic variation patterns associated with *Ranunculus* genotype and/or lighting conditions, as evidenced also for other species (Carillo et al., 2019 a, b).

In conclusion, under natural light, the two *R. asiaticus* L. hybrids showed intrinsic differences in photosynthetic behaviour, plant growth and flowering rate. Hybrid-specific response to photoperiodic light spectrum in flowering time and quality should be taken into account to optimize lighting protocols and production scheduling.

**Author´s contributions**

RP and SDP proposed the experiment GCM carried out plant cultivation GP customized the LEDs panels RP, CA and GCM performed photosynthesis and Chlorophyll a fluorescence measurements PC, EDA and GMF performed pigments and metabolic analyses All the Authors contributed to writing the manuscript.

# Chapter 4

# Blue and Red light modulation and different FR light intensities affect growth, physiology and quality of leafy vegetables

Giuseppe Carlo Modarelli[1], Charlotte Grootaert[2], John Van Camp[2], Roberta Paradiso[1], Carmen Arena[3], Marie Christine Van Labeke[4*]

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici, Naples (Italy)

[2] Faculty of Bioscience Engineering, Department of Food Technology, Safety and Health, Ghent University, Coupure Links 653, B-9000, Ghent (Belgium)

[3] Department of Biology, University of Naples Federico II, Via Cinthia 4, 80126, Naples (Italy)

[4] Faculty of Bioscience Engineering, Department of Plant and Crops, Ghent University, Coupure links 653, B-9000, Ghent (Belgium)

*Correspondence: mariechristine.vanlabeke@ugent.be

*Keywords:* vertical farming, antioxidant compounds, leaf vegetation indexes, photosynthesis

***Abstract***

Light is a key factor for plants development because it drives the photosynthetic process, growth and morphogenesis. The aim of these experiments was to assess 1) the influence of different blue (B): red (R) ratios (25:75; 20:80 and 10:90 ratios) and 2) the effect of increasing far red (FR) intensity (0, 25, 50, 75 µmol µmol $m^{-2}$ $s^{-1}$) over a fixed BR spectrum at 20:80 ratio on plant growth, photosynthesis and leaf phenolic content in *Chicorium endivia* L. 'Lindo' and *Lactuca sativa* L. 'Blackhawk'.

Different B:R ratio did not affect photochemistry, namely PSII maximal photochemical efficiency (Fv/Fm) and linear electron transport rate (ETR), in both species. On the contrary, gas exchange was promoted in both species grown under the different R:B ratios compared to controls under white light (CW) and in particular under the ratio $B_{20\%}R_{80\%}$ in both species. The ratio $B_{20\%}R_{80\%}$ also modulated the content of photosynthetic pigments and polyphenols in both species. The addition of different intensities of FR light over the $B_{20\%}R_{80\%}$ spectrum determined a different behaviour in the two species: FR intensity at 50 and 75 μmol $m^{-2}$ $s^{-1}$ determined a reduction of gas exchange in endive, but not in lettuce. Contextually, FR addition at 25 and 50 μmol $m^{-2}$ $s^{-1}$ also triggered a decline of $ETR_{max}$ and light saturation point ($I_k$) in both species compared to the respective controls, while, the addition of 75 μmol $m^{-2}$ $s^{-1}$ promoted $ETR_{max}$ and $I_k$ only in endive. On the contrary the ETR efficiency at low light intensity (α) improved in lettuce plants grown with additional FR light.

FR light differently influenced the polyphenolic profile and promoted plant leaf expansion and dry matter accumulation only in endive.

Our results highline the importance of a correct balance between B and R light to promote photosynthetic process and the need of correct amount of FR to promote the plant growth and the biosynthesis of phenolic compounds. However, the species-specific response to light modulation should be taken into account to optimize its efficacy.

**Introduction**

Light plays a crucial role in plant life. It has several effects on plants (Pattison et al., 2018), affecting photosynthetic process (Johnson, 2016) and regulating seeds germination, stem elongation, leaf expansion, flowering and the biosynthesis of hormones and several metabolites (Fukuda, 2019). Light is perceived by plants through specific photoreceptors able to detect and distinguish the different wavelengths present in the light spectrum.

Apart from photosynthetic pigments, chlorophylls and carotenoids, plant photoreceptors regulate plant photomorphogenesis response and the biosynthesis of

several metabolites (Fukuda, 2019) and they are classified in five families: Phytochromes, Phototropins, Cryptochromes, ZTL/FKF1/LKP2 and UVR8 (Fukuda, 2019; Paik and Huq, 2019). Phytochromes are present in all plant tissues; they absorb red (R) and far red (FR) light and are present in two forms: the Pr form absorbs R light and is converted to the Pfr form, that is converted back to Pr under FR light. The ratio between the Pfr/Ptot is named Phytochrome Photo-Equilibrium (PPE) (Sager et al., 1988) Phytochromes regulate functions like seed germination, seedlings de-etiolation, stem elongation and leaf expansion (also as shade avoidance response), tuberization, bud dormancy, day-length perception and hence circadian rhythm, transition from vegetative to flowering stage (Demotes-Mainard et al., 2016 and reference therein). Cryptochromes absorb mainly blue (B) light and, together with the phytochromes, regulate plant photomorphogenesis response, stomatal aperture and hence transpiration and photosynthesis (Fukuda, 2019). Phototropins absorb mainly B and ultraviolet A (UV-A) radiation and control a wide range of response to optimize plant photosynthetic efficiency (Okajima, 2016), moreover, they are involved in the genetic control of anthocyanin synthesis pathway (Kadomura-Ishikawa et al., 2013).

An unfavourable environment in terms of light intensity/quality or low nutrients experiences plants through a resource imbalance, under these conditions, plants drive photosynthate carbon into the biosynthesis of photo-protective phytochemicals, such as phenolic compounds, to cope unfavourable conditions (Dudt and Shure, 1994). Phenolic compounds are a large group of phytochemicals widespread in the plant kingdom (Cartea et al., 2011), classified in phenols, phenolic acids, hydroxynnamic acid derivates and flavonoids (Holopainen et al., 2018). Many phenolic compounds present in plants have been demonstrated to have a marked antioxidant activity, and hence health benefits with anti-inflammatory effects, or to reduce the risk of diabetes or cancer diseases (Taulavuori et al., 2013 and reference therein).

Since the introduction in the market of monochromatic light emitting diodes (LEDs) photobiology research increased exponentially, as LEDs enabled to accurately investigate the spectral effects on plant physiology and morphology, defining in better way the synergistic mechanism between different wavelengths (Pattison et al., 2018).

Light modulation in terms of spectral composition has been proved to be effective in modulating the biosynthesis and the content of phenolic compounds in many species; for instance, supplemental B light was effective to increase phenolic compounds concentration in parsley, basil, tomatoes and lettuce (Taulavuori et al., 2013, 2018; Hyöky et al., 2016). The addition of UV-A, B, G, R of FR light to a white fluorescent lamp spectrum was also effective in modifying the qualitative attributes in terms of ascorbic acid, anthocyanin and phenolics concentration in lettuce plants grown in a controlled environment (Li and Kubota, 2009). A B:R ratio of 1 increased betacyanin content in *Atriplex hortensis* (Izzo et al., 2019), while different B:R ratios affected nutrients uptakes and the aromatic profile in basil (Pennisi et al., 2019). FR light negatively influenced the anthocyanin synthesis pathway and determined a decrease in chlorophylls content in tomato, potato and lettuce plants (Kerckhoffs et al., 1992; Yanovsky et al., 1998; Demotes-Mainard et al., 2016), on the contrary, increasing the R:FR ratio enhanced chlorogenic and caffeic acids content in lettuce (Lee et al., 2016).

Lettuce is the most consumed *Asteraceae* species cultivated worldwide for leafy salad. It is low in calories, fat and sodium, rich of fibers, iron, folate vitamin C and in particular it is a good source for human of different health-beneficial bioactive compounds (Kim et al., 2016 and reference therein).

Curly endive, like lettuce, belongs to the *Asteraceae* family. It is cultivated in greenhouse or open field and is highly appreciated in the European markets (Lucchin et al., 2008). It is rich in phenolic compounds, such as kaempferol, and its consumption has beneficial effect on human-health (DuPont et al., 2004).

In the light of these consideration we aimed to investigate light quality effect on leafy vegetables in two experiments. In the first experiment, we focused our attention on the effect of different percentages of R and B light on plant growth and photosynthesis in two leafy crops: lettuce and endive. These species are largely consumed and lettuce cultivation in plant factories under artificial light is increasing. To assess the effect of light quality on their physiology and growth is a fundamental step to increase their edible biomass as well as the production of functional compounds like phenols. The second experiment aimed to study the consequences of the addition of different FR

intensity to a fixed B:R spectrum on photosynthesis, growth and polyphenols amount in lettuce and endive grown in a controlled environment.

## Materials and Methods

### *Plant material and growth conditions*

Seedlings of *Lactuca sativa* L. 'Blackhawk' and *Chicorium endivia* L. 'Lindo' at the 3rd-4th leaf stage, delivered in peat cubes, were gently washed with tap water to remove the peat from the root system and then transplanted into a floating system filled with a Hoagland solution, with pH 5.8 and EC 1.2 dS $m^{-1}$. Planting density was 75 plants $m^{-2}$ (Petropoulos et al., 2016), and aeration in the solution media was provided by oxygen pipes.

Cultivation was carried out in growth chamber, under a day/ night temperature of 20.0/17.5 °C and 60/80 % relative humidity. Seedlings were acclimated under a white LED spectrum [Control White (CW), 40% B (460 nm), 20% G (560 nm), 40% R (660 nm) and 2% FR (736 nm)] (GreenPower LED dynamic module, Signify, Eindhoven, The Netherlands), at a photosynthetic flux density (PPFD) at canopy level of 245 µmol $m^{-2}$ $s^{-1}$, under 16 hours photoperiod, for two days prior to the start the lighting treatments.

### *Lighting treatments*

Two experiments were carried out to assess the influence of different B:R ratios (Experiment 1) and the effect of increasing FR intensity over a B:R ratio 20:80 (Experiment 2) on plant growth, photosynthetic process and leaf phenolic content. Each experiment was conducted on 15 plants per combination *Species x Lighting treatment*.

The lighting treatments were provided by LEDs (GreenPower LED dynamic module, Signify, Eindhoven, The Netherlands); the light spectra used in the two experiments are reported in Table 1. The light spectral distribution of the different treatments was assessed by a spectrometer (JAZ-ULM-200, Ocean Optics, USA) and converted to µmol $m^{-2}$ $s^{-1}$ with Spectrasuite software (Ocean Optics, USA) (Figure 1). The lighting

treatments, in both the experiments, started 3 days after transplanting and lasted until the harvest (35 days). In Experiment 2, phytochrome photo-equilibrium (PPE) at plant level was estimated according to Sager et al. (1988).

Table 1: Spectral composition of the light treatments used in the Experiments 1 and 2 to growth endive and lettuce plants in a growth chamber under 16 hours photoperiod; light was provided by GreenPower LED dynamic module (Signify, Eindhoven, The Netherlands). Spectrum was measured at the plant canopy level with a JAZ-ULM-200 spectrometer (Ocean Optics, FL, US); Phytochrome photoequilibrium (PPE) was estimated according to Sager et al. (1988).

| | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | CW | $B_{25\%}R_{75\%}$ | $B_{20\%}R_{80\%}$ | $B_{10\%}R_{90\%}$ | $B_{20\%}R_{80\%}$ | $B_{20\%}R_{80\%}+FR_{25}$ | $B_{20\%}R_{80\%}+FR_{50}$ | $B_{20\%}R_{80\%}+FR_{75}$ |
| Blue (B) % | 40 | 25 | 20 | 10 | 20 | 20 | 20 | 20 |
| Green (G) % | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Red (R) % | 40 | 75 | 80 | 90 | 80 | 80 | 80 | 80 |
| Far Red (FR) (µmol/s/m²) | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 25.0 | 50.0 | 75.0 |
| PPFD (PAR) | 245 | 245 | 245 | 245 | 245 | 245 | 245 | 245 |
| TPFD (400-800) | 247.6 | 245.0 | 245.0 | 245.0 | 245.0 | 270.0 | 295.0 | 320.0 |
| B:R ratio | 1.00 | 0.33 | 0.25 | 0.11 | 0.25 | 0.25 | 0.25 | 0.25 |
| PPE | n.a | n.a | 0.88 | n.a | 0.88 | 0.85 | 0.82 | 0.80 |

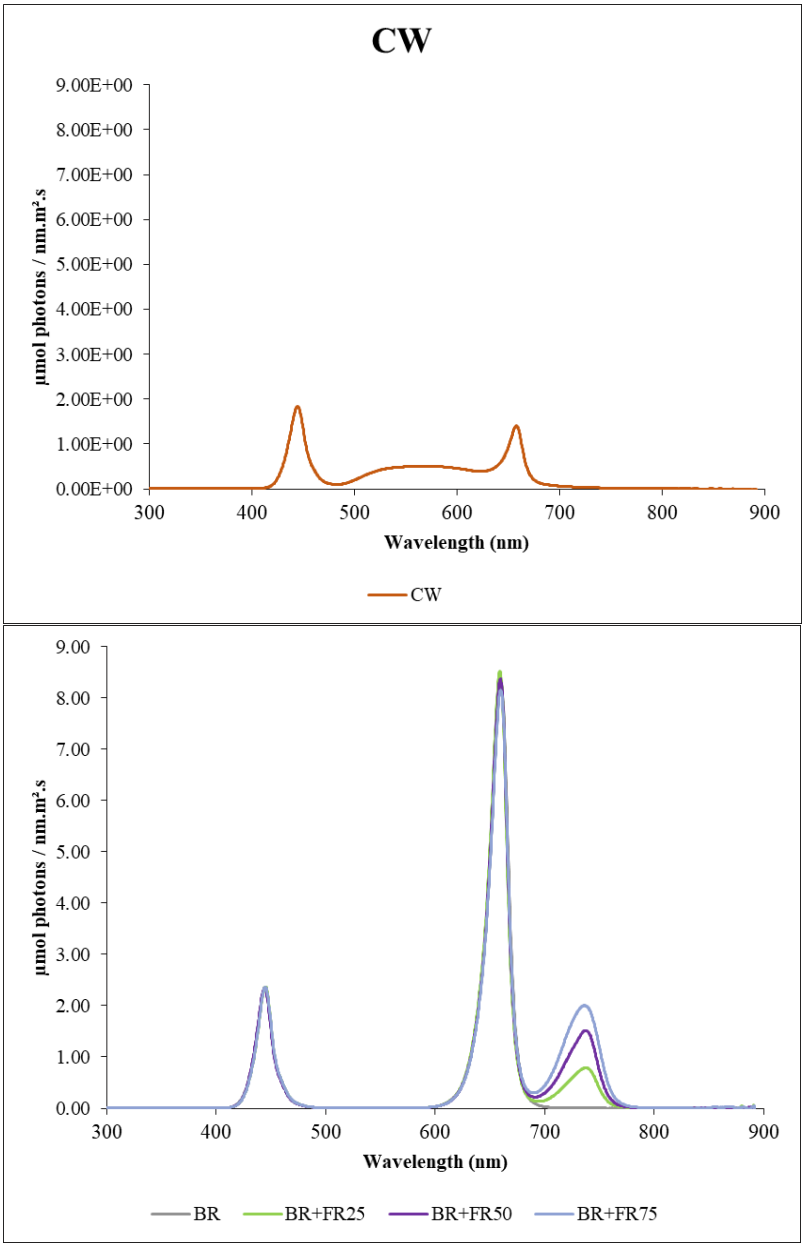



Figure 1 - Spectral distribution (µmol photons $^{-1}$ nm$^{-1}$ m$^{-2}$ s) of the light under the treatments CW used to acclimatate after transplanting the seedlings of endive 'Lindo' and lettuce 'Blackhawk' for 48 hours prior the start of the lighting treatments in both the experiments and the light treatments BR+FR used in the experiment 2.

***Measurements***

All physiological, morphological and qualitative attributes measurements were performed on five plants per combination *Species x Lighting treatment*.

***Gas exchanges, chlorophyll a fluorescence and leaf photosynthetic pigments***

Gas exchanges and chlorophyll *a* fluorescence were measured on fully expanded leaves. In both the experiments, gas exchanges in term of leaf net photosynthesis (*Pn*) and stomatal conductance (*gs*) were determined 31 days after the beginning of lighting treatments by a portable photosynthesis system Licor 6400 XT (Licor, Lincoln, USA), equipped with the 6400-40 Leaf Chamber Fluorometer (LCF) (Licor, Lincoln, USA). In Experiment 1, the LCF blue actinic light percentage used to measure on plants was 50% in CW, 25% in $B_{25\%}R_{75\%}$, 20% in $B_{20\%}R_{80\%}$, 10% in $B_{10\%}R_{90\%}$; in Experiment 2, the B % in the LCF was 20% in all treatments. All the measurements were carried out at a light intensity of 245 μmol $m^{-2}$ $s^{-1}$ and the $CO_2$ concentration was fixed at 400 ppm.

On the same leaves used for gas exchanges, chlorophyll *a* fluorescence was measured using a portable fluorometer PAM 2500 (Walz, Effeltrich, Germany). In Experiment 1, slow induction kinetics curves were performed every 6 days since the start of the lighting treatments. In Experiment 2, light intensity response curves were performed 30 days after the beginning of the lighting treatments.

For the measurements in the dark, leaves were dark adapted with a dark leaf clip DLC-8 (Walz, Effeltrich, Germany) for 30 min, then a 0.6 s a saturating light pulse (3450 μmol $m^{-2}$ $s^{-1}$) was given to obtain $F_m$ and $F_0$ values. A continuous actinic light (245 μmol $m^{-2}$ $s^{-1}$) was applied for 5 min to allow leaf light adaption and a saturating pulses of 2968 μmol $m^{-2}$ $s^{-1}$ every 25 s was given, to obtain the maximum light adapted fluorescence ($F_m'$) and steady state fluorescence ($F_s$). The minimal fluorescence after the PSII excitation ($F_0'$) was obtained turning off the actinic light and applying a far-red pulse. The PSII maximum photochemical efficiency ($F_v/F_m$) was calculated as $F_v/F_m = (F_m - F_0)/F_m$. The quantum yield of PSII electron transport ($\Phi_{PSII}$) was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ following Genty et al. (1989); the non-photochemical quenching (NPQ) was estimated according to Baker (2008). The

electron transport rate (ETR) was calculated as ETR = $\Phi_{PSII}$ × PAR × 0.84 × 0.5, considering the absorbed photon energy (PAR) equally distributed between PSI and PSII and 0.84 the assumed light absorbance of the leaf.

The fluorescence light response curves were built by increasing the actinic light from 0 to 2968 μmol $m^{-2}$ $s^{-1}$ in eleven steps, applying saturation pulses of 0.6 s at each step. The ETR was calculated at each illumination step. The plant response to the increasing light intensity was obtained by fitting each ETR curve according to the model by Platt et al. (1980), estimating the maximal electron transport rate ($ETR_{max}$), electron transport rate efficiency at low light intensity (α, electron photons $^{-1}$) and the light saturation point ($I_k$, μmol photons $m^{-2}$ $s^{-1}$) calculated as $I_k = ETR_{max}/\alpha$.

Photosynthetic pigments content was analyzed according to Lichtenthaler and Buschmann (2001). Freeze powdered leaf samples of 150 mg were dissolved in 10 mL of acetone (80% in vol.), incubated at –20°C for 24 hours; after that an aliquot of 300 μL per sample was inserted in a 96 well plate and the absorbance was read at 470 nm, 647 nm and 663 nm with Tecan 200 Infinite spectrophotometer (Infinite 200, Tecan Group Ltd., Switzerland).

***Leaf optical properties***

Every 6 days from the beginning of lighting treatments, the following parameters were measured, using a PolyPen RP 400 UVIS (PSI, Photon Systems Instruments, Drasov, Czech Repubblic) with a spectral response range from 380 nm to 780 nm: leaf absorbance and transmittance, Greenness Index (G), Modified Chlorophyll Absorption Reflectance Index (MCARI), Photochemical Reflectance Index (PRI), Anthocyanin Reflectance Index 1 (ARI1), Carotenoid Reflectance Index 1 (CR1). For both experiments, in this study, we present only the results obtained the last week of growing as a result of the whole growing cycle and in line with the other measurements presented in this study.

***Plant growth***

Plant growth was determined on 5 plants per combination *Species x Lighting treatment* at harvest time, measuring leaf number and total leaf area, root and leaf

fresh and dry weight. Leaf area was determined by ImageJ software 1.50i version (Wayne Rasband National Institute of Health, USA); dry weight was measured after drying the samples for 48 hours at 70 °C.

***Polyphenol content***

Polyphenol content was measured in leaves on 5 plants per combination *Species x Lighting treatment*, collected at harvest time and immediately frozen into liquid nitrogen, and stored at –80°C. The plant material was ground into fine powder and homogenized using liquid nitrogen into an IKA-A11 analytical mill (IKA®-Werke GmbH & Co. KG, Staufen, Germany).

*Solvent extraction*

The solvent extraction was based on the protocol of Nguyen et al. (2018), with the following modifications. An aliquot of 2 g of freeze-plant powder was weighted and placed in 100 mL glass tube. 10 mL of 100% methanol (MeOH) was added, and the sample washomogenized with an IKA T25 digital Ultraturrax at 10,000 rpm for 45 s. After, the tubes were placed on ice for 15 min and centrifuged at 3,000 x g for 5 min at 4°C. The supernatant (1) was collected in a 10 mL glass tube using a glass Pasteur pipette, while the residue was re-extracted with 8 mL of 80% MeOH (2) The supernatants (2) was added to the supernatant (1) and the residue was dried under a light stream of nitrogen, and then stored.

*Compounds identification and quantification using UHPLC-DAD*

Quantification was done using a Thermo Dionex Ultimate 3000 UHPLC (ThermoScientific, Landsmeer, The Netherlands), equipped with diode array detector (DAD). Chromatographic separation was done by a Waters Acquity BEHC18 column (2.1 mm x 150 mm, 1.7µm particle size). The mobile phase was composed as follows: phase A MilliQ water (0.1% vol. formic acid); phase B MeOH (0.1% vol. formic acid). The column temperature was maintained at 40°C. Twenty µL of sample was injected at a flow rate of 250 µL $min^{-1}$during the elution multi-step gradient. The

elution program was: 0 min, 2% B; 0-2 min, 2% B; 2-8 min, 2-10% B linear; 8-23 min, 10-50% B linear; 23-24 min, 50-95% B linear; 24-26 min, 95% B isocratic; 26-28 min, 95-2% B, linear; 28-30 min, 2% B isocratic the eluent was then directed to the DAD detector and read at a wavelength of 250, 320, 350 and 520 nm. A spectrum scan was used as additional identification of the compounds.

*Reagents and standards*

UHPLC grade MeOH and formic acid were acquired from Biosolve (Valkenswaard, The Netherlands). Analytical grade MeOH used for the extraction was purchased from VWR International (Leuven, Belgium). Kaempferol, quercetin, quercetin glucoside, chicoric acid, chlorogenic acid, ferulic acid and cyanidin 3-glucoside chloride standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

***Statistical analysis***

Eco-physiological and growth data were analysed by ONE WAY ANOVA using the SPSS 25 software package (www.ibm.com/software/analytics/spss), performed separately per each species under the different lighting treatments; means were compared by Tukey HSD *post-hoc* ($P \leq 0.05$).

Polyphenolic profile was analysed by principal component analysis (PCA) and cluster analysis conducted using Primer 6 software package (PRIMER-e, Albany, New Zealand) to highline variation in plants grown through the different light treatments. Cluster analysis was performed normalizing variables considering Euclidean distance as the similarity measure and hierarchical clustering with complete linkage between samples. The PCA analysis extracted 5 components and the PCA outputs include treatment component scores as well as variable loadings with cluster analysis between samples overloaded in the plot.

**Results**

***Gas exchanges, Chlorophyll a fluorescence and leaf photosynthetic pigments***

In Experiment 1, the different lighting treatments did not influence the Fv/Fm ratio and ETR values of the endive plants, that were on average 0.760±0.004 and 69.66±0.39, respectively (Figure 2). Compared to CW light, lighting treatment $B_{20\%}R_{80\%}$ increased *Pn* and *gs* by 39 and 41 % respectively, even if *gs* increase was not significant (Figure 2).

Similarly, in lettuce plants grown under different lighting treatments, no difference was observed in both Fv/Fm and ETR values, that were on average 0.774±0.002 and 70.48±0.50, respectively (Figure 2). Compared to CW light treatment, in plants grown under $B_{20\%}R_{80\%}$ light *Pn* and *gs* increased significantly by 38 and 64 % respectively (Figure 2).

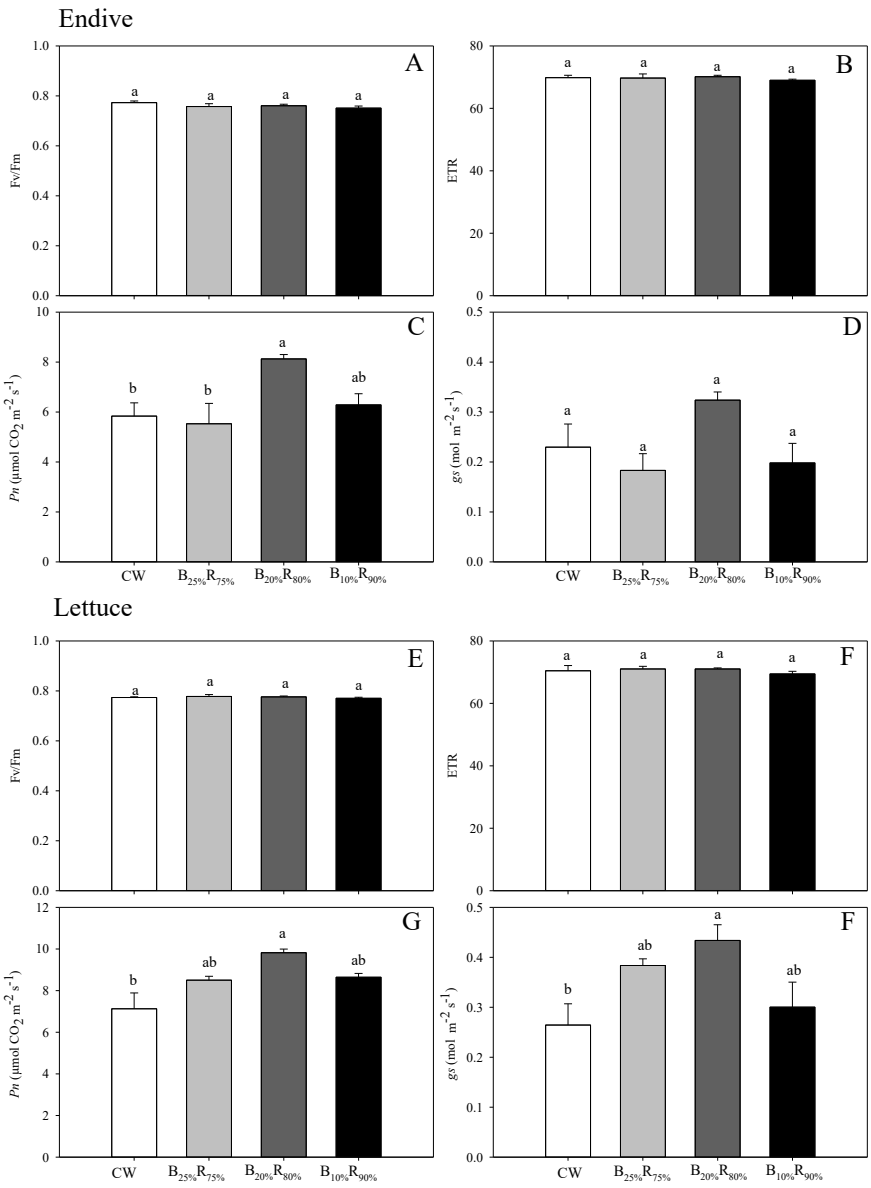



Figure 2- Maximal photochemical efficiency of PSII ($F_v/F_{m;}$ A, E), electron transport rate (ETR; B, F), net photosynthesis (*Pn*; C, G), stomatal conductance (*gs*; D, H) respectively in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 1 in a growth chamber in floating system under artificial light provided by LEDs white (CW) or different blue and red ratios at a PPFD of 245 µmol $m^2$ $s^{-1}$. Mean values ± Standard errors; n=5 followed by different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* ($P<0.05$).

In the second experiment, endive and lettuce plants showed no difference in Fv/Fm values under different FR light intensities added to the fixed spectrum of $B_{20\%}R_{80\%}$.

Compared to endive plants grown under $B_{20\%}R_{80\%}$ light, in plants grown with the addition of 50 and 75 μmol $m^{-2}$ $s^{-1}$ of FR light to the BR light spectrum, *Pn* decreased by 34 and 38 %, respectively (Figure 3). No difference was observed for $ETR_{max}$ between plants under $B_{20\%}R_{80\%}$ and $B_{20\%}R_{80\%}$+$FR_{75}$ lighting treatments; conversely the addition of 25 and 50 μmol $m^{-2}$ $s^{-1}$ of FR light determined a *Pn* decrease of 32 and 54 %, respectively (Figure 3).

Compared to plant subjected to $B_{20\%}R_{80\%}$ spectrum, in plants where 50 μmol $m^{-2}$ $s^{-1}$ of FR light was added a decrease ($P<0.05$) of α and $I_k$ by 22 and 37% respectively was found, while no significant difference was observed under the other treatments (Figure 3).

The ETR response curves at growing irradiance showed no difference among plants grown under all the lighting treatments between 0 to 99 μmol photons $m^{-2}$ $s^{-1}$, while a clear separation between the light treatments was observed for irradiance equal and higher than196 μmol photons $m^{-2}$ $s^{-1}$, with significantly higher values recorded in plants under the $B_{20\%}R_{80\%}$ and $B_{20\%}R_{80\%}$+$FR_{75}$ treatments (Figure 4).

In lettuce plants grown with the addition of 25 μmol $m^{-2}$ $s^{-1}$ of FR, *Pn* was 5 % higher, compared to plants grown under $B_{20\%}R_{80\%}$ light, while, it decreased ($P<0.05$) by 14% with 50 μmol photons $m^{-2}$ $s^{-1}$ of additional FR light (Figure 3). Compared to $B_{20\%}R_{80\%}$ plants, the addition of 25, 50 and 75 μmol $m^{-2}$ $s^{-1}$ of FR to the BR spectrum decreased the $ETR_{max}$ by 22, 45 and 43 % respectively; while the α was 16 % higher in plants grown with the addition of 50 μmol $m^{-2}$ $s^{-1}$ of FR light (Figure 3). $I_k$ decreased by 24, 51 and 42 % respectively in plants grown with the addition 25, 50 and 75 μmol $m^{-2}$ $s^{-1}$ of FR (Figure 3).

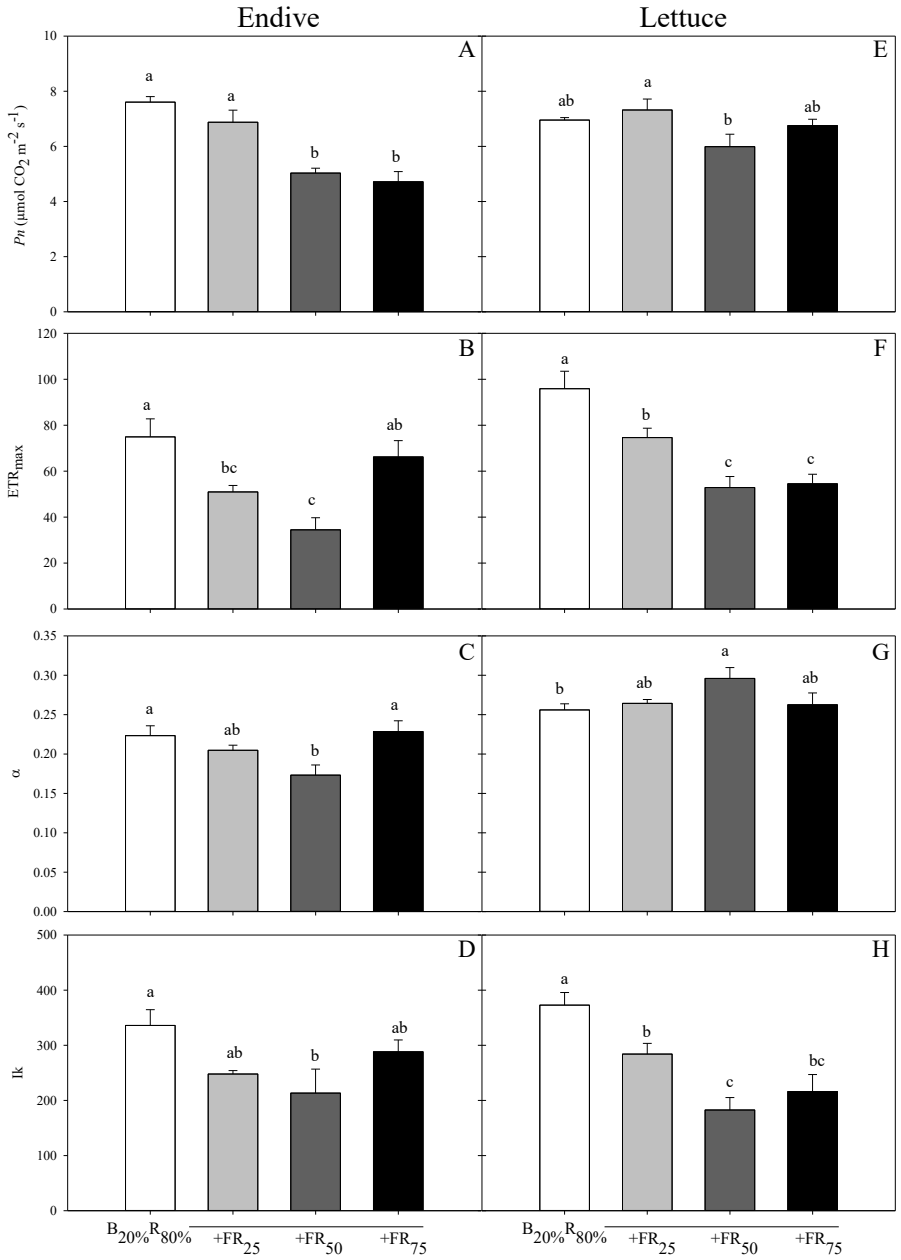



Figure 3- Net photosynthesis (*Pn*; A, E), maximal electron transport rate ($ETR_{max;}$;B, F), electron transport rate efficiency at low light intensity (α; C, G) and light saturation point ($I_k$; D, H) in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light. Mean values ± Standard errors; n=5 followed by different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* ($P<0.05$).

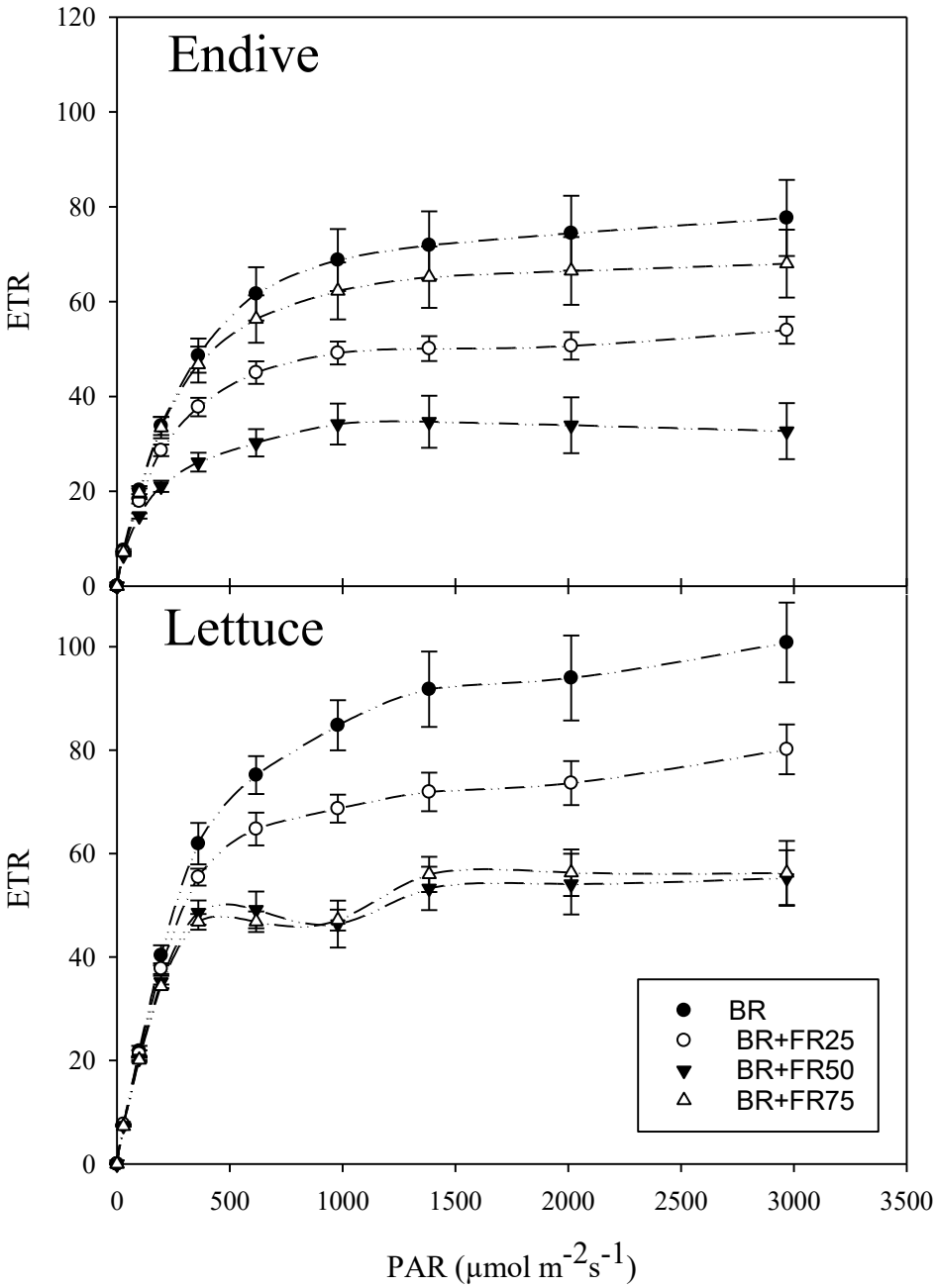



Figure 4- Electron transport rate (ETR) response curve at growing irradiances in plants of endive 'Lindo' and lettuce 'Blackhawk' grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light. Mean values ± Standard errors; n=5.

ETR response curves at growing irradiance showed no differences among plants grown under all the lighting treatments at low irradiances between 0 to 196 μmol $m^{-2}$ $s^{-1}$, while a clear separation was observed for irradiance equal and higher than 361 μmol $m^{-2}$ $s^{-1}$, with significantly higher values in plants under $B_{20\%}R_{80\%}$ light and lower values with additional 50 and 75 μmol $m^{-2}$ $s^{-1}$ of FR (Figure 4).

Compared to endive plants grown under CW treatments, in plants grown in the first experiment, no difference was found in chlorophyll *a* and *b*, total carotenoid content and chlorophylls/carotenoid ratio, while, chlorophylls *a*/*b* ratio was higher in plants grown under $B_{25\%}R_{75\%}$ and $B_{10\%}R_{90\%}$ treatments (Table 2).Compared to lettuce plants grown under CW light, plants grown under $B_{10\%}R_{90\%}$ showed high ($P<0.05$) chlorophyll *a* content; chlorophyll *b* content increased under $B_{25\%}R_{75\%}$ light while the total chlorophyll content increased in plants under $B_{25\%}R_{75\%}$ and $B_{20\%}R_{80\%}$ light. Carotenoids content increased only in plants grown under $B_{25\%}R_{75\%}$ light whereas no differences were observed in chlorophyll *a*/*b* and total chlorophyll/carotenoid ratios (Table 2).

Table 2- Chlorophyll *a*, Chlorophyll *b*, Chlorophyll total, Carotenoids, Chlorophyll a/*b* ratio and Chlorophyll total/ Carotenoids ratio after 35 days from planting in plants of in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 1 in a growth chamber in floating system under artificial light provided by LEDs white (CW) or different blue and red ratios at a PPFD of 245 µmol $m^2$ $s^{-1}$. Photosynthetic pigments are expressed as µg/g of fresh material. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | Chl *a* | Chl *b* | Chl tot | Carotenoids | Chl *a*/*b* | Chl tot/Crtn |
|---|---|---|---|---|---|---|
| Endive | | | | | | |
| CW | 152.233 ± 13.100 | 99.312 ± 6.007 | 251.545 ± 19.019 | 31.423 ± 4.705 | 1.5239 ± 0.045 b | 8.459 ± 0.792 |
| $B_{25\%}R_{75\%}$ | 184.743 ± 6.660 | 109.279 ± 3.425 | 294.022 ± 10.063 | 39.388 ± 2.763 | 1.690 ± 0.012 a | 7.544 ± 0.309 |
| $B_{20\%}R_{80\%}$ | 156.067 ± 7.034 | 96.550 ± 3.307 | 252.617 ± 10.316 | 34.852 ± 1.569 | 1.6141 ± 0.020 ab | 7.278 ± 0.281 |
| $B_{10\%}R_{90\%}$ | 170.448 ± 15.147 | 100.144 ± 6.158 | 270.592 ± 21.275 | 31.218 ± 2.694 | 1.689 ± 0.056 a | 8.692 ± 0.121 |
| *mean* | *165.873* | *101.321* | *267.194* | *34.220* | *1.629* | *7.994* |
| Significance | ns | ns | ns | ns | * | ns |
| Lettuce | | | | | | |
| CW | 229.595 ± 7.526 b | 123.555 ± 4.256 b | 353.151 ± 11.523 b | 59.322 ± 2.899 b | 1.859 ± 0.027 | 5.975 ± 0.127 |
| $B_{25\%}R_{75\%}$ | 297.327 ± 13.743 ab | 156.669 ± 10.230 a | 453.996 ± 22.651 a | 80.634 ± 4.512 ab | 1.910 ± 0.069 | 5.663 ± 0.287 |
| $B_{20\%}R_{80\%}$ | 238.629 ± 27.359 b | 127.134 ± 10.055 ab | 365.763 ± 37.401 ab | 65.261 ± 8.502 ab | 1.852 ± 0.080 | 5.715 ± 0.217 |
| $B_{10\%}R_{90\%}$ | 310.821 ± 14.886 a | 149.728 ± 5.770 ab | 460.548 ± 20.526 a | 87.404 ± 5.092 a | 2.074 ± 0.031 | 5.289 ± 0.120 |
| *mean* | *269.093* | *139.272* | *408.364* | *73.155* | *1.924* | *5.660* |
| Significance | * | * | * | * | ns | ns |

In the second experiment, endive plants grown with the addition of 50 μmol $m^{-2}$ $s^{-1}$ of FR light to the spectrum showed a significant lower chlorophyll *a* and total chlorophyll content compared to plants grown under $B_{20\%}R_{80}$; Furthermore, the Chlorophyll *a*/*b* ratio decreased in plants grown under additional 50 and 75 μmol $m^{-2}$ $s^{-1}$ of FR light (Table 3).

In the second experiment, lettuce plants grown under the addition of 75 μmol $m^{-2}$ $s^{-1}$ of FR light showed a decrease ($P<0.05$) of total carotenoid and chlorophyll *a* content compared to plants grown under $B_{20\%}R_{80\%}$ and a rise of the total chlorophyll/carotenoid ratio (Table 3).

Table 3- Chlorophyll *a*, Chlorophyll *b*, Chlorophyll total, Carotenoids, Chlorophyll a/b ratio and Chlorophyll total/ Carotenoids ratio after 35 days from planting in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light.. Photosynthetic pigments are expressed as µg/g of fresh material. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | Chl *a* | Chl *b* | Chl tot | Carotenoids | Chl *a*/*b* | Chl tot/Crtn |
|---|---|---|---|---|---|---|
| Endive | | | | | | |
| $B_{20\%}R_{80\%}$ | 223.624 ± 15.963 a | 116.605 ± 5.688 | 340.229 ± 21.611 a | 45.898 ± 3.878 | 1.910 ± 0.045 a | 7.47 ± 0.20 |
| $B_{20\%}R_{80\%}+FR_{25}$ | 183.319 ± 12.177 ab | 102.469 ± 5.138 | 285.788 ± 17.229 ab | 39.658 ± 3.668 | 1.783 ± 0.042 ab | 7.32 ± 0.32 |
| $B_{20\%}R_{80\%}+FR_{50}$ | 160.465 ± 15.520 b | 96.892 ± 4.969 | 257.356 ± 20.461 b | 32.301 ± 4.286 | 1.641 ± 0.076 b | 8.24 ± 0.56 |
| $B_{20\%}R_{80\%}+FR_{75}$ | 180.032 ± 9.925 ab | 110.451 ± 3.745 | 290.483 ± 13.331 ab | 39.204 ± 2.472 | 1.627 ± 0.047 b | 7.45 ± 0.22 |
| *mean* | *186.860* | *106.604* | *293.464* | *39.265* | *1.740* | *7.62* |
| Significance | * | ns | * | ns | * | ns |
| Lettuce | | | | | | |
| $B_{20\%}R_{80\%}$ | 207.317 ± 16.154 | 111.902 ± 5.842 | 319.220 ± 21.966 | 57.648 ± 5.369 a | 1.843 ± 0.051 a | 5.59 ± 0.16 b |
| $B_{20\%}R_{80\%}+FR_{25}$ | 153.891 ± 13.188 | 95.502 ± 5.686 | 249.394 ± 18.789 | 40.076 ± 4.079 ab | 1.602 ± 0.047 ab | 6.29 ± 0.20 ab |
| $B_{20\%}R_{80\%}+FR_{50}$ | 169.322 ± 19.723 | 104.932 ± 11.316 | 274.254 ± 30.141 | 42.056 ± 5.233 ab | 1.611 ± 0.090 ab | 6.61 ± 0.30 ab |
| $B_{20\%}R_{80\%}+FR_{75}$ | 151.492 ± 16.550 | 96.726 ± 6.928 | 248.218 ± 23.300 | 37.295 ± 4.979 b | 1.550 ± 0.074 b | 6.86 ± 0.42 a |
| *mean* | *170.506* | *102.266* | *272.771* | *44.269* | *1.651* | *6.34* |
| Significance | ns | ns | ns | * | * | * |

***Leaf optical properties***

In endive plants grown in the first experiment, no difference was observed between the lighting treatments for the Greenness Index (G), Modified Chlorophyll Absorption Reflectance Index (MCARI), Photochemical Reflectance Index (PRI), Anthocyanin Reflectance Index 1 (ARI 1) and Carotenoid Reflectance Index 1 (CR1) (Table 4).

Table 4- Greenness Index (G), Modified Chlorophyll Absorption in Reflectance Index (MCARI), Photochemical Reflectance Index (PRI), Anthocyanin Reflectance Index 1 (ARI1), Carotenoid Reflectance Index 1 (CR1), after 35 days from planting in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 1 in a growth chamber in floating system under artificial light provided by LEDs white (CW) or different blue and red ratios at a PPFD of 245 µmol $m^2$ $s^{-1}$. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | G | MCARI | PRI | ARI 1 | CRI 1 |
|---|---|---|---|---|---|
| Endive | | | | | |
| CW | 2.083 ± 0.050 | 0.284 ± 0.020 | 0.035 ± 0.003 | 0.300 ± 0.059 | 3.078 ± 0.212 |
| $B_{25\%}R_{75\%}$ | 1.810 ± 0.101 | 0.231 ± 0.035 | 0.024 ± 0.005 | 0.366 ± 0.052 | 2.876 ± 0.445 |
| $B_{20\%}R_{80\%}$ | 1.859 ± 0.059 | 0.253 ± 0.014 | 0.026 ± 0.004 | 0.347 ± 0.066 | 2.694 ± 0.394 |
| $B_{10\%}R_{90\%}$ | 1.965 ± 0.125 | 0.277 ± 0.023 | 0.027 ± 0.004 | 0.346 ± 0.046 | 3.052 ± 0.555 |
| *mean* | *1.929* | *0.261* | *0.028* | *0.34* | *2.925* |
| Significance | ns | ns | ns | ns | ns |
| Lettuce | | | | | |
| CW | 2.273 ± 0.231 | 0.394 ± 0.053 | 0.033 ± 0.010 ab | 1.141 ± 0.552 | 4.695 ± 0.524 |
| $B_{25\%}R_{75\%}$ | 1.771 ± 0.184 | 0.306 ± 0.044 | 0.005 ± 0.002 b | 2.958 ± 0.674 | 4.570 ± 0.549 |
| $B_{20\%}R_{80\%}$ | 2.088 ± 0.120 | 0.302 ± 0.042 | 0.036 ± 0.006 a | 1.359 ± 0.344 | 5.697 ± 1.046 |
| $B_{10\%}R_{90\%}$ | 2.487 ± 0.267 | 0.464 ± 0.042 | 0.020 ± 0.012 ab | 2.062 ± 1.138 | 5.185 ± 0.421 |
| *mean* | *2.155* | *0.366* | *0.023* | *1.88* | *5.037* |
| Significance | ns | ns | * | ns | ns |

In lettuce, compared to plants grown under CW light, the lighting treatments $B_{25\%}R_{75\%}$ and $B_{20\%}R_{80\%}$ respectively decreased and increased (-96% and +9%, respectively) the Photochemical Reflectance Index (PRI), while no difference was observed for the other indexes (Table 4). In the second experiment, compared to endive plants grown under the $B_{20\%}R_{80\%}$ light, the Greenness Index (G) and the Carotenoid Reflectance Index 1 (CRI 1) decreased by 15 and 42% in plants grown under $B_{20\%}R_{80\%}$+$FR_{75}$ treatment, while no difference was observed for the other indexes (Table 5).

Compared to plants grown under the $B_{20\%}R_{80\%}$ treatment, in lettuce the addition of 25, 50 and 75 μmol photons $m^{-2}$ $s^{-1}$ of FR determined, respectively, an increase of 57, 78 and 71% of the Greenness Index (G), and of 271, 363 and 302% of Photochemical Reflectance Index (PRI) (Table5). The Modified Chlorophyll Absorption Reflectance Index (MCARI) was significantly higher (+81%) only in plants treated with FR 75 μmol photons $m^{-2}$ $s^{-1}$, on the contrary, the addition of 25, 50 and 75 μmol photons $m^{-2}$ $s^{-1}$ of FR light to the spectrum decreased the Anthocyanin Reflectance Index 1 (ARI 1) by 83, 93 and 82%, respectively (Table 5).

Table 5- Greenness Index (G), Modified Chlorophyll Absorption in Reflectance Index (MCARI), Photochemical Reflectance Index (PRI), Anthocyanin Reflectance Index 1 (ARI1), Carotenoid Reflectance Index 1 (CR1), after 35 days from planting in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | G | MCARI | PRI | ARI 1 | CRI 1 |
|---|---|---|---|---|---|
| Endive | | | | | |
| $B_{20\%}R_{80\%}$ | 2.095 ± 0.074 a | 0.284 ± 0.014 | 0.028 ± 0.006 | 0.268 ± 0.058 | 3.096 ± 0.242a |
| $B_{20\%}R_{80\%}+FR_{25}$ | 2.184 ± 0.066 a | 0.338 ± 0.008 | 0.031 ± 0.004 | 0.307 ± 0.042 | 3.175 ± 0.212 a |
| $B_{20\%}R_{80\%}+FR_{50}$ | 2.025 ± 0.067 ab | 0.334 ± 0.017 | 0.020 ± 0.004 | 0.295 ± 0.051 | 2.390 ± 0.207 ab |
| $B_{20\%}R_{80\%}+FR_{75}$ | 1.780 ± 0.034 b | 0.288 ± 0.016 | 0.019 ± 0.004 | 0.298 ± 0.053 | 1.796 ± 0.256 b |
| *mean* | *2.021* | *0.311* | *0.024* | *0.292* | *2.614* |
| Significance | * | ns | ns | ns | * |
| Lettuce | | | | | |
| $B_{20\%}R_{80\%}$ | 1.504 ± 0.193 b | 0.316 ± 0.011 b | -0.014 ± 0.009 b | 5.248 ± 1.543 a | 3.626 ± 0.851 |
| $B_{20\%}R_{80\%}+FR_{25}$ | 2.367 ± 0.072 a | 0.367 ± 0.033 ab | 0.024 ± 0.010 a | 0.901 ± 0.193 b | 5.029 ± 0.496 |
| $B_{20\%}R_{80\%}+FR_{50}$ | 2.684 ± 0.077 a | 0.507 ± 0.030 ab | 0.037 ± 0.005 a | 0.391 ± 0.035 b | 4.025 ± 0.388 |
| $B_{20\%}R_{80\%}+FR_{75}$ | 2.578 ± 0.140 a | 0.573 ± 0.104 a | 0.028 ± 0.006 a | 0.942 ± 0.273 b | 4.623 ± 0.468 |
| *mean* | *2.283* | *0.441* | *0.019* | *1.87* | *4.325* |
| Significance | * | * | * | * | ns |

**Plant growth**

In the first experiment, the different lighting treatments did not determine any differences in growth parameter in endive plants (Table 6). Conversely, lettuce, plants grown under $B_{25\%}R_{75\%}$ and $B_{20\%}R_{80\%}$ treatments, compared to those grown under CW developed a higher number of leaves per plant (+26% and +45% respectively) (Table 6).

In the second experiment, compared to endive plants grown under $B_{20\%}R_{80\%}$, the leaf number and the leaf fresh weight were similar in plants under all the treatments. On the contrary the addition of 25 μmol $m^{-2}$ $s^{-1}$ of FR increased significantly plant leaf area (+79%) (Table 7). The total dry weight increased significantly in plants grown with additional 25 or 75 μmol $m^{-2}$ $s^{-1}$ of FR light (+54 and 62%, respectively), the root: shoot ratio was similar in plants grown without and with 25 μmol $m^{-2}$ $s^{-1}$ of FR light, while it was significantly lower (-36%) in plants under additional 50 μmol $m^{-2}$ $s^{-1}$ of FR light (Table 7).

Lettuce plant growth was not affected by the lighting treatments (Table 7).

Table 6 - Plant growth after 35 days from planting in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 1 in a growth chamber in floating system under artificial light provided by LEDs white (CW) or different blue and red ratios at a PPFD of 245 µmol $m^2$ $s^{-1}$. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | Number of leaves (N plant$^{-1}$) | Plant leaf area (cm$^2$ plant$^{-1}$) | Leaves fresh weight (g plant$^{-1}$) | Total dry weight (g plant$^{-1}$) | Root/Shoot ratio |
|---|---|---|---|---|---|
| Endive | | | | | |
| CW | 76.80±2.03a | 1,557.7±172.2a | 106.69±3.43a | 6.71±0.28a | 0.135±0.004a |
| $B_{25\%}R_{75\%}$ | 87.80±2.82a | 1,481.6±161.7a | 102.39±4.64a | 8.36±0.45a | 0.159±0.014a |
| $B_{20\%}R_{80\%}$ | 86.20±2.63a | 1,483.5±140.4a | 113.48±5.83a | 8.11±0.39a | 0.137±0.009a |
| $B_{10\%}R_{90\%}$ | 77.60±5.41a | 1,480.2±122.4a | 103.30±10.62a | 8.09±0.65a | 0.160±0.009a |
| *mean* | *82.10* | *1,700.7* | *106.46* | *7.82* | *0.15* |
| Significance | ns | ns | ns | ns | ns |
| Lettuce | | | | | |
| CW | 18.80±1.39c | 1,148.6±90.6a | 82.49±6.18a | 5.24±0.37a | 0.116±0.009a |
| $B_{25\%}R_{75\%}$ | 23.60±0.75ab | 1,138.2±103.4a | 94.40±7.29a | 5.85±0.41a | 0.118±0.006a |
| $B_{20\%}R_{80\%}$ | 27.20±1.16a | 1,300.9±62.7a | 96.42±2.35a | 6.45±0.24a | 0.130±0.007a |
| $B_{10\%}R_{90\%}$ | 22.60±0.87bc | 1,176.8±59.6a | 91.48±4.60a | 5.68±0.37a | 0.114±0.001a |
| *mean* | *23.05* | *1,191.1* | *91.20* | *5.81* | *0.12* |
| Significance | * | ns | ns | ns | ns |

Table 7 - Plant growth after 35 days from planting. in endive 'Lindo' and lettuce 'Blackhawk' plants grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light. Mean values ± Standard errors; n=5. Significance and non-significance differences at P<0.05 are indicated as * and ns respectively. Different letters within each parameter and species are significantly different based on Tukey HSD *post-hoc* (P<0.05).

| | Number of leaves (N plant$^{-1}$) | Plant leaf area (cm$^2$ plant$^{-1}$) | Leaves fresh weight (g plant$^{-1}$) | Total dry weight (g plant$^{-1}$) | Root/Shoot ratio |
|---|---|---|---|---|---|
| Endive | | | | | |
| $B_{20\%}R_{80\%}$ | 61.20±5.12 | 918.22±46.32b | 58.61±4.07 | 5.73±0.46b | 0.26±0.02a |
| $B_{20\%}R_{80\%}+FR_{25}$ | 64.20±3.22 | 1639.06±180.16a | 91.43±12.40 | 8.83±0.75a | 0.27±0.03a |
| $B_{20\%}R_{80\%}+FR_{50}$ | 58.00±1.52 | 1373.27±103.72ab | 84.36±7.68 | 8.08±0.18ab | 0.16±0.02b |
| $B_{20\%}R_{80\%}+FR_{75}$ | 52.80±4.26 | 1299.29±124.95ab | 83.31±9.62 | 9.31±0.85a | 0.20±0.01ab |
| *mean* | *59.05* | *1307.46* | *79.43* | *7.99* | *0.22* |
| Significance | ns | * | ns | * | * |
| Lettuce | | | | | |
| $B_{20\%}R_{80\%}$ | 23.80±0.66 | 1244.11±95.21 | 99.40±9.60 | 7.48±0.15 | 0.13±0.01 |
| $B_{20\%}R_{80\%}+FR_{25}$ | 21.401.44 | 1175.64±146.66 | 82.81±13.46 | 6.98±0.42 | 0.16±0.02 |
| $B_{20\%}R_{80\%}+FR_{50}$ | 22.20±1.20 | 1377.35±104.12 | 97.98±10.22 | 7.03±0.78 | 0.15±0.02 |
| $B_{20\%}R_{80\%}+FR_{75}$ | 21.40±0.93 | 970.79±91.40 | 75.00±9.41 | 5.58±0.38 | 0.17±0.02 |
| *mean* | *22.20* | *1191.97* | *88.80* | *6.76* | *0.15* |
| Significance | ns | ns | ns | ns | ns |

***Polyphenolic profile***

Principal component analysis (PCA) showed no clear separation between the two species grown in the Experiment 1 in the polyphenolic profile. However, the 66.1% of the total variance (PC1) was determined by the lighting treatments, with high similarity between both species grown under CW or under $B_{25\%}R_{75\%}$ light, moreover the $B_{10\%}R_{90\%}$ treatment determined a different profile compared to CW, while the $B_{20\%}R_{80\%}$ treatment influenced differently endive and lettuce polyphenolic profile (Figure 5).

In the Experiment 2, PCA analysis of the polyphenolic profile of endive and lettuce plants revealed that the species accounted for the 65.1% of the total variance (PC1) and the light treatments accounted for the 14% of the total variance (PC2). The cluster analysis, integrated in the PCA output at Euclidean distance considering complete linkage between samples, highlines a different polyphenolic profile in the two species grown under a BR spectrum and a similar effect of FR on the profile of plants of the same species grown with the addition of 75 μmol $m^{-2}$ $s^{-1}$ of FR light to a fixed BR spectrum (Figure 6).

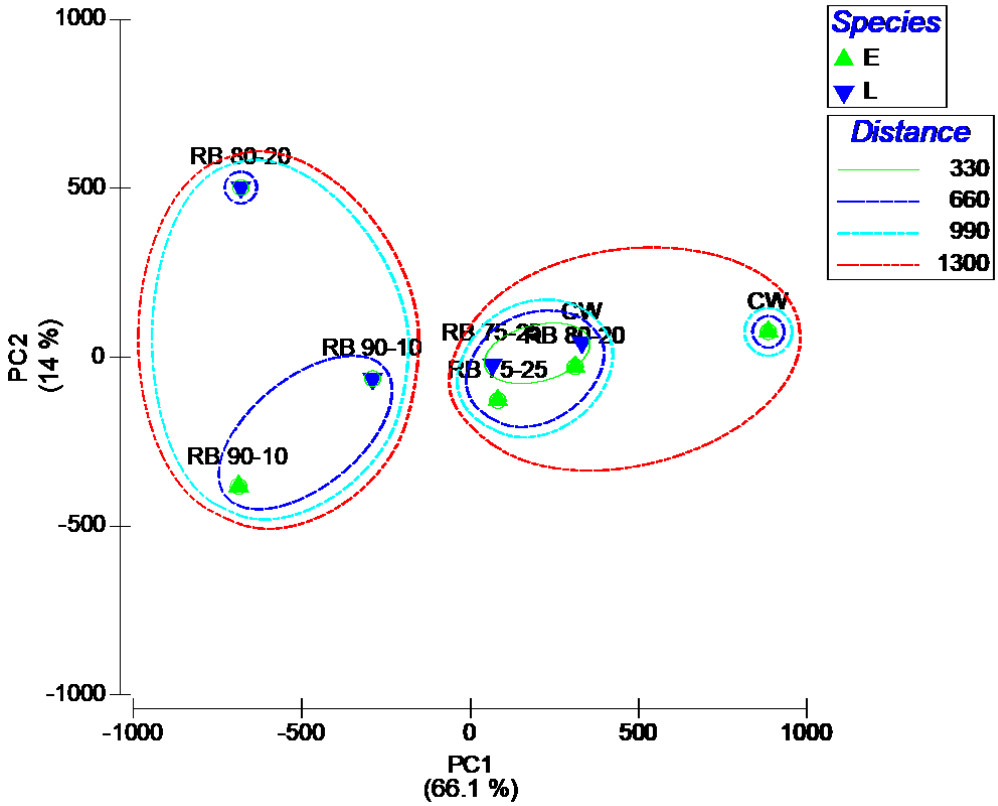



Figure 5- Principal component loading plot and scores of principal component analysis (PCA) integrated with cluster analysis at Euclidean distance with complete linkage of the polyphenols profile between 8 and 28 min of retention time, detected at 250 nm using a Thermo Dionex Ultimate 3000 UHPLC (ThermoScientific, Landsmeer, The Netherlands), equipped with diode array detector (DAD) and Waters Acquity BEHC18 column. in samples of endive 'Lindo' (E) and lettuce 'Blackhawk' (L) plants grown in the Experiment 1 in a growth chamber in floating system under artificial light provided by LEDs white (CW) or red and blue ratios RB 75-25, RB 80-20 or RB 90-10 at a PPFD of 245 µmol $m^2$ $s^{-1}$.

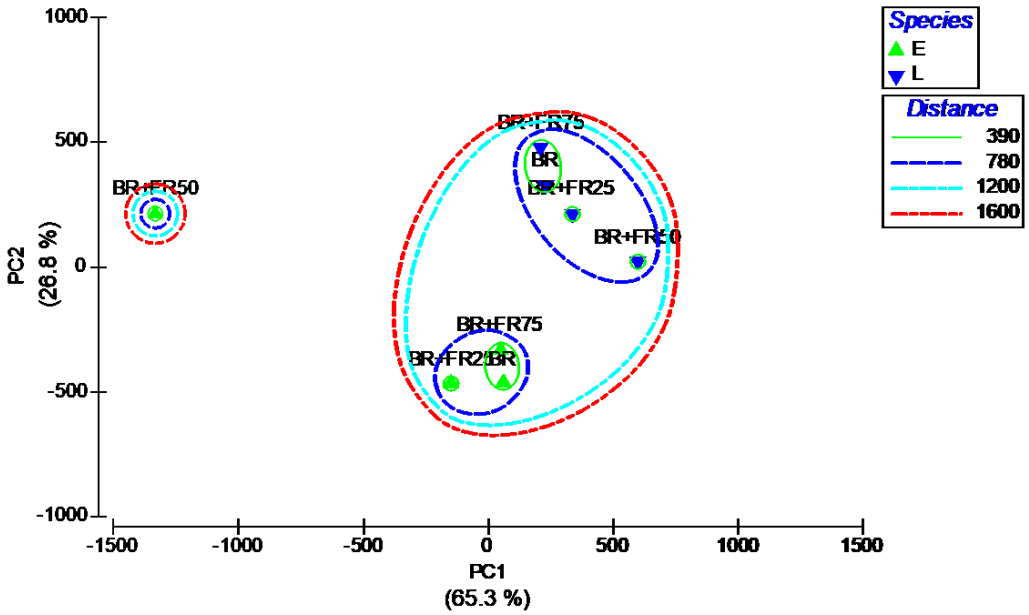



Figure 6- - Principal component loading plot and scores of principal component analysis (PCA) integrated with cluster analysis at Euclidean distance with complete linkage of the polyphenols profile between 8 and 28 min of retention time, detected at 250 nm using a Thermo Dionex Ultimate 3000 UHPLC (ThermoScientific, Landsmeer, The Netherlands), equipped with diode array detector (DAD) and Waters Acquity BEHC18 column, in samples of endive 'Lindo' (E) and lettuce 'Blackhawk' (L) plants grown in the Experiment 2 in a growth chamber in floating system under artificial light provided by LEDs BR 20:80 ratio (BR) at a PPFD of 245 µmol $m^2$ $s^{-1}$ or BR plus 25, 50 or 75 µmol $m^2$ $s^{-1}$ of FR light.

**Discussion**

The use of lettuce varieties for indoor production under artificial light has been well studied by several authors (Li and Kubota, 2009; Bantis et al., 2016; Meng et al., 2019).

The measured photochemical indexes indicate a good health status of the photosynthetic apparatus in both species, being the Fv/Fm ratio of both endive and lettuce plants close to 0.8 under all the different lighting treatments (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004). In fact, endive plants grown in the Experiment 1 under CW or different B:R ratio showed no difference in the linear ETR indicating similar capacity to absorb photons, however the different B:R light treatments determined different antenna capability to intercept light, as indicated by the higher Chloropyll *a*/*b* ratio (Evans, 1995; Vogelmann and Evans, 2002). However, these differences did not influence the leaf optical properties and the relative vegetation indexes G, MCARI and PRI that were similar between plants grown under different lighting treatment. In endive plants, as the linear ETR does not change in response to the different light treatments, conversely to *Pn*, we suppose a different utilization of reductive power under the diverse light treatments. More specifically, it may be hypothesised that in the treatment $B_{20\%}R_{80\%}$ more electrons were driven into photosynthetic process as showed by the higher *Pn* and *gs* values, while under the other light treatments the electron transport power is likely diverted toward other photochemical processes such as photorespiration or water-water cycle (Asada, 1999; Johnson, 2016; Friedland et al., 2019). This could explain the absence of difference in plant growth: in fact, no biomass accumulation was found in these plants.

Photochemistry and gas exchanges in lettuce plants grown in the Experiment 1 were influenced by the lighting treatments similarly to endive plants. However, compared to endive they synthesized a higher content of photosynthetic pigments especially under the specific B:R ratio of $B_{25\%}R_{75\%}$ and $B_{10\%}R_{90\%}$. It is known that chlorophylls absorb light in the B and in R spectrum region and that light treatments with B and R light induce modification in leaf thickness that may affect leaf absorbance and transmittance within cell layers (Arena et al., 2016; Zheng and Van Labeke, 2017),

moreover, change in leaf optical properties, may be the results of variation in specific pigments profile that result in different sensitivity of indexes like the PRI associated with xanthophyll content and to variation in the chlorophylls : carotenoids ratio (Sims and Gamon, 2002). The interplay between assimilative and dissipative process, in lettuce grown under BR light treatment, determined an higher number as well as an higher biomass of leaves. This results is consistent with data reported by Amitrano et al. (2013) which found in mung bean an increase of leaf number and leaf biomass. It is noteworthy that the increase of the B fraction into a BR spectrum even if it is species dependent (Hernández and Kubota, 2016; Wang et al., 2016; Naznin et al., 2019) may interact at both plant physiological level, promoting stomata conductance (Arena et al., 2016) and at biochemical level affecting gibberellic acid biosynthesis and differentiation in the meristematic tissues (Fukuda, 2019).

In the experiment 2, endive and lettuce plants showed a different behaviour in response to the addition of FR light to the spectrum despite the Fv/Fm ratio was similar and close to 0.8 in all plants from the different treatments. In fact, the ETR light response curves shows a FR intensity-dependent response decreasing photochemical capacity at high irradiance in lettuce, on the contrary, in endive, the addition of 75 μmol $m^{-2}$ $s^{-1}$ of FR light to the spectrum promotes the electron transport, increasing the $ETR_{max}$, the $I_k$ and α, with values comparable with plants grown without FR (Platt et al., 1980). Moreover, endive plants resulted less green as indicated by the Greenness index in line with the decrease of photosynthetic pigment content. This results is consistent with findings of other authors (Li and Kubota, 2009; Chen et al., 2016; Lee et al., 2016; Meng and Runkle, 2019) which attributed the chlorophyll decline to a reduced expression of Phytochrome B inducing chloroplast modification by modifying thylakoid structures and protein composition (Demotes-Mainard et al., 2016; and references therein). Lettuce plants instead resulted greener under FR light, despite the absence of difference in the total chlorophyll content, this result can be due to changes in leaves absorbance mainly in the green region of the spectrum as shown by the decrease of anthocyanin content indicated by the ARI 1 index. It is noteworthy that anthocyanin synthesis is mediated by Phytochromes A and B antagonistic effect and by their complex interaction with Cryptochromes on plant

photomorphogenesis and pigmentation (Kerckhoffs et al., 1992; Li and Kubota, 2009; Meng and Runkle, 2019). The addition of FR light to the light environment may have determined at antenna level an adaptive response inducing a reduction in the antenna size in both the species, as indicated by the lower Chloropyll *a*/*b* ratio (Dale and Causton, 1992; Vogelmann and Evans, 2002). In lettuce this adaptive response determined variation in the PRI index, related to changes in xanthophylls content and to chlorophyll /carotenoids ratio (Sims and Gamon, 2002) and a decrease in total carotenoid content in accordance with other studies reporting a negative effect of the increasing FR light (decreasing the PPE) on carotenoid content (Li and Kubota, 2009; Kalaitzoglou et al., 2019). The effect of FR on photosynthesis is not completely clear, however different morpho-physiological traits involved in the photosynthetic process from stomata opening regulation, leaf morphological and anatomical traits and down regulation of some enzyme in the Calvin cycle are regulated by phytochrome B and R and FR light (Demotes-Mainard et al., 2016). Moreover despite FR photons are not active photosynthetic radiation, they can contribute to increase *Pn* thanks to the Emerson enhancement effect (Emerson et al., 1957; Pettai et al., 2005). In our experiment, at growing irradiance, *Pn* decreased, in both species, in response to the increasing of FR light intensity. A recent study Kalaitzoglou et al. (2019) on tomatoes, reported a positive effect of FR on photosynthesis at a PPE of 0.80 while the lowering of PPE, increasing the FR component in the spectrum, did not contributed to increase leaf photosynthesis. Furthermore, contrasting information on FR influence on photosynthesis are reported on *Phaseolus vulgaris* L. (Holmes et al., 1986; Barreiro et al., 1992). Photo-morphogenetic variations at plant level are induced by the interaction between different wavelengths of the spectrum (Devlin et al., 2007; Dueck et al., 2016). B and FR radiation interacts each other causing opposite morphological changes at plant level. B radiation regulates cryptochromes to suppress extension of growth, leaf expansion and hence total plant weight, on the other hand, FR radiation through phytochromes result in the opposite effect and interact with R light to activate or suppress shade-avoidance response mediated by phytochromes (Franklin, 2008b), B and FR effect is mainly related to the B intensity, in our experiment, as expected the addition of FR, and hence lowering the R:FR ratio promoted, only in endive, leaf

expansion than new leaves formation, resulting in an higher leaf area accomplish to increase the total dry weight compared to plants grown without FR (Demotes-Mainard et al., 2016; Lee et al., 2016; Park and Runkle, 2018), on the contrary in lettuce we did not observe any differences.

**Conclusions**

Plant response to light quality and FR addition to light spectrum is species-dependent; in our experiments we report for the first time light effects on curly endive and showed its feasibility to be considered as a promising crop for the cultivation in plant factory under artificial light. Our results highlight the different response of endive compared to lettuce to light quality modulation in photosynthetic process as well as in the production of polyphenols. In particular, a good balance between B and R light is needed to promote gas exchanges and different BR ratios have a direct effect on the polyphenolic profile of both species. The addition of FR photons to a fixed BR spectrum does not contribute to the Emerson enhancement effect and does not reduce leaf net photosynthesis. However, FR light at above a certain irradiance promotes photochemical process and changes in leaf optical properties as indicated by different vegetation indexes and polyphenolic profile. Further analysis focused on the quantification of the different phenolic compounds are ongoing and will clarify the effect of BR light modulation and FR light on the biosynthesis of these compounds.

To conclude, light quality modulation may be considered a powerful mean to modify qualitative attributes of leafy vegetables in the view of their production on large scale even if the specific response typical of each species should be considered in the application of light protocols for plant growth.

**Authors contribution statement**

GCM and MCVL conceived and designed the experiments. GCM and CG developed the polyphenol protocol and CG performed the polyphenol analysis. GCM performed the experiments, analyzed the data and drafted the

manuscript. MCVL, CA and RP critically revised the manuscript. All authors reviewed and approved the final manuscript.

# Overall conclusions

During the PhD program, the effect of light modulation was investigated on ornamentals and leafy vegetables species taking into account the tree dimensions of the light: duration, quality and intensity. In particular, the attention was focused on photosynthetic behaviour, primary and secondary metabolites production and plant photo-morphogenetic response to light.

The choice of *R. asiaticus* as ornamental species for our studies was due to the growing demand on the market for this species as well as to the missing information on its physiology in literature. The first experiment was addressed to enlarge the knowledge on *R. asiaticus* physiology, considering the influence of the preparation procedures, especially cold treatments/vernalization and their specific control on plant growth, photosynthesis and metabolic profile.

The results of the first experiment highlighted the occurrence of different morpho-physiological and biochemical traits in the selected *R. asiaticus* hybrids (MDR - medium earliness; MBO - early flowering) and a different response of these hybrids to the preparation procedure of the tuberous roots. Our data indicated that the dissimilar behaviour between the two hybrids could be linked to a genotype specific response in terms of cold requirement (duration and temperature) as well as to the different size of the tuberous roots, confirming that the size of propagation material should be considered in the selection of the hybrid (Meynet, 1974; Beruto et al., 2018).

In case of long vernalization treatment the cold stress could induce dentrimental outcomes on plant growth, flowering earliness and quality of the flower stems as we observed in the hybrid MDR, while the hybrid MBO, in response to vernalization showed a fine tuning of selected sugars and amino acids in tuberous roots, which prepared plants for flowering. In particular, the action of protectant metabolites in tuberous roots helped plants to cope with the vernalization related-cold stress. In rosette tissues the metabolism is diverted toward the utilization or accumulation of particular amino acids and sucrose functions to promote flowering. At photosystem level, chlorophyll *a* fluorescence measurements indicated the efficacy of tuberous roots vernalization treatments to promote plant growth and flowering. In particular, in the hybrid MBO, in which vernalization showed a positive effect, PSII photochemical efficiency was promoted as well as an higher photosynthetic pigment content resulting in a faster growth and early flowering for this hybrid.

A further experiment on *R. asiaticus* aimed to assess the influence of light quality in photoperiodic treatments to promote flowering. This issue was never studied before on this species. Previous experiments on the effect of photoperiodic lighting carried out considered only the response to photoperiod variation excluding the influence of the light spectral composition used to extend the day length (Farina et al., 1985; Ohkawa, .1986; De Pascale and Scogliamiglio, 1998)

Our results demonstrated that the significant changes in morphological and physiological traits of *R. asiaticus* plants were related to both genotype and light quality of photoperiodic treatments. The photoperiodic lighting promoted in both hybrids photochemistry and reduced the thermal dissipation processes, especially under the photoperiodic treatment R:FR 3:1, as shown by the ΦPSII decline and NPQ rise. Moreover, the different light spectrum of photoperiodic lighting directly influenced the metabolic profile in both hybrids. In particular, the treatment R:FR 3:1 increased chlorophyll content in both hybrids, and fructose, sucrose and ethanolamine (MEA) content only in MDR. On the contrary, the treatment R:FR 1:3 promoted polyphenols content in both hybrids. MBO and MDR plants showed a different metabolic profile: the total amino acid content increased in MBO compared to MDR independently of the applied light treatment. Contextually, in MBO, the increase in total amino acids observed in the treatment R:FR 3:1 was mainly due an increased in minor amino acids and Branched-Chain Amino Acids (BCAAs) without no difference in the hexose content. Conversely, the higher amino acid content observed in MBO grown under R:FR 1: 3 treatment was mainly due to an increase in primary amino acids and GABA. As result, the metabolic and physiological differences related to the genotype and/ or to the light treatment regulated plant growth and positively flowering earliness only in MBO plants. Photoperiodic lighting with R:FR LEDs, compared to NL and FL, promoted leaf expansion in plants; contributing to increase whole plant photosynthetic rate. These results may explain the reason why photoperiodic lighting anticipated the time of appearance of the flower buds only in MBO with no effect on MDR compared to plants grown under NL.

Our data also demonstrated that in *R. asiaticus* flowering promotion by the photoperiodic lighting was mainly related to the spectral composition of the light source rather than the relative PPE value.

The last part of this PhD thesis aimed to evaluate the effect of light modulation and the addition of different intensity of FR light to improve the biosynthesis of antioxidant compound in leafy vegetables for indoor cultivation. The research contributes to improve the knowledge on *Chicorium endivia* L. (endive) physiology and for the first time the effect of light quality on this species.

Light modulation in terms of BR ratios highlight different photosynthetic response and polyphenols profile between endive and lettuce plants., however, among the different BR ratios the addition of 20% of blue light in the light source spectrum triggered promoted gas exchanges and plant growth in both species. The addition of FR photons to a fixed BR spectrum did not contribute to the Emerson enhancement effect; generally the Emerson enhancement effect on photosynthesis occurs when plants are exposed to both R and FR radiation and the rate of photosynthesis is higher than the sum of the photosynthetic rate caused by R and FR radiation alone (Emerson et al., 1957). In fact, the addition of FR mitigated the photosynthesis decline in both species. On the contrary, the addition of FR modified in both species leaf optical properties, polyphenolic profile and under high irradiance conditions promoted photochemical process only in endive. Photomorphological variation were induced by the addition of FR only in endive where FR promoted biomass allocation to the shoot increasing leaf expansion and the total dry matter content.

To conclude, light modulation in terms of duration, quality and intensity can be considered a powerful tool to modify qualitative attributes in ornamentals and leafy vegetables.

From our studies on *R. asiaticus* in response to photoperiodic lighting we can state that a high R:FR may be more effective on flowering promotion without negative effect on flower stems quality, but its efficacy depends on different genotypes.

Qualitative attributes in leafy vegetables can be modified modulating the spectral composition of the light source and the addition of FR light can contribute to improve plant growth and polyphenolic profile; however, further studies on the precise amount of FR photons are needed to elucidate its role in the biosynthesis of antioxidant compounds.

The results of this thesis may have scientific as well as practical significance. They may be transferred into the production sector and applied at more large scale in greenhouses or in systems of indoor cultivation to implement the yield of crop and ornamental species.

# Literature cited

Amitrano, C., Vitale, E., Micco, V. De, and Arena, C. (2013). Light Fertilization Affects Growth and Photosynthesis in Mung Bean (*Vigna radiata*) *Plants Journal of Environmental Accounting and Management Plants*. 7. doi:10.5890/JEAM.2018.12.002.

Annunziata, M.G., Apelt, F., Carillo, P., Krause, U., Feil, R., Mengin, V., Lauxmann, M.A., Köhl, K., Nikoloski, Z., Stitt, M., and Lunn, J.E. (2017). Getting back to nature: a reality check for experiments in controlled environments. *J. Exp. Bot*. 68, 4463–4477. doi: https://doi.org/10.1093/jxb/erx220

Annunziata, M.G., Carillo, P., Fuggi, A., Troccoli, A. and Woodrow, P. (2013). Metabolic profiling of cauliflower under traditional and reduced tillage systems. *Aust. J. Crop Sci*. 7, 1317–1323.

Arena, C., Tsonev, T., Doneva, D., Micco, V. De, Michelozzi, M., and Brunetti, C. (2016). The effect of light quality on growth, photosynthesis, leaf anatomy and volatile isoprenoids of a monoterpene-emitting herbaceous species (*Solanum lycopersicum* L.) and an isoprene-emitting tree (*Platanus orientalis* L.*). Environ. Exp. Bot*. 130, 122–132. doi:10.1016/j.envexpbot.2016.05.014.

Asada, K. (1999). THE WATER-WATER CYCLE IN CHLOROPLASTS: Scavenging of Active Oxygens and Dissipation of Excess Photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 50, 601–639. doi:10.1146/annurev.arplant.50.1.601.

Baçzek-Kwinta, R., Serek, B., and Wator, A. (2007). Effect of chilling on total antioxidant capacity and growth processes of basil (*Ocimum basilicum* L.) cultivars. *Herba Pol*. 53, 75–84.

Baker, N. R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annu. Rev. Plant Biol*. 59, 89–113. doi:10.1146/annurev.arplant.59.032607.092759.

Baker, N. R., and Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot*. 55, 1607–1621. doi: 10.1093/jxb/erh196

Bantis, F., Ouzounis, T., and Radoglou, K. (2016). Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success. *Sci. Hortic*. (Amsterdam). 198, 277–283.

Barrera-Aguilar, E., Valdez-Aguilar, L. A., Castillo-González, A. M., Cartmill, A. D., Cartmill, D. L., Avitia-García, E., et al. (2013). Potassium nutrition in *Lilium*: critical concentrations, photosynthesis, water potential, leaf anatomy, and nutrient status. *HortScience* 48, 1537–1542. doi: 10.21273/HORTSCI.48.12. 1537

Barreiro, R., Guiamét, J. J., Beltrano, J., and Montaldi, E. R. (1992). Regulation of the photosynthetic capacity of primary bean leaves by the red:far red ratio and photosynthetic photon flux density of incident light. *Physiol. Plant*. 85, 97–101. doi:10.1111/j.1399-3054.1992.tb05269.x.

Bekhradi, F., Delshad, M., Marín, A., Luna, M. C., Garrido, Y., Kashi, A., et al. (2015). Effects of salt stress on physiological and postharvest quality characteristics of different Iranian genotypes of basil. *Hortic. Environ. Biotechnol*. 56, 777–785. doi: 10.1007/s13580-015-1095-9

Bendeck de Cantú, L., and Kandeler, R. (1989). Significance of polyamines for flowering in *Spirodela punctata*. *Plant Cell Physiol*. 30, 455–458. doi: 10.1093/ oxfordjournals.pcp.a077762

Bernier, G., Havelange, A., Houssa, C., Petitjean, A., and Lejeune, P. (1993). Physiological signals that induce flowering. *Plant Cell*. 5, 1147–1155. doi: 10. 1105/tpc.5.10.1147

Beruto, M., Fibiani, M., Rinino, S., Lo Scalzo, R., and Curir, P. (2009). Plant development of *Ranunculus asiaticus* L. tuberous roots is affected by different temperature and oxygen conditions during storage period. *Israel J. Plant Sci*. 57, 377–388. doi: 10.1560/IJPS.57.4.377

Beruto, M., Rabaglio, M., Viglione, S., Van Labeke, M. C., and Dhooghe, E. (2018). "*Ranunculus*," in Ornamental Crops. *Handbook of Plant Breeding*, Vol. 11, ed. J. Van Huylenbroeck (Cham, CH: Springer International Publishing AG), 649–671. doi: 10.1007/978-3-319-90698-0-25

Bilger, W., and Björkman, O. (1990). Role of the xantophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and

photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res*. 25, 173–185. doi: 10.1007/BF00033159

Björkman, O., and Demmig, B. (1987). Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170, 489–504. doi: 10.1007/BF00402983

Blanchard, M. G., and Runkle, E. S. (2009). Use of a cyclic high-pressure sodium lamp to inhibit flowering of chrysanthemum and velvet sage. *Sci. Hortic. (Amsterdam).* 122, 448–454. doi:10.1016/j.scienta.2009.06.016.

Boccalandro, H.E., Rugnone, M.L., Moreno, J.E., Ploschuk, E.L., Serna, L., Yanovsky, M.J. and Casal, J.J. ( 2009). Phytochrome B enhances photosynthesis at the expense of water-use efficiency in *Arabidopsis*. *Plant Physiol*. 150, 1083 – 1092. doi: https://doi.org/10.1104/pp.109.135509

Borghi, M., and Fernie, A. R. (2017). Floral metabolism of sugars and amino acids: implications for pollinators' preferences and seed and fruit set. *Plant Physiol*. 175, 1510–1524. doi: 10.1104/pp.17.01164

Borowski, E., and Blamowski, Z. K. (2009). The effects of triacontanol 'TRIA' and Asahi SL on the development and metabolic activity of sweet basil (*Ocimum basilicum* L.) plants treated with chilling. *Folia Hort*. 21, 39–48. doi: 10.2478/ fhort-2013-0124

Bouché, N., Fait, A., Bouchez, D., Møller, S. G., and Fromm, H. (2003). Mitochondrial succinic-semialdehyde dehydrogenase of the g-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants. *Proc. Natl. Acad. Sci. U.S.A*. 100, 6843–6848. doi: 10.1073/pnas.10375 32100

Carillo, P. (2018). GABA Shunt in durum wheat. *Front. Plant Sci*. 9:100. doi: 10.3389/fpls.2018.00100

Carillo, P., Cacace, D., De Pascale, S., Rapacciuolo, M., and Fuggi, A. (2012). Organic vs. traditional potato powder. *Food Chem*. 133, 1264–1273. doi: 10. 1016/j.foodchem.2011.08.088

Carillo, P., Arena, C., Modarelli, G.C., De Pascale, S. and Paradiso, R. (2019a). Photosynthesis in *Ranunculus asiaticus* L.: The influence of the hybrid and the preparation procedure of tuberous roots. *Front. Plant Sci*. 10, 241. doi: https://doi.org/10.3389/fpls.2019.00241

Carillo, P., Cacace, D., De Pascale, S., Rapacciuolo, M. and Fuggi, A. (2012). Organic vs. traditional potato powder. *Food Chem*. 133, 1264–1273. doi: https://doi.org/10.1016/j.foodchem.2011.08.088

Carillo, P., Kyriacou, M. C., El-Nakhel, C., Pannico, A., Dell'Aversana, E., D'Amelia, L., Colla, G., Caruso, G., De Pascale, S. and Rouphael, Y. (2019b). Sensory and functional quality characterization of protected designation of origin 'Piennolo del Vesuvio' cherry tomato landraces from Campania-Italy. *Food Chem.* 292, 166–175. doi:10.1016/j.foodchem.2019.04.056.

Cartea, M. E., Francisco, M., Soengas, P., and Velasco, P. (2011). Phenolic compounds in Brassica vegetables. *Molecules* 16, 251–280. doi:10.3390/molecules16010251.

Casal, J. J. (2013). Photoreceptor Signaling Networks in Plant Responses to Shade. *Annu. Rev. Plant Biol.* 64, 403–427. doi:10.1146/annurev-arplant-050312-120221.

Chen, M., Galvão, R. M., Li, M., Burger, B., Bugea, J., Bolado, J., et al. (2010). *Arabidopsis* HEMERA/pTAC12 Initiates Photomorphogenesis by Phytochromes. *Cell* 141, 1230–1240. doi:10.1016/j.cell.2010.05.007.

Chen, X., Xue, X., Guo, W., Wang, L., and Qiao, X. (2016). Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental light provided by light-emitting diode. *Sci. Hortic*. (Amsterdam). 200, 111–118. doi:10.1016/J.SCIENTA.2016.01.007.

Ciarmiello, L. F., Piccirillo, P., Carillo, P., De Luca, A., and Woodrow, P. (2015). Determination of the genetic relatedness of fig (*Ficus carica* L.) accessions using RAPD fingerprint and their agro-morphological characterization. *S. Afr. J. Bot*. 97, 40–47. doi: 10.1016/j.sajb.2014.11.012

Craig, D. S., and Runkle, E. S. (2016). An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants. *Environ. Exp. Bot.* 121, 132–138. doi:10.1016/j.envexpbot.2015.04.004.

Dale, A. M. P., and Causton, D. R. (1992). Use of the Chlorophyll a / b Ratio as a Bioassay for the Light Environment of a Plant. *Funct. Ecol*. 6, 190–196.

De Hertogh, A. (1996). *Holland Bulb Forcer's Guide*. 5th ed. , ed. A. De Hertogh Alkemade Printing BV.

De Micco, V., Paradiso, R., Aronne, G., De Pascale, S., Quarto, M. and Arena, C. (2014). Leaf anatomy and photochemical behaviour of *Solanum lycopersicum* L. plants from seeds irradiated with low-LET ionising radiation. *Scientific World Journal*. 2014, 428141. doi: https://doi.org/10.1155/2014/428141

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., et al. (2016). Plant responses to red and far-red lights, applications in horticulture. *Environ. Exp. Bot.* 121, 4–21. doi:10.1016/j.envexpbot.2015.05.010.

De Pascale, S., and Scognamiglio, D. (1998). Effetti del fotoperiodo e dell'acido gibberellico su *Ranunculus asiaticus*. *Colture Protette*. 9, 107–112.

Devlin, P. F., Christie, J. M., and Terry, M. J. (2007). Many hands make light work. *J. Exp. Bot.* 58, 3071–3077. doi:10.1093/jxb/erm251.

Dudt, F. J., and Shure, D. J. (1994). The Influence of Light and Nutrients on Foliar Phenolics and Insect Herbivory. *Ecology* 75, 86–98.

Dueck, T., van Ieperen, W. and Taulavuori, K. (2016). Light perception, signalling and plant responses to spectral quality and photoperiod in natural and horticultural environments. *Environ. Exp. Bot*. 121, 1–3. https://doi.org/10.1016/j.envexpbot.2015.06.012

DuPont, M. S., Day, A. J., Bennett, R. N., Mellon, F. A., and Kroon, P. A. (2004). Absorption of kaempferol from endive, a source of kaempferol-3-glucuronide, in humans. *Eur. J. Clin. Nutr*. 58, 947–954. doi:10.1038/sj.ejcn.1601916.

Eilers, P. H. C., Peeters, J. C. H., Vranová, E., Coman, D., Gruissem, W., Dieleman, J. A., et al. (2019). Integrating Morphological and Physiological Responses of Tomato Plants to Light Quality to the Crop Level by 3D Modeling. *Mol. Plant*. 5, 318–333. doi:10.1093/mp/sss015.

Emerson, R., Chalmers, R., and Cederstrand, C. (1957). Some factors influencing the long-wave limit of photosynthesis. *Proc. Natl. Acad. Sci*. 43, 133–143. doi:10.1073/pnas.43.1.133.

Evans, J. R. (1995). Carbon fixation profiles do reflect light absorption profiles in leaves. *Funct. Plant Biol*. 22, 865–873. doi:10.1071/PP9950865.

Farina, E., Paterniani, T., and Suffia, G. (1985). Prove sperimentali sulla reattività del ranuncolo ibrido al fotoperiodo. *Annali dell'Istituto Sperimentale per la Fioritura San Remo*. 16, 93–99.

Ferchichi, S., Hessini, K., Dell'Aversana, E., D'Amelia, L., Woodrow, P., Ciarmiello, L.F., Fuggi, A. and Carillo, P. (2018). *Hordeum vulgare* and *Hordeum maritimum* respond to extended salinity stress displaying different temporal accumulation pattern of metabolites. *Funct. Plant Biol*. 45, 1096. https://doi.org/10.1071/fp18046

Franklin, K. A. (2008). Shade avoidance. *New Phytol*. 179, 930–944. doi:10.1111/j.1469-8137.2008.02507.x.

Frederic Dudt, J., and Shure, D. J. (1994). The Influence of Light and Nutrients on Foliar Phenolics and Insect Herbivory. *Ecology* 75, 86–98.

Friedland, N., Negi, S., Vinogradova-Shah, T., Wu, G., Ma, L., Flynn, S., et al. (2019). Fine-tuning the photosynthetic light harvesting apparatus for improved photosynthetic efficiency and biomass yield. *Sci. Rep*. 9, 1–12. doi:10.1038/s41598-019-49545-8.

Fritz, C., Mueller, C., Matt, P., Feil, R. and Stitt, M. (2006). Impact of the C-N status on the amino acid profile in tobacco source leaves. *Plant, Cell Environ*. 29, 2055–2076. https://doi.org/10.1111/j.1365-3040.2006.01580.x

Fritz, C., Palacios-Rojas, N., Feil, R., and Stitt, M. (2006). Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. *Plant J*. 46, 533–548. doi: 10.1111/j. 1365-313X.2006.02715.x

Fukuda, N. (2019). Plant Growth and Physiological Responses to Light Conditions. *Plant Fact. Using Artif. Light*, 71–77. doi:10.1016/B978-0-12-813973-8.00008-7.

Fukuda, N., Ajima, C., Yukawa, T., and Olsen, J. E. (2016). Antagonistic action of blue and red light on shoot elongation in petunia depends on gibberellin, but the effects on flowering are not generally linked to gibberellin. *Environ. Exp. Bot.* 121, 102–111. doi:10.1016/j.envexpbot.2015.06.014.

Geigenberger, P., Lerchi, J., Stitt, M., and Sonnewald, U. (1996). Phloem specific expression of pyrophosphatase inhibits long distance transport of carbohydrates and amino acids in tobacco plants. *Plant Cell Environ*. 19, 43–55. doi: 10.1111/j.1365-3040.1996.tb00225.x

Genty, B., Briantais, J.-M. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta - Gen. Subj*. 990, 87–92. doi:10.1016/S0304-4165(89)80016-9.

Hamrick, D. (2003). *Ball Redbook*, 17th Edn, Vol. 2. Batavia, IL: Ball Publishing.

Haroun, S. A., Shukryshy, W. M., Abbas, M. A., and Mowafy, A. M. (2011). Growth and physiological responses of *Solanum lycopersicum* to atonik and benzyl adenine under vernalized conditions. *J. Ecol. Nat. Environ*. 3, 319–331.

Hassan, H. A., Agina, E. A., Koriesh, E. M., and Mohamed, S. M. (1985). Physiological studies on *Anemone coronaria* L. and *Ranunculus asiaticus* L. 2. Effect of gibberellic acid. *Ann. Agric. Sci. Moshtohor*. 22, 583–595.

Horovitz, A. (1985). "Ranunculus," in *Handbook of Flowering*, Vol. 4, ed. A. Halevy (Boca Raton, FL: CRC Press), 155–161.

Hernández, R., and Kubota, C. (2016). Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot*. 121, 66–74. doi:10.1016/J.ENVEXPBOT.2015.04.001.

Holmes, M. G., Sager, J. C., and Klein, W. H. (1986). Sensitivity to far-red radiation in stomata of *Phaseolus vulgaris* L.: Rhythmic effects on conductance and photosynthesis. *Planta* 168, 516–522. doi:10.1007/BF00392271.

Holopainen, J. K., Kivimäenpää, M., and Julkunen-Tiitto, R. (2018). New Light for Phytochemicals. *Trends Biotechnol.* 36, 7–10. doi:10.1016/J.TIBTECH.2017.08.009.

Horovitz, A., 1985. *Ranunculus*, in: Handbook of Flowering Vol.4. CRC Press, Boca Raton FL, pp. 155–161.

Horton, P., Ruban, A. V., and Walters, R. G. (1996). The dissipation of excess excitation energy in British plant species. *Plant Cell Environ*. 16, 673–679.

Huché-Thélier, L., Crespel, L., Gourrierec, J. Le, Morel, P., Sakr, S., and Leduc, N. (2016). Light signaling and plant responses to blue and UV radiations - Perspectives for applications in horticulture. *Environ. Exp. Bot.* 121, 22–38. doi:10.1016/j.envexpbot.2015.06.009.

Hyöky, V., Oksanen, J., Taulavuori, E., Julkunen-Tiitto, R., Taulavuori, K., Hyöky, V., et al. (2016). Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light. *Environ. Exp. Bot.* 121, 145–150. doi:10.1016/j.envexpbot.2015.04.002.

Ishizaki, T., Ohsumi, C., Totsuka, K., and Igarashi, D. (2010). Analysis of glutamate homeostasis by overexpression of Fd-GOGAT gene in *Arabidopsis thaliana*. *Amino Acids*. 38, 943. doi: 10.1007/s00726-009-0303-2

Izzo, L. G., Arena, C., De Micco, V., Capozzi, F., and Aronne, G. (2019). Light quality shapes morpho-functional traits and pigment content of green and red leaf cultivars of *Atriplex hortensis*. *Sci. Hortic. (Amsterdam).* 246, 942–950. doi:10.1016/J.SCIENTA.2018.11.076.

Jang, I.C., Henriques, R., Seo, H. S., Nagatani, A., and Chua, N.-H. (2010). *Arabidopsis* PHYTOCHROME INTERACTING FACTOR Proteins Promote Phytochrome B Polyubiquitination by COP1 E3 Ligase in the Nucleus. *Plant Cell* 22, 2370–2383. doi:10.1105/tpc.109.072520.

Jenkins, G. I. (2009). Signal Transduction in Responses to UV-B Radiation. *Annu. Rev. Plant Biol.* 60, 407–431. doi:10.1146/annurev.arplant.59.032607.092953.

Johnson, G.N., Young, A.J., Scholes, J.D. and Horton, P. (1993). The dissipation of excess excitation energy in British plant species. *Plant. Cell Environ*. 16, 673–679. doi: https://doi.org/10.1111/j.1365-3040.1993.tb00485.x

Johnson, M. P. (2016). Photosynthesis. *Essays Biochem*. 60, 255–273. doi:10.1042/EBC20160016.

Kadomura-Ishikawa, Y., Miyawaki, K., Noji, S., and Takahashi, A. (2013). Phototropin 2 is involved in blue light-induced anthocyanin accumulation in *Fragaria x ananassa* fruits. *J. Plant Res*. 126, 847–857. doi:10.1007/s10265-013-0582-2.

Kalaitzoglou, P., van Ieperen, W., Harbinson, J., van der Meer, M., Martinakos, S., Weerheim, K., et al. (2019). Effects of continuous or end-of-day far-red light on tomato plant growth, morphology, light absorption, and fruit production. *Front. Plant Sci*. 10, 1–11. doi:10.3389/fpls.2019.00322.

Kalamaki, M. S., Merkouropoulos, G., and Kanellis, A. K. (2009). Can ornithine accumulation modulate abiotic stress tolerance in *Arabidopsis*? *Plant Signal. Behav*. 4, 1099–1101. doi: 10.4161/psb.4.11.9873

Kalisz, A., Jezdinský, A., Pokluda, R., Sêkara, A., Grabowska, A., and Gil, J. (2016). Impacts of chilling on photosynthesis and chlorophyll pigment content in juvenile basil cultivars. *Hortic. Environ. Biote*. 57, 330–339. doi: 10.1007/ s13580-016-0095-8

Kamenetsky, R., Peterson, R. L., Melville, L. H., Machado, C. F., and Bewley, D. (2005). Seasonal adaptations of the tuberous roots of *Ranunculus asiaticus* to desiccation and resurrection by changes in cell structure and protein content. *New Phytol*. 166, 193–204. doi: 10.1111/j.1469-8137.2004.01306.x

Kami, C., Lorrain, S., Hornitschek, P., and Fankhauser, C. (2010). "Chapter Two – Light-Regulated Plant Growth and Development," in *Current Topics in Developmental Biology*, 29–66. doi:10.1016/S0070-2153(10)91002-8.

Karlsson, M. (2003) Producing ravishing *Ranunculus*. *Greenhouse Product News*, January 2003: 44–48

Kerckhoffs, L. H. J., Kendrick, R. E., Whitelam, G. C., and Smith, H. (1992). Extension growth and anthocyanin responses of photomorphogenic tomato mutants to changes in the phytochrome-photoequulibrium during the daily photoperiod. *Photochem. Photobiol.* 56, 611–615. doi:10.1111/j.1751-1097.1992.tb02210.x.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., and Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. Food Compos. Anal*. 49, 19–34. doi:10.1016/j.jfca.2016.03.004.

Kitajima, K., Mulkey, S. S., Samaniego, M., and Wright, S. J. (2002). Decline of photosynthetic capacity with leaf age and position in two tropical pioneer tree species. *Am. J. Bot*. 89, 1925–1932. doi: 10.3732/ajb.89.12.1925

Kogan, M. J., Kristoff, G., Benavides, M. P., and Tomaro, M. L. (2000). Effect of pre-treatment with ethanolamine on the response of *Helianthus annuus* L. to salt stress. *Plant Growth Regul*. 30, 87–94. doi: 10.1023/A:1006360912089

Krall, J., and Edwards, G. (1992). Relationship between photosystem II activity and CO2 fixation in leaves. *Physiol. Plant*. 86, 180–187. doi: 10.1111/j.1399-3054. 1992.tb01328.x

Lea, P. J., and Forde, B. G. (2007). Glutamate in plants: metabolism, regulation, and signalling. *J. Exp. Bot*. 58, 2339–2358. doi: 10.1093/jxb/erm121

Lee, M. J., Son, K. H., and Oh, M. M. (2016). Increase in biomass and bioactive compounds in lettuce under various ratios of red to far-red LED light supplemented with blue LED light. *Hortic. Environ. Biotechnol.* 57, 139–147. doi:10.1007/s13580-016-0133-6.

Li, Q., and Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67, 59–64. doi:10.1016/J.ENVEXPBOT.2009.06.011.

Lichtenthaler, H. K., and Buschmann, C. (2001). Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Curr. Protoc. Food Anal. Chem*. 1, F4.3.1-F4.3.8. doi:10.1002/0471142913.faf0403s01.

Liu, J. H., Honda, C., and Moriguchi, T. (2006). Involvement of polyamine in floral and fruit development. *JARQ*. 40, 51–58. doi: 10.6090/jarq.40.51

Liu, F., Zhang, H., Wu, G., Sun, J., Hao, L., Ge, X., et al. (2011a). Sequence Variation and Expression Analysis of Seed Dormancy- and Germination-Associated ABA- and GA-Related Genes in Rice Cultivars. *Front. Plant Sci.* 2, 1–13. doi:10.3389/fpls.2011.00017.

Liu, H., Liu, B., Zhao, C., Pepper, M., and Lin, C. (2011b). The action mechanisms of plant cryptochromes. *Trends Plant Sci.* 16, 684–691. doi:10.1016/j.tplants.2011.09.002.

Lucchin, M., Varotto, S., and Barcaccia, G. (2008). Handbook of plant breeding volume I "Vegetables I.". doi:10.1007/978-0-387-30443-4.

Luo, P.G., Deng, K.J., Hu, X.Y., Li, L.Q., Li, X., Chen, J.B., Zhang, H.Y., Tang, Z.X., Zhang, Y., Sun, Q.X., Tan, F.Q. and Ren, Z.L. (2013). Chloroplast ultrastructure regeneration with protection of photosystem II is responsible for the functional 'stay-green' trait in wheat. *Plant. Cell Environ*. 36, 683–696. https://doi.org/doi:10.1111/pce.12006

Madsen, T. V. (1993). Growth and photosynthetic acclimation by *Ranunculus aquatilis* L. in response to inorganic carbon availability. *New Phytol*. 125, 707–715. doi: 10.1111/j.1469-8137.1993.tb03919.x

Madsen, T. V., and Brik, H. (1997). Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia*. 110, 320–327. doi: 10.1007/s004420050165

Martínez-García, J.F., Gallemí, M., Molina-Contreras, M.J., Llorente, B., Bevilaqua, M.R.R. and Quail, P.H. (2014). The shade avoidance syndrome in *Arabidopsis*: The antagonistic role of phytochrome A and B differentiates vegetation proximity and canopy shade. *PLoS One*. 9, e109275. https://doi.org/10.1371/journal.pone.0109275

Maxwell, K., and Johnson, G. (2000). Chlorophyll fluorescence-a practical guide. *J. Exp. Bot*. 51, 659–668. doi: 10.1093/jexbot/51.345.659

Mayoli, R. N., Isutsa, D. K., and Tunya, G. O. (2009). Growth of *Ranunculus* cut flower under tropical high-altitude conditions.1. Effects of GA3 and shade. *Afr. J. Hort. Sci*. 2, 13–28.

McCree, K. J. (1971). The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agric. Meteorol.* 9, 191–216. doi:10.1016/0002-1571(71)90022-7.

Meng, Q., Kelly, N., and Runkle, E. S. (2019). Substituting green or far-red radiation for blue radiation induces shade avoidance and promotes growth in lettuce and kale. *Environ. Exp. Bot*. 162, 383–391. doi:https://doi.org/10.1016/j.envexpbot.2019.03.016.

Meng, Y., Li, H., Wang, Q., Liu, B., and Lin, C. (2013). Blue light-dependent interaction between cryptochrome2 and CIB1 regulates transcription and leaf senescence in soybean. *Plant Cell* 25, 4405–4420. doi:10.1105/tpc.113.116590.

Meng, Q., and Runkle, E. S. (2018). Regulation of flowering by green light depends on its photon flux density and involves cryptochromes. *Physiol. Plant*., 762–771. doi:10.1111/ppl.12832.

Meng, Q. and Runkle, E. S. (2019). Far-red radiation interacts with relative and absolute blue and red photon flux densities to regulate growth, morphology, and pigmentation of lettuce and basil seedlings. *Sci. Hortic.* 255, 269–280. doi:10.1016/J.SCIENTA.2019.05.030.

Meynet, J. (1974). Research on *Ranunculu*s hybrid varieties for growing under protection. *Acta Hortic*. 43, 191–195. doi: 10.17660/ActaHortic.1974.43.21

Meynet, J. (1993). "*Ranunculus*" in Physiology of Flower Bulbs: A Comprehensive Treatise on the Physiology and Utilization of Ornamental Flowering Bulbous and Tuberous Plants, eds A. A. De Hertogh and M. Le Nard (Amsterdam: Elsevier), 603–610.

Moe, R., and Berland, M. (1986). Effect of various corm treatments on flowering of *Liatris spicata* wild. *Acta Hortic*. 177, 197–201. doi: 10.17660/ActaHortic.1986. 177.26

Mott K, Gibson A and O'Leary J. (1982). The adaptative significance of amphistomatic leaves. *Plant Cell Environ*. 5, 455-460.

Muhlemann, J. K., Klempien, A., and Dudareva, N. (2014). Floral volatiles: from biosynthesis to function. *Plant Cell Environ*. 37, 1936–1949. doi: 10.1111/pce. 12314

Muhlemann, J. K., Maeda, H., Chang, C.-Y., San Miguel, P., Baxter, I., Cooper, B., et al. (2012). Developmental changes in the metabolic network of snapdragon flowers. *PLoS One*. 7: e40381. doi: 10.1371/journal.pone.0040381

Naznin, M., Lefsrud, M., Gravel, V., and Azad, M. (2019). Blue Light added with Red LEDs Enhance Growth Characteristics, Pigments Content, and Antioxidant Capacity in Lettuce, Spinach, Kale, Basil, and Sweet Pepper in a Controlled Environment. *Plants*. 8, 93. doi:10.3390/plants8040093.

Nelson, J. A., and Bugbee, B. (2014). Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. *PLoS One*. 9, e99010. doi:10.1371/journal.pone.0099010.

Nguyen, N.M.P., Le, T.T., Vissenaekens, H., Gonzales, G.B., Van Camp, J., Smagghe, G. and Raes, K. (2019). In vitro antioxidant activity and phenolic profiles of tropical fruit by-products. *Int J Food Sci Technol*, 54: 1169-1178. doi:10.1111/ijfs.14093

Ohkawa, K. (1986). Growth and flowering of *Ranunculus asiaticus*. *Acta Hort*. 177, 165–177. doi: 10.17660/ActaHortic.1986.177.22

Okajima, K. (2016). Molecular mechanism of phototropin light signaling. *J. Plant Res*. 129, 149–157. doi:10.1007/s10265-016-0783-6.

Ouzounis, T., Rosenqvist, E., and Ottosen, C. O. (2015). Spectral effects of artificial light on plant physiology and secondary metabolism: A review. *HortScience* 50, 1128–1135.

Paik, I., and Huq, E. (2019). Plant photoreceptors: Multi-functional sensory proteins and their signaling networks. *Semin. Cell Dev. Biol.* 92, 114–121. doi:10.1016/j.semcdb.2019.03.007.

Paradiso, R., Arena, C., Rouphael, Y., d'Aquino, L., Makris, K., Vitaglione, P. and De Pascale, S. (2019). Growth, photosynthetic activity and tuber quality of two potato cultivars in controlled environment as affected by light source. *Plant Biosyst*. 153(5), 725-735. doi: 10.1080/11263504.2018.1549603

Park, Y. G., and Jeong, B. R. (2019). Night interruption light quality changes morphogenesis, flowering, and gene expression in *Dendranthema grandiflorum*. *Hortic. Environ. Biotechnol*. 60, 167–173. doi:10.1007/s13580-018-0114-z.

Park, Y., and Runkle, E. S. (2017). Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. *Environ. Exp. Bot*. 136, 41–49. doi:10.1016/j.envexpbot.2016.12.013.

Park, Y., and Runkle, E. S. (2018). Far-red radiation and photosynthetic photon flux density independently regulate seedling growth but interactively regulate flowering. *Environ. Exp. Bot*. 155, 206–216. doi:10.1016/j.envexpbot.2018.06.033.

Partelli, F. L., Vieira, H. D., Viana, A. P., Batista-Santos, P., Rodrigues, A. P., Leitão, A. E., et al. (2009). Low temperature impact on photosynthetic parameters of coffee genotypes. *Pesq. Agropec. Bras*. 44, 1404–1415. doi: 10.1590/S0100- 204X2009001100006

Pattison, P. M., Tsao, J. Y., Brainard, G. C., and Bugbee, B. (2018). LEDs for photons, physiology and food. *Nature* 563, 493–500. doi:10.1038/s41586-018-0706-x.

Pennisi, G., Blasioli, S., Cellini, A., Maia, L., Crepaldi, A., Braschi, I., et al. (2019). Unraveling the Role of Red:Blue LED Lights on Resource Use Efficiency and Nutritional Properties of Indoor Grown Sweet Basil. *Front. Plant Sci.* 10, 305. doi:10.3389/fpls.2019.00305.

Petropoulos, S. A., Chatzieustratiou, E., Constantopoulou, E., and Kapotis, G. (2016). Yield and quality of lettuce and rocket grown in floating culture system. *Not. Bot. Horti Agrobot. Cluj-Napoca*. 44, 603–612. doi:10.15835/nbha44210611.

Pettai, H., Oja, V., Freiberg, A., and Laisk, A. (2005). Photosynthetic activity of far-red light in green plants. 1708, 311–321. doi:10.1016/j.bbabio.2005.05.005.

Platt, T., Gallegos, C. L., and Harrison, W. G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res*.

Putterill, J., Laurie, R., and Macknight, R. (2004). It's time to flower: The genetic control of flowering time. *BioEssays* 26, 363–373. doi:10.1002/bies.20021.

Rashad, E. S. M., and El-Soud, M. E. S. A. (2009). Comparative study on the effect of pre-sowing seed treatments and Ethrel foliar application on growth, fruiting and essential oil content of *Foeniculum vulgare* Mill. plants. *Med. Aromat. Plant. Sci. Biotechnol*. 3, 14–20.

Runkle, E. S., Padhye, S. R., Oh, W., and Getter, K. (2012). Replacing incandescent lamps with compact fluorescent lamps may delay flowering. *Sci. Hortic. (Amsterdam).* 143, 56–61. doi:10.1016/j.scienta.2012.05.028.

Sager, J. C., and McFarlane, J. C. (1997). "Radiation," in *Plant growth chamber handbook*, eds. R. W. Langhans and T. W. Tibbits, 1–29.

Sager, J. C., Smith, W. O., Edwards, J. L., and Cry, K. L. (1988). Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. Asae* 31, 1882–1889.

Sakr, M. M., Gowayed, S. M., Kadasa, N. M. S., Azab, E. S., and Harabawy, A. S. (2013). The relationship between sowing dates and vernalization treatments and growth characters and some chemical components of *Beta vulgaris* L. cv. Pleno. *Life Sci. J*. 10, 1846–1849.

Sims, D. A., and Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ*. 81, 337–354. doi:10.1016/S0034-4257(02)00010-X.

Singh, A. (1984). Promotion in chlorophyll formation and hill activity by chilling of seedlings and seeds of *Cicer arietinum* and *Luffa aegyptiaca*. *Indian J. Plant Physiol*. 17, 401–404.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*. 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sorrentino, M. C., Capozzi, F., Amitrano, C., Giordano, S., Arena, C., and Spagnuolo, V. (2018). Performance of three cardoon cultivars in an industrial heavy metal-contaminated soil: effects on morphology, cytology and photosynthesis. *J. Haz. Mat*. 351, 131–137. doi: 10.1016/j.jhazmat.2018.02.044

Stapleton, J., and Jones, M. (1987). Effect of vernalization on the subsequent rates of leaf extension and photosynthesis of perennial ryegrass (*Lolium perenne* L.). *Grass Forage Sci*. 42, 27–31. doi: 10.1111/j.1365-2494.1987.tb02087.x

Taïbi, K., Del Campo, A. D., Vilagrosa, A., Bellés, J. M., López-Gresa, M. P., LópezNicolás, J. M., et al. (2018). Distinctive physiological and molecular responses to cold stress among cold-tolerant and cold-sensitive *Pinus halepensis* seed sources. *BMC Plant Biol*. 18:236–236. doi: 10.1186/s12870-018-1464-5

Taiz, L., and Zeiger, E. (2002). "Photosynthesis: physiological and ecological consideration," in *Plant Physiology* (Sinauer Associates Inc. Publishers), 171–192.

Taulavuori, K., Julkunen-Tiitto, R., Valtteri, H., and Taulavuori, E. (2013). Blue Mood for Superfood. *Nat. Prod. Commun.* 8, 791–794. doi:10.1177/1934578X1300800627.

Taulavuori, K., Pyysalo, A., Taulavuori, E., and Julkunen-Tiitto, R. (2018). Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. *Environ. Exp. Bot.* 150, 183–187. doi:10.1016/j.envexpbot.2018.03.016.

Thakur, P., and Nayyar, H. (2013). "Facing the cold stress by plants in the changing environment: sensing, signaling, and defending mechanisms," in *Plant Acclimation to Environmental Stress*, eds N. Tuteja and S. Singh Gill (New York, NY: Springer). doi: 10.1007/978-1-4614-5001-6_2

Thomas, B., and Vince-Prue, D. (1997). *Photoperiodism in Plants*. Second. San Diego CA: Academic Press.

Tuteja, N., and Gill, S. S. (2016). Abiotic Stress Response in Plants. Hoboken, NJ: John Wiley & Sons. doi: 10.1002/9783527694570

Tzin, V., and Galili, G. (2010). The biosynthetic pathways for shikimate and aromatic amino acids in *Arabidopsis thaliana*. *Arabidopsis Book*. 8:e0132. doi: 10.1199/tab.0132

Vogelmann, T. C., and Evans, J. R. (2002). Profiles of light absorption and chlorophyll within spinach. *Plant. Cell Environ*. 25, 1313–1323.

Wahl, V., Ponnu, J., Schlereth, A., Arrivault, S., Langenecker, T., Franke, A., et al. (2013). Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. *Science*. 339, 704–707. doi: 10.1126/science.1230406

Wang, J., Lu, W., Tong, Y., and Yang, Q. (2016). Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomatal development of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. *Front. Plant Sci*. 7, 1–10. doi:10.3389/fpls.2016.00250.

Wang, W., Su, X., Tian, Z., Liu, Y., Zhou, Y., and He, M. (2018). Transcriptome profiling provides insights into dormancy release during cold storage of *Lilium pumilum*. *BMC Genomics* 19, 1–17. doi:10.1186/s12864-018-4536-x.

Whitman, C. M., Heins, R. D., Cameron, A. C., and Carlson, W. H. (1998). Lamp type and irradiance level for daylength extensions influence flowering of *Campanula carpatica* "blue clips", *Coreopsis grandiflora* "Early Sunrise", and *Coreopsis verticillata*

"Moonbeam." *J. Am. Soc. Hortic. Sci.* 123, 802–807. doi:https://doi.org/10.21273/JASHS.123.5.802.

Woodrow, P., Ciarmiello, L.F., Annunziata, M.G., Pacifico, S., Iannuzzi, F., Mirto, A., D'Amelia, L., Dell'Aversana, E., Piccolella, S., Fuggi, A. and Carillo, P. (2017). Durum wheat seedling responses to simultaneous high light and salinity involve a fine reconfiguration of amino acids and carbohydrate metabolism. *Physiol. Plant*. 159, 290-312. https://doi.org/10.1111/ppl.12513

Yadav, U. P., Ivakov, A., Feil, R., Duan, G. Y., Walther, D., Giavalisco, P., et al. (2014). The sucrose–trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. *J. Exp. Bot*. 65, 1051–1068. doi: 10.1093/jxb/ert457

Yadegari, L. Z., Heidari, R., and Carapetian, J. (2008). The influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (*Glycine max*) seedling. *Res. J. Biol. Sci*. 3, 74–79.

Yang, Y., Zhang, L., Chen, P., Liang, T., Li, X., and Liu, H. (2019). UV-B photoreceptor UVR8 interacts with MYB73/MYB77 to regulate auxin responses and lateral root development. *EMBO J.* doi:10.15252/embj.2019101928.

Yanovsky, M. J., Alconada-Magliano, T. M., Mazzella, M. A., Gatz, C., Thomas, B., and Casal, J. J. (1998). Phytochrome A affects stem growth, anthocyanin synthesis, sucrose-phosphate-synthase activity and neighbour detection in sunlight-grown potato. *Planta* 205, 235–241. doi:10.1007/s004250050316.

Yoshie, F., and Yoshida, S. (1987). Seasonal changes in photosynthetic characteristics of *Anemone raddeana*, a spring-active geophyte, in the temperate region of Japan. *Oecologia*. 72, 202–206. doi: 10.1007/BF00379268

Zhang, M., and Runkle, E. S. (2019). Regulating Flowering and Extension Growth of Poinsettia Using Red and Far-red Light-emitting Diodes for End-of-day Lighting. *HortScience* 54, 323–327. doi:10.21273/hortsci13630-18.

Zhang, M., Whitman, C. M., and Runkle, E. S. (2019). Manipulating growth, color, and taste attributes of fresh cut lettuce by greenhouse supplemental lighting. *Sci. Hortic. (Amsterdam)*. 252, 274–282.

Zheng, L., and Van Labeke, M. C. (2017). Long-term effects of red- and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. *Front. Plant Sci*. 8. doi:10.3389/fpls.2017.00917.

Zoltowski, B. D., and Imaizumi, T. (2014). "Chapter Nine – Structure and Function of the ZTL/FKF1/LKP2 Group Proteins in *Arabidopsis*," in *The Enzymes*, 213–239. doi:10.1016/B978-0-12-801922-1.00009-9.

# Appendix 1

Table S1. Free amino acid content in tuberous roots of *Ranunculus asiaticus* L. hybrids MBO, obtained by two preparation procedures, only rehydration (Control, C) and rehydration plus vernalization (V), at three plant phenological stages: planting, leaf rosette, beginning of flowering. Plants grown in climatic chamber under controlled environment. Mean values ± standard errors; n=3.

| | Planting | | | Leaf rosette | | | Beginning of flowering | | |
|---|---|---|---|---|---|---|---|---|---|
| | MBO-C | MBO-V | *mean* | MBO-C | MBO-V | *mean* | MBO-C | MBO-V | *mean* |
| Alanine | 3,87±0,26 | 5,64±0,37 | *4,76* | 1,52±0,17 | 1,52±0,31 | *1,52* | 1,79±0,41 | 1,60±0,39 | *1,70* |
| Arginine | 0,36±0,05 | 0,91±0,06 | *0,64* | 1,06±0,25 | 0,38±0,11 | *0,72* | 0,97±0,27 | 0,44±0,06 | *0,70* |
| Asparagine | 17,68±0,51 | 21,37±2,28 | *19,53* | 35,35±7,04 | 39,01±0,68 | *37,18* | 85,46±13,97 | 88,53±16,71 | *86,99* |
| Aspartate | 0,69±0,08 | 1,03±0,07 | *0,86* | 0,35±0,04 | 0,29±0,05 | *0,32* | 0,91±0,21 | 1,10±0,18 | *1,01* |
| Ethanolamine | 0,32±0,07 | 0,68±0,03 | *0,50* | 0,55±0,09 | 0,37±0,06 | *0,46* | 0,37±0,08 | 0,62±0,03 | *0,49* |
| Glycine | 0,69±0,03 | 1,11±0,01 | *0,90* | 0,46±0,09 | 0,78±0,18 | *0,62* | 0,80±0,17 | 0,89±0,14 | *0,84* |
| Glutamate | 10,95±0,59 | 15,30±1,02 | *13,13* | 1,42±0,28 | 2,24±0,22 | *1,83* | 7,10±1,02 | 8,62±0,90 | *7,86* |
| Glutamine | 34,49±1,99 | 26,90±2,20 | *30,70* | 7,24±1,71 | 16,67±1,36 | *11,95* | 33,06±8,57 | 47,80±3,17 | *40,43* |
| Isoleucine | 2,74±0,22 | 3,81±0,09 | *3,28* | 0,56±0,13 | 3,39±0,32 | *1,97* | 0,76±0,18 | 0,74±0,15 | *0,75* |
| Histidine | 3,83±0,22 | 3,68±0,18 | *3,76* | 0,99±0,12 | 3,40±0,30 | *2,19* | 1,66±0,22 | 2,03±0,38 | *1,85* |
| Leucine | 2,34±0,12 | 3,64±0,02 | *2,99* | 0,61±0,12 | 2,98±0,34 | *1,80* | 0,84±0,12 | 0,72±0,13 | *0,78* |
| Lysine | 2,28±0,17 | 2,92±0,30 | *2,60* | 1,68±0,25 | 3,18±0,52 | *2,43* | 2,56±0,03 | 1,80±0,34 | *2,18* |
| Methionine | 0,15±0,02 | 0,36±0,03 | *0,25* | 0,28±0,05 | 0,07±0,01 | *0,17* | 0,36±0,04 | 0,12±0,03 | *0,24* |
| Ornithine | 2,14±0,19 | 1,96±0,05 | *2,05* | 3,19±0,82 | 1,57±0,23 | *2,38* | 3,33±0,44 | 2,38±0,35 | *2,85* |
| Phenylalanine | 1,45±0,20 | 2,22±0,05 | *1,83* | 1,16±0,18 | 2,58±0,62 | *1,87* | 0,97±0,18 | 0,87±0,19 | *0,92* |
| Proline | 0,57±0,12 | 1,07±0,08 | *0,82* | 0,24±0,05 | 1,05±0,06 | *0,64* | 0,51±0,00 | 0,36±0,01 | *0,44* |
| Serine | 4,35±0,30 | 5,22±0,27 | *4,79* | 1,26±0,21 | 3,99±0,49 | *2,63* | 1,48±0,20 | 1,69±0,31 | *1,59* |
| Tyrosine | 2,10±0,14 | 2,20±0,31 | *2,15* | 0,81±0,14 | 1,75±0,36 | *1,28* | 1,38±0,10 | 1,26±0,31 | *1,32* |
| Threonine | 1,44±0,07 | 1,98±0,36 | *1,71* | 2,15±0,20 | 1,12±0,40 | *1,64* | 3,62±0,29 | 3,69±0,40 | *3,66* |
| Tryptophan | 1,03±0,09 | 1,93±0,05 | *1,48* | 1,24±0,21 | 2,75±0,58 | *2,00* | 1,09±0,14 | 0,73±0,11 | *0,91* |
| Valine | 4,59±0,47 | 5,98±0,18 | *5,29* | 1,06±0,23 | 4,89±1,19 | *2,97* | 2,12±0,17 | 1,52±0,36 | *1,82* |
| BCAAs | 9,68±0,78 | 13,44±0,26 | *11,56* | 2,23±0,46 | 11,26±1,77 | *6,75* | 3,73±0,47 | 2,98±0,61 | *3,36* |
| Total AA | 98,07±3,24 | 109,95±5,88 | *104,01* | 63,15±11,69 | 93,99±3,84 | *78,57* | 151,15±9,58 | 167,52±17,19 | *159,34* |
| Minor AA | 19,84±1,03 | 25,72±0,52 | *22,78* | 8,20±1,42 | 22,62±3,34 | *15,41* | 11,63±1,08 | 9,51±1,75 | *10,57* |

Table S2. Starch, glucose, fructose and sucrose content in tuberous roots of *Ranunculus asiaticus* L. hybrids MBO, obtained by two preparation procedures, only rehydration (Control, C) and rehydration plus vernalization (V), at three plant phenological stages: planting, leaf rosette and beginning of flowering. Plants grown in climatic chamber under controlled environment. Mean values ± standard errors; n=3.

| | Planting | | | Leaf rosette | | | Beginning of flowering | | |
|---|---|---|---|---|---|---|---|---|---|
| | MBO-C | MBOV | *mean* | MBO-C | MBOV | *mean* | MBO-C | MBOV | *mean* |
| Starch | 5,91±0,44 | 4,05±0,47 | *4,98* | 0,61±0,08 | 1,16±0,11 | *0,89* | 3,69±1,26 | 5,53±0,23 | *4,61* |
| Glucose | 5,21±0,43 | 5,10±0,39 | *5,15* | 1,64±0,16 | 4,27±0,86 | *2,96* | 1,05±0,55 | 0,25±0,07 | *0,65* |
| Fructose | 5,74±0,23 | 4,33±0,42 | *5,04* | 0,59±0,02 | 2,80±0,36 | *1,70* | 2,10±0,62 | 2,46±0,31 | *2,28* |
| Sucrose | 2,49±0,28 | 2,19±0,29 | *2,34* | 0,87±0,11 | 3,00±0,21 | *1,93* | 2,09±0,34 | 2,36±0,24 | *2,23* |

# Appendix 2

## List of publications

Complete list of articles published during the Ph.D. period:

- Modarelli G.C., Arena, C., Carillo, P., De Pascale, S. and Paradiso, R. (2018). Studi sulla fotosintesi in *Ranunculus asiaticus L.* XII Giornate Scientifiche della Società Ortoflorofrutticoltura Italiana (S.O.I.) - Innovazione e meccanismi per favorire la sua adozione e applicazione in orto-floro-frutticoltura 19-22 June 2018; Bologna (Italy). *Acta Italus Hortus*, 23:32.
- Modarelli G.C., Arena, C., De Pascale, S. and Paradiso, R. (2018). Photosynthesis in *Ranunculus asiaticus* L.*:* characterization in two Mediterranean hybrids under fluorescent white light. Acta Hort: III International Symposium on Innovation and New Technologies in Protected Cultivation, Istanbul (Turchia), in press.
- Carillo, P., Arena, C., Modarelli, G.C., De Pascale, S. and Paradiso, R. (2019). Photosynthesis in *Ranunculus asiaticus* L.: the influence of the hybrid and the preparation procedure of tuberous roots. Front. Plant Sci. 10: 241. ISSN:1664-462X. https://doi.org/10.3389/fpls.2019.00241
- Modarelli G.C., Arena, C., De Pascale, S. and Paradiso, R. (2019). The influence of the hybrid and the preparation procedure of tuberous roots on plant growth and flowering in *Ranunculus asiaticus* L. Acta Hort. GreenSys 2019 International Symposium on Advanced Technologies and Management for Innovative Greenhouses. Angers (France), in press.
- Modarelli G.C., Arena, C., De Pascale, S. and Paradiso, R. (2019). LEDs photoperiodic lighting to promote flowering of two *Ranunculus asiaticus* L. hybrids. Poster presentation at VertiFarm2019 - International workshop on vertical farming. Wageningen university, Wageningen (The Netherlands), 13-15 October 2019.

- Modarelli, G.C., Arena, C., Pesce, G., Dell´Aversana, E., Fusco, G.M., Carillo, P., De Pascale, S. and Paradiso, R. (2019). Unravelling the role of light quality of photoperiodic lighting on photosynthesis, flowering and metabolic profiling in two *Ranunculus asiaticus* L. hybrids. Submitted to Physiologia Plantarum.
- Modarelli, G.C., Grootaert, C., Paradiso, R., Arena, C., and van Labeke, M.C. (2020). Blue and Red light modulation and different Far Red light intensities affect growth, physiology and quality of leafy vegetables. In preparation.

# Participation to other research projects

Convenzione Biolchim - Dipartimento di Agraria: Valutazione dell'efficacia di formulati commerciali biostimolanti e correttivi in specie floricole allevate fuori suolo. Project manager: prof. Roberta Paradiso.

# Conferences

- XII Giornate Scientifiche SOI, Innovazione e meccanismo per favorire la sua adozione e applicazione in ortoflorofrutticoltura. *Alma Mater Studiorum* - University of Bologna, Bologna, 19-22 June 2018.

- III International Symposium on Innovation and New Technologies in Protected Cultivation, Istanbul (Turkey), 12-16 August 2018.

- GreenSys 2019 International Symposium on Advanced Technologies and Management for Innovative Greenhouses. Angers (France), 16-20 June 2019.

- VertiFarm2019 - International workshop on vertical farming. University of Wageningen, Wageningen (The Netherlands), 13-15 October 2019

# Oral presentations

XII Giornate Scientifiche SOI, Innovazione e meccanismo per favorire la sua adozione e applicazione in ortoflorofrutticoltura. *Alma Mater Studiorum* – University of Bologna, Bologna, 19-22 June 2018. Oral comunication: Studi sulla fotosintesi di *Ranunculus asiaticus* L. Authors: Modarelli G.C., Arena C. De Pascale S., Paradiso R.

# Posters

- III International Symposium on Innovation and New Technologies in Protected Cultivation, Istanbul (Turkey), 12-16 August 2018. Poster presented: Photosynthesis in *Ranunculus asiaticus* L.: characterization in two Mediterranean hybrids under fluorescent white light. Authors: Modarelli G.C., Arena C., De Pascale S., Paradiso R.
- GreenSys 2019 International Symposium on Advanced Technologies and Management for Innovative Greenhouses. Angers (France), 16-20 June 2019. Poster presented: The influence of the hybrid and the preparation procedure of tuberous roots on plant growth and flowering in *Ranunculus asiaticus* L. Authors: Modarelli G.C., Arena C., De Pascale S., Paradiso R.
- VertiFarm2019 - International workshop on vertical farming. University of Wageningen, Wageningen (The Netherlands), 13-15 October 2019. Poster presented: LEDs photoperiodic lighting to promote flowering of two *Ranunculus asiaticus* L. hybrids. Authors: Modarelli G.C., Arena C., De Pascale S., Paradiso R.

# Courses and seminars

**Courses:**

- Laboratorio di analisi dati agro ambientali; Dr. Paolo Nasta (6 CFU, 50 hours)
- How to write a scientific paper; Prof. Domenico Carputo (9 CFU, 75 hours)
- Gestione e progettazione di progetti di ricerca; Prof Albino Maggio (6 CFU, 50 hours)

Total CFU: 21

**Courses abroad:**

- Lighting in greenhouses and vertical farms 2018. University of Wageningen, Wageningen (The Netherlands), 7- 9 February 2018

**Seminars:**

- "Tolerance strategy adopted by plant cells to face photoinhibition". Department of Biology, University of Naples Federico II. Prof. Arena, C. 2 hours.
- "Beyond photosynthesis". Department of Biology, University of Naples Federico II. Prof. Loreto, F. 2 hours
- "Behind sustainable agriculture, research and innovation by Valagro". Department of Agricultural Sciences, University of Naples Federico II. Dr. Piaggesi A. 2 hours.
- "Le colture fuori suolo: dai giardini di Babilonia alle plant factory" Department of Agricultural Sciences, University of Naples Federico II. Prof. De Pascale, S. 1 hours.

**Seminars held by foreigner speaker:**

- Biogenic isoprene beyond non-trivial carbon loss by plants: new insights from transgenic poplar; Prof. Velikova, V.
- General overview of projects on environmental research in Fukushima and behavior of radionuclides in the Fukushima environment; Dr. Iijima, K.

# Side activities

Seminar: Orticultura urbana e sistemi fuori suolo (2 hours) in the course: Laboratorio di colture orto-floricole fuori suolo (Other learning activities); Professor Roberta Paradiso.

Department of Agricultural Science, University of Naples Federico II, Portici (NA), 21th April 2017.

- Participation to experimental master thesis activities. Students: Raffaele Esposito Matr. N09-361, Emilio Starace Matr. N09-380. Master degree: Scienze e tecnologie agrarie. Supervisor: prof. Roberta Paradiso.

**Department divulgation activities:**

- Futuro Remoto – "Connessioni" 2017., Naples. 28 May 2017.
- "Natale in Reggia: Scienze Agrarie sotto l'albero". Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), 10 December 2017.
- Futuro Remoto – "Rigenerazioni" 2018., Naples. 8-10 November 2018.
- Notte europea dei ricercatori 2018, Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Portici, 28 September 2018.

# Scientific collaborations

**Scientific institutions:**

- Prof. Petronia Carillo, Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta (Italy)
- Dr. Giuseppe Pesce, Department of Physic, University of Naples Federico II, Laboratory of Laser spectrometry and optical manipulation. Naples (Italy)
- Prof. Marie-Christine Van Labeke, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent (Belgium).
- Post doc. Charlotte Grootaert, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University (Belgium).

**Private institutions**

- Biancheri creazioni, Camporosso (Imperia, Italy)
- Biolchim S.P.A, Medicina (Bologna, Italy)
- Azienda agricola fratelli Esposito, Castellammare di Stabia (Naples, Italy)

# Acknowledgements

The authors thanks Mario Rabaglio (Biancheri Creazioni, Italy) for *R. asiaticus* L. propagation material and technical asistance in *R. asiaticus* L the experiments, Raffaele Garramone (University of Naples Federico II) for his help in setting up the experiments detailed in Chapter 2 and 3, Giuseppe Nenna (ENEA Portici Research Center) for light emission spectra of light source used in the experiment detailed in Chapter 3, Christophe Petit for his help setting up the experiments detailed in Chapter 4 and Paulien De Clerq for photosynthetic pigment analysis in the experiments detailed in Chapeter 4.