
DROUGHT STRESS RESPONSE IN LONG STORAGE TOMATOES: MORPHOLOGICAL, PHYSIOLOGICAL, YIELD AND QUALITY TRAITS

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PhD THESIS – SCIENCE AND TECHNOLOGY OF FOOD-AGRICULTURE PRODUCTION XXVI CICLO



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To Ale, my little man, my heart, my life and my soul

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Preface

This thesis is submitted in partial fulfilment of the requirements for the Doctor of Philosophy (PhD) degree at University of Naples, Federico II, Faculty of Agriculture, Italy.

The PhD project consisted in an experiment conducted in a greenhouse of the Faculty of Agriculture, University of Naples located in Portici and it was part of the CISIA project (Integrate knowledge for sustainability and innovation of the agro-food made in Italy) of the Italian National Research Council.

The PhD program was carried out in collaboration between Faculty of Agriculture of the University of Naples and CNR-ISAFoM (Italian National Research Council - Institute for Mediterranean Agriculture and Forest Systems).

During the PhD program were collected the following research products:

- n°13 manuscripts on ISI (International Scientific Indexing) journals;
- n°1 manuscript on Scopus journal;
- n°2 book chapters;
- n°12 abstracts/posters/oral dissertations in international conferences;
- n°10 abstracts/posters/oral dissertations in national conferences;
- n°1 registered software.

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Lists of symbols and abbreviations

ABA	abscissic acid
ANOVA	analysis of variance
AsA	ascorbic acid
C_a	carbon dioxide concentration in the Li-6400 leaf chamber
CEC	cation exchange capacity
DHA	dehydroascorbic acid
ds	drought stressed plants
DW	dry weight
t_{ds1}	end of the 1 st drought stress period
t_{rw1}	end of the 1 st re-watering period
t_{ds2}	end of the 2 nd drought stress period
t_{rw2}	end of the 2 nd re-watering period
F.C.	field capacity
FW	fresh weight
FBPase	fructose-1,6-bisphosphatase
F_0	initial/minimal fluorescence
C_i	intercellular carbon dioxide concentration
LA	leaf area
RWC	leaf relative water content
Ψ_{leaf}	leaf water potential
F_m	maximal fluorescence
F_v/F_m	maximum quantum yield of PSII
NADP-ME	NADP-malic enzyme
A	net photosynthetic rate
PEPCase	phosphoenolpyruvate carboxylase
PS I	photosystem I
PS II	photosystem II
PPDK	pyruvate orthophosphate dikinase
ROS	reactive oxygen species
rw	re-watering
RuBisCo	ribulose-1,5 -bisphosphate carboxylase/oxygenase
Root/shoot	root to shoot ratio
SWC	soil water content
g_s	stomatal conductance
TS	total solids
TSS	total soluble solids
TW	turgid weight
F_v	variable fluorescence
ww	well-watered plants or control plants
W.P.	wilting point

ABSTRACT

Long-storage tomato (*Lycopersicon esculentum* Mill.) is a product cultivated in the Mediterranean area whose fruits combine a good taste and extended shelf-life to excellent nutritional properties. Furthermore, due to the high drought tolerance of the plant, traditionally cultivated under no water supply, long-storage tomato may represent an interesting genetic source in breeding programs for water stress resistance in both fresh-market and processing tomatoes. It could be considered useful for the environmental sustainability and cultivation in countries with hot and dry climate where the deficiency of water for irrigation purposes strongly limits the agriculture.

The aim of this work was to identify the main adaptive physiological and morphological mechanisms that regulate the maintenance of the plant's activity under drought conditions and the effects on quantitative and qualitative aspects of the production.

The study was carried out in a greenhouse of the Faculty of Agriculture, University of Naples Federico II, during the 2012 season. Two long storage tomato landraces (Pizzutello di Sciacca and Locale di Salina) collected at CNR-ISAFoM of Catania with indeterminate growth, were used. A processing tomato cultivar (Brigade) was also considered as control. Five weeks-old seedlings were transplanted into plastic pots, at the 4th true leaf stage. The plants were watered daily to container capacity prior to starting the treatments. Plants before flowering were submitted to two different treatments. Control plants were irrigated daily and drought stressed plants received no water until stomata conductance reached value near to zero. Recovery of plants was carried out by re-irrigation of pots daily. So during the experiment two periods of drought stress and subsequent re-watering were considered. For both stressed and control plants were evaluated leaf gas exchange parameters, leaf abscissic acid (ABA) and proline content, plant water status aspects, growth behavior, yield components and qualitative characteristics of the fruits.

Based on the physiological study, Pizzutello di Sciacca and Locale di Salina maintained higher values of the leaf photosynthetic rate (A) with fewer reductions in stomatal conductance (g_s) respect to Brigade and they showed a rapid and full recovery of all gas exchange parameters after re-watering suggesting a good tolerance to water stress. The rates of change in g_s , with ABA content were greater for Pizzutello di Sciacca and Locale di Salina respect to Brigade and was cause of the early stomatal closure during drought, limiting further water loss and subsequent damages. Leaf water status was affected by drought with lower values recorded in Brigade that accumulate in the leaves higher proline contents.

Drought caused a reduction in plant growth parameters of all genotypes also if the magnitudes of the changes were greater for Brigade than Pizzutello di Sciacca and Locale di Salina. Furthermore, water stress increased root length and the root to shoot ratio and together decrease the specific leaf area (SLA) in Pizzutello di Sciacca in order to diminish water consumption and increase absorption of water. Highest total yield reduction was recorded in Brigade correlated to a reduced fruit number per plants and lower fruits weight. Fruit firmness was significantly improved in the absence of irrigation in Pizzutello di Sciacca and Locale di Salina respect to Brigade. Fruit thinning has been proposed as a feasible strategy to compensate the loss in fruit size caused by water stress. Drought produced solid soluble solids (TSS), total solids, reducing sugars, vitamin C and total phenols contents increase in the fruits of all genotypes with higher rates in Pizzutello di Sciacca and Locale di Salina. In addition a higher β -carotene content was found in fruits of Pizzutello di Sciacca and Locale di Salina developed under soil water deficit.

In conclusion, Pizzutello di Sciacca and Locale di Salina were characterized by less severe decline in physiological, growth and yield parameters and greater improvement of fruit quality under drought conditions. They had more efficient stress protection mechanisms to survive under drought conditions.

1. STATE OF THE ART

Faced with scarcity of water resources, drought is the single most critical threat to world food security. It was the catalyst of the great famines of the past. Because the world's water supply is limiting, future food demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville and Briscoe, 2001). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery et al., 1994).

Under the climatic changing context, drought has been, and is becoming an acute problem most constraining plant growth, terrestrial ecosystem productivity, in many regions all over the world, particularly in arid and semi-arid area (Knapp et al., 2001; Fischlin et al., 2007).

Based on the fourth assessment report by the Intergovernmental Panel on Climate Change global surface average temperature will have a 1.1-6.4 °C range increase by the end of this century. With global warming, it is expected that water deficit would be escalated by increasing evapotranspiration, increasing the frequency and intensity of drought with an increase from 1% to 30% in extreme drought land area by 2100 (Fischlin et al., 2007).

The degree of drought effects on plants depends on the impact on plant physiological, biochemical, as well as molecular biological process and the ability of plant to adapt in these conditions (Bulbotko, 1973; Atkinson et al., 2000; Massonnet et al., 2007).

Plants express various responses to drought stress and develop a wide range of mechanisms (Yamaguchi-Shinozaki and Shinozaki, 2006; Ahuja et al., 2010; Skirycz and Inze, 2010; Osakabe et al., 2011; Nishiyama et al., 2013; Ha et al., 2014).

1.1. Plant water relations and mechanisms of drought tolerance

Water movement through a plant is a passive process, where it is driven by water potential differences between the soil, plant and atmosphere and the hydraulic conductivities between each component (Lobet *et al.*, 2014). Water moves from high water potential to a lower potential. Plants absorb water from soil through roots and absorbed water moves to xylem vessels through radial water movement. After entering to xylem vessels, water moves from roots to leaves through the xylem and release to atmosphere as water vapor through stomata (Fig. 1).

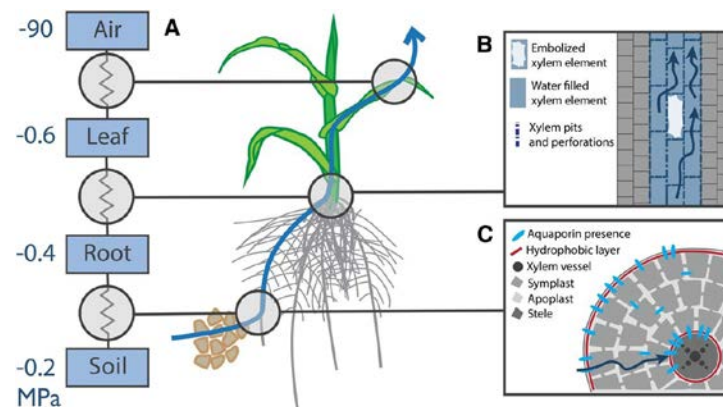


Figure 1 - A schematic diagram showing water movement through a plant (from Lobet *et al.*, 2014). **A.** Water movement inside the plant. **B.** Axial water movement in xylem vessels. **C.** Radial water movement in roots.

Plants prefer adequate water supply for functioning. However, under drought conditions, create high water pressure difference between soil and plant roots initially and it continues towards canopy with the progression of the stress. This is not favorable to its normal functioning. Plants have different adaptive mechanisms to overcome drought stress: escape (drought escape), avoidance (drought avoidance) and tolerance (drought tolerance)

Drought escape is the ability of a plant to complete its life cycle before the onset of drought and to undergo dormancy before the onset of the dry season. It is a general phenomenon in some desert plants, which exhibit extremely short life cycles and produce seeds during short rainy seasons in order to save them from extinction (Levitt, 1980). Some extremely short duration crops and even genotypes within a species exhibit drought escape, but this always taxes yield (Turner *et al.*, 2001). Drought escape is more advantageous where chances of terminal drought are more frequent; as short-duration cultivars frequently escape terminal drought compared with late maturing cultivars (Meyre *et al.*, 2001).

Drought avoidance, instead, is the ability of a plant to sustain high plant water status or cellular hydration under drought. Plants accomplish this mechanism either by catching more water from the soil or by minimizing water loss through transpiration (Blum, 2005).

Plants undergo certain morphological and physiological changes to minimize stress-induced losses; and considerable flexibility among crop species and even within species exists. Reduced biomass is a common phenomenon under drought; however, the extent of the reduction varies as different plant organs are affected by drought.

Crops plants more able to extract water from the entire soil profile can better resist drought. Root plasticity is the ability of a genotype to regulate its root growth pattern in accordance with prevailing constraints (Yamauchi et al., 1996). More rooting depth, root proliferation, and root length density commensurate with fetching more water are considered drought avoidance traits (Matsui and Singh, 2003; Wang and Yamauchi, 2006). A more prolific and deeper root system is imperative under drought as roots are the only organ capable of extracting water from the soil profile (Kavar et al., 2007; Gowda et al., 2011). Genotypes with more root growth in stress-prone environments are preferred. Drought stress can inhibit root growth in general, even in tolerant genotypes, but the effect was more prominent on sensitive genotypes due to an overall decrease in newly synthesized cell wall polysaccharides such as pectins, hemicelluloses, and cellulose (Piro et al., 2003). Generally, when water availability is limited, the root to shoot ratio (root/shoot) of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000). Reduced leaf size is well correlated with drought stress; many xerophytes have small leaves as their adaptation to survive in harsh environments. A small leaf area (LA) is advantageous to restricted water use and it is also responsible for low productivity of crops (Sinclair and Muchow, 2001) but different crops or genotypes behave differently. Reduced transpiration water loss is another important adaptation of drought avoidance. To attain that, plants have developed several morphological adaptations such as higher root–shoot ratios with fewer and smaller leaves to withstand water deficit conditions by maintaining water budget with higher uptake and minimal loss (Lei et al., 2006). Increased stomatal and cuticular resistance, less small stomata, smaller LA, and vertical leaf orientation are among the major drought avoidance traits to minimize transpiration in order to save water under stress conditions (Sinclair and Muchow, 2001; Wang and Yamauchi, 2006). However, reduced LA and plant stature are advantageous for restricted water use but may also result in low productivity of crop plants (Sinclair and Muchow, 2001). On the other hand, in drought tolerance strategy, plant endures drought without undergoing injury, retaining the capacity of normal growth and development when rehydrated. In tolerance, plants mitigate the stress by maintaining high water potential through accumulation of compatible osmotic solutes. The accumulation of compatible solutes (amino acids, glycine betaine, sugars, or sugar alcohols, proline) is well regarded as a basic strategy for the protection and survival of plants under abiotic stress conditions (Chen et al., 2007). The osmotic adjustment allows cell enlargement and plant growth during severe drought stress, and allows stomata to remain partially open and CO₂ assimilation to continue during drought stress (Hare et al. 1998). Apart from playing a primary role of turgor maintenance, these compatible solutes may also be involved in stabilizing proteins and cell structures. Of these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It influences protein solvation and preserves the quaternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photoinhibition (Demiral and Turkan, 2004). Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007).

1.2. Drought effects on physiological functions

Plant water potential influences physiological functions of plants, including photosynthesis, transpiration, stomatal conductance (Chaves et al., 2002; Blanke and Cooke, 2004; Flexas et al., 2004). With optimal water supply, plants functions, such as photosynthesis, transpiration and stomatal conductance, happen well. Moreover, continuous water supply allows high transpiration and low leaf to air water vapor pressure difference (VPD). Low VPD, under drought stress, reduces photosynthesis (Brunce, 1988). In tomatoes, high transpiration rates reduce photosynthetic capacity and induce drought stress (Blanke and Cooke, 2004). Large water potential gradients between the xylem and the site of evaporation (leaves) result in reduced photosynthesis (Sharkey, 1984, Blanke and Cooke, 2004). It was observed that the net photosynthetic rate (*A*) and transpiration rate declined with an increase in drought stress (Teraza et al., 1999; Rao et al., 2000; Flexas et al., 2004). Hui Lian (1997) carried out an experiment with greenhouse tomato cv. Capello in a peat moss-based substrate (70% sphagnum peat + 30% perlite, (v/v)) subjected to low soil water content (SWC) to observe the effects of a prolonged substrate water deficit on photosynthesis and plant water relations. Net photosynthetic rate (*A*) decreased by 24% compared with the control one day after SWC was depleted to 55%. Under drought stress, stomata close and this affects CO₂ flux. Stomatal closure is one of the first responses to drought stress (Hommel et al., 2014; Xie et al., 2014). Stomata close when plant water potential reduces or if the leaf turgor reduces. The response limits CO₂ exchange in leaves (Chaves et al., 2002) and the rate of photosynthesis decreases. Photosynthetic system in plants depends on the availability of CO₂, especially in photosystem II (PSII). Under drought stress, the primary reason of the decline in *A* is the CO₂ deficiency (Meyer et al., 1998). The closure of stomata under drought stress leads to the decrease in intracellular CO₂ levels (*C_i*), which in turns results in over-reduction of electron transport chain

components. Thus, the electrons are transferred to oxygen at photosystem I (PSI) generating reactive oxygen species [ROS, (Mahajan and Tutja, 2005)]. Water deficiency also results in decreasing of ribulose-1.5-bisphosphate carboxylase/oxygenase (RuBisCo), phosphoenolpyruvate carboxylase (PEPCase), NADP-malic enzyme (NADP-ME), fructose-1.6-bisphosphatase (FBPase) and pyruvate orthophosphate dikinase (PPDK) (Bota et al., 2004). On the other hand, plant tissue water potential is reduced by drought. Both ROS production and reduced activity of enzymes lower the carboxylation. Further, drought causes a down-regulation of non-cyclic electron transport, which negatively affects ATP synthesis. Because of low carboxylation and low ATP levels, photosynthesis drops under drought conditions (Fig. 2) (Farooq et al., 2009).

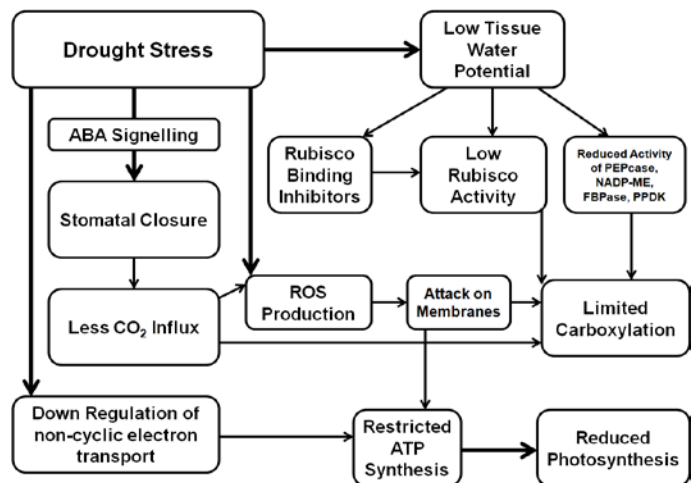


Figure 2 - A schematic diagram showing the photosynthesis reduction under drought stress (from Farooq et al., 2009).

As drought stress progresses, it reduces the leaf water potential (Ψ_{leaf}) and stomatal conductance (Medrano et al., 2002; Bota et al., 2004; Flexas et al., 2004; Miyashita et al., 2005). Blanke and Cooke (2004) found that the Ψ_{leaf} reduced under severe drought stress, but Miyashita et al. (2005) discovered that the Ψ_{leaf} stayed constant for a period after the onset of water and then reduced rapidly.

Furthermore, the recovery after re-watering declined gradually when the drought stress progressed. Reduction of Ψ_{leaf} with stress also affected leaf relative water content [RWC (Teraza et al., 1999; Bota et al., 2004; Turkan et al., 2005)].

1.3. Chemical and hydraulic signalling under drought stress

Root-to-leaf signalling is one of the mechanisms used by crops to regulate stomatal closure under drought conditions (Schachtman and Goodger, 2008; Wilkinson and Davies, 2002). This signal has been shown to be the abscisic acid (ABA), which is synthesized in the roots (Davies and Zhang, 1991) and is translocated via the xylem stream to the leaves, where it causes the closure of stomata (Zhang et al., 1987; Davies and Zhang, 1991). Another source of ABA is the leaves (Taylor, 1991) where it is synthesized and loaded into the phloem, transported to the roots and is either deposited in root tissues or re-circulated to the leaves (Hartung et al., 2002). This drought induced root-to-leaf signaling through the transpiration stream results in stomatal closure, which is an important adaptation to limited water supply in the field. When a plant is subjected to drought, there is an increase in ABA biosynthesis, leading to elevated ABA levels in the tissues (Plant et al., 1991). The increase in the ABA concentration in leaves results in stomatal closure and minimal water loss from the plant. However, the stomatal closure reduces photosynthesis (Bray, 1988; Plant et al. 1991; Zegzouti et al., 1997). High ABA concentration in root tips was observed in plants subjected to drought stress (-1.6 MPa) (Sharp and LeNoble, 2002). Bray (1988) studied the role of ABA in drought, using an ABA deficient tomato. In optimal growth conditions, ABA concentration in this mutant was 50% of the wild type plant. When both the wild type and mutant were exposed to drought stress, there was a significant increase in the synthesis of ABA in the wild type but reduced in the mutant. It was recorded that the ABA concentration of the mutant under drought stress was 6% of its ABA concentration grown under optimal conditions. In addition to chemical signalling by ABA, control of stomatal conductance (g_s) may also occur through hydraulic signalling, such as Ψ_{leaf} and xylem hydraulic conductivity (Nardini et al., 2003). Such a signal is induced by the difference in water potential somewhere in the plant, meaning that when the availability of water in the soil diminishes, the gradient-driven uptake of water ceases and the internal water potential in the root decreases. This effect results in a decrease in the Ψ_{leaf} resulting in stomatal closure (Wakrim et al., 2005). Chemical signals most probably dominate during early stages of stress before hydraulic signals are produced (Goodger et al., 2005), and become less important under severe drought when Ψ_{leaf} declines and leaves wilt (Christmann et al., 2007; Wakrim et al., 2005).

1.4. Effect of drought on yield and quality

Drought-induced yield reduction has been reported in many crop species, which depends upon the severity and duration of the stress period. Water deficit leads to decrease in the number of flowers and consequently the number of fruit and ultimately to less marketable yield (Losada and Rincaon, 1994; Colla et al., 1999; Rahman et al., 1999; Veit-Kohler et al., 1999). Chiaranda and Zerbi (1981) conducted an experiment with lysimeter-grown greenhouse tomatoes and observed a remarkable sensitivity of the crop to water stress during the vegetative and the flowering periods, with respect to early and late harvesting records. Doorenbos and Kassam (1979) reported that the highest demand for water in tomato plant is during flowering. Ripening is the most sensitive stage and any heterogeneous distribution of irrigation leads to fruit cracking (Losada and Rincaon, 1994). Blossom-end rot of tomatoes is a common problem occurring under conditions of water stress and heavy fruit load (Hodges and Steinegger, 1991). It appears as brown to black lathery spots of the underside/blossom end of the fruit of tomatoes, which eventually leads to decay of fruits (Sanders et al., 1989). Even a temporary water stress during early fruit enlargement can cause blossom-end rot because the fruits are the last to receive adequate calcium (Hodges and Steinegger, 1991). Franco et al. (1999) showed that at higher irrigation levels there was a high yield potential and less blossom-end rot affected fruit. Young Hah et al. (1999) found that total and marketable yields were increased by increasing soil water tension and by varying night temperature (14 ± 1 °C to 10 ± 1 °C). Fruit cracking decreased with increasing soil water tensions.

Adams (1990) observed a reduction in the size of the fruit in tomato under water stress. Lapushner et al. (1986) observed that the fruit weight of tomato was reduced by water stress.

Quality of the fruit in terms of total soluble solids, acidity (Shinohara et al., 1995; Colla et al., 1999; Veit-Kohler et al., 1999), viscosity, and vitamin C is improved by water deficit (Rudich et al., 1977; Veit-Kohler et al., 1999; Zushi and Matsuzoe, 1998). High sugar content in tomato is a desirable character, which can be achieved by decreased irrigation (Imada et al., 1989; Veit-Kohler et al., 1999). A decrease up to 20% irrigation or even lesser percentage of irrigation shows significant improvement in tomato fruit flavor components (Veit-Kohler et al., 1999), in addition accelerated development of color and increased amount of β -carotene content in cherry tomato due to water deficit is observed. Also Naothke et al. (1998) studied the effect of SWC on fruit coloring and carotene formation using four cherry tomato varieties. It was found that the soil water deficit effect on the fruit coloring was more evident during the fall cropping season than in the spring season and that the amount of β -carotene increased in case of cv. Yellow Carol.

Veit-Kohler et al. (1999) investigated whether even a small reduction in water supply (without visible symptoms of water stress) results in high fruit quality together with high marketable fruit productions. In the treatment with lower water supply plant growth, and in particular the number of fruit were decreased and the sugar and vitamin C concentrations of the fruits were significantly increased, especially during fruit ripening.

Zushi and Matsuzoe (1998) observed the effects of soil water deficit on vitamin C content varied depending on the cultivar. They found that vitamin C content increased in some cultivars whereas it remained unchanged in others. In almost all cultivars under water-stressed plants, glucose and fructose were found in higher proportions than in plants receiving full irrigation. This indicates that the soil water deficit merely reduced water accumulation by the fruits. The amount of organic acid and free amino acids both increased under water stress.

Shinohara et al. (1995) observed that water stress caused decreasing yield but increasing treatable acidity.

Lapushner et al. (1986) observed that fruit weight was reduced by water stress but marketable yield, fruit color and contents of total soluble solids (TSS) and reducing sugar were improved.

Matsuzoe et al. (1998) investigated the effects of SWC on fruit color and β -carotene content in cherry tomato cultivars: Mini Carol (red), Cherry Pink (pink), Yellow Carol (yellow), and Orange Carol (yellow-tangerine), in Japan. They observed that soil water deficit accelerated fruit coloring in spring and autumn crops of Mini Carol, and in autumn crops of Cherry Pink. Soil water deficit increased the amount of β -carotene in Yellow Carol, but had no effect on the β -carotene content of Orange Carol in spring or autumn crops.

1.5. Long-storage tomato

Long-storage tomato (*Solanum lycopersicum* L.) is a niche product typical of the Mediterranean area; in Italy, it is diffused mainly in the southern regions (Campania, Apulia, Basilicata, Calabria, Sicily), where its cultivation and consumption have been handed down for many generations. In the past, long-storage tomato had significant importance, especially because was the only source of tomatoes during the colder months of the year due to thick and elastic skin which limits fruit's dehydration and ensures a long shelf life.

Long-storage tomato, traditionally, is cultivated under no water supply, the only watering (about $300\text{--}400\text{ m}^3\text{ ha}^{-1}$) occurs at transplant (late March - early April) to ensure seedling rooting. This aspect, in addition to high genetic variability of these tomatoes that confer them resistance to abiotic stress, is particularly interesting for the environmental sustainability. Furthermore, long-storage tomato cultivation is interesting in arid or semi-arid countries where the irrigation water shortage severely limits the agriculture, also in consideration of the competitive use of water for civil and industrial destination.

Long-storage tomato, being cultivated with no irrigations, shows higher nutritional properties (higher antioxidant content, vitamin C, β -carotene and Lycopene) respect to well-known processing tomato cultivars.

Over the years, there has been a significant reduction of the area cultivated with long-storage tomato, caused by a lower yield (cultivation without watering) respect to irrigated processing tomato and subsequent ripening with higher labor costs.

Recently long-storage tomato has been subjected of a more careful consideration, because of the characteristic of drought-resistance that guarantee its implication in breeding programs to improve the shelf-life, the drought resistant and nutritional value of table and processing tomato cultivars.

2. AIMS OF THE STUDY

Limited water resources cause severe problems in arid and semi-arid regions in terms of crop productivity. Therefore, necessity of more attention to sustainable solutions in agricultural field and performing operations to reduce the effects of this factor is very important. It should be recommended the identification of crops tolerant to water stress and with greater ability to recover after a drought period for cultivation in these areas.

Most commercial cultivars of *Lycopersicon esculentum* are sensitive to abiotic stresses during all stages of plant development. The cultivated species of tomato has a limited genetic variability due to several genetic bottlenecks during its domestication and evolution for drought tolerance. In this specie, the best source of resistance is from genotypes that are indigenous to arid and semiarid environments. Hence, this current study was carried out with the main aim of investigate on the performances of long-storage tomato under greenhouse drought stress conditions. The hypothesis of the experiment was that long-storage tomato can produce yield under extreme soil-drying conditions. The specific objectives of study were to:

1. examine the effect of soil-drying on stomatal conductance, photosynthesis, leaf water status, plant growth parameters;
2. evaluation of yield and quality responses of long-storage tomato;
3. identify the mechanisms used by long-storage tomato to tolerate soil-drying.

3. MATERIALS AND METHODS

3.1. Experimental Setup

The study was conducted on two landraces of long-storage tomato, Pizzutello di Sciacca and Locale di Salina, originally from Sicily, belonging to the germplasm collection of the CNR-ISAFoM of Catania (Italy). The commercial hybrid Brigade (Asgrow Italia Vegetable Seeds, Lodi, Italy) of processing tomato was also included in the experiments as control. The experiment was carried out in a greenhouse of the University of Naples located in Portici (40° 49' N, 14° 20' E, 29 m above sea level) from March to July 2012.

Tomato seeds were sown in seedlings trays on the 28th of February 2012, and transplanted 30 days later when the plants had four true leaves. Seedlings were transplanted as individuals into pots with 22 cm height, 24 cm upper diameter. Pots were filled with a silt clay loam soil, which was air dried and sieved through a 2 mm sieve. The soil had the following general characteristics: sand - 5.8%, silt - 60.2%, clay - 34.0%, field capacity (F.C.) - 38%, permanent wilting point (W.P.) - 10%, maximum water holding capacity - 52%, bulk density - 1.27g cm⁻³, pH - 6.8, organic matter - 1.14%, CEC (cation exchange capacity) - 17.9 meq/100g soil and N - 0.06%.

Before transplanting, the equivalent of 75, 100 and 100 kg ha⁻¹ of N, P and K respectively were applied to the soil as liquid fertilizer. A month after transplanting (before flowering), a further 75 kg ha⁻¹ of N were supplied.

The experiment was built up of 180 pots with 60 pots per genotype in two complete sets (30 control and 30 stressed pots for each genotype) that were arranged according to a completely randomized design.

Harvesting was started 100 days after transplanting and there were two harvests at about 15 days intervals (at beginning and mid-July). The first harvest was performed when ripe fruit rate on the first and second trusses reached about 90%. Instead, at second harvest, were considered the ripe fruits of the higher trusses.

3.2. Treatments

All pots were irrigated with water to F.C. until the stage of pre-flowering, four weeks after transplanting, after which water restriction treatment was imposed.

Half of the seedlings of each genotype (30 plants) were kept for regular watering as control plants and the remaining half were submitted to drying and re-watering cycles according to the scheme reported in Figure 3. Control plants (well-watered plants – ww) were irrigated daily in the evening, with an amount of water to replenish F.C. Instead drought stressed plants (ds) received no water for 10 days as soon as the stomatal conductance reached values near zero and plants started wilting.

Recovery of plants was carried out by re-watering (rw) of pots to F.C. for 7 days. A second cycle of drought stress was imposed by withholding irrigation for 6 days followed by a final re-watering.

Irrigation was given manually using a measuring cylinder. Careful attention was taken for homogenous application of irrigation water in the pots throughout the whole growing period of the plants.

3.3. Measurements

SWC, physiological, biochemical and biometric measurements on control and stressed plants were performed during the drying re-watering cycles (Fig. 3). Non-destructive determinations such as stomatal conductance (g_s), photosynthetic rate (A), intercellular carbon dioxide concentration (C_i), maximum quantum yield of PSII (F_v/F_m), were carried out daily. Leaf water potential (ψ_{leaf}) and abscisic acid content (ABA) were measured before stress application (0 d), at 4 day (d) and 10 d of the 1st ds at 2 d and 7 d of the 1st rw, at 2 d and 6 d of the 2nd ds and at 6 d of the 2nd rw. Leaf relative water content (RWC), proline content and growth parameters such as plant height, stem diameter, root length, dry weights (DW - roots, leaves and stems) and leaf area (LA) were determined at the end of the drought stress periods (t_{ds1} and t_{ds2}) and re-watering phases (t_{rw1} and t_{rw2}). The total yield and number of fruits was evaluated as sum of the result of both harvests. Quality parameters of tomato fruits such as biometric (fruit weight, fruit length, fruit width and fruit shape), physiochemical (firmness, skin colour, pH, treatable acidity, total soluble solids (TSS), total solids (TS), and reducing sugars) and antioxidant traits (Vitamin C, Lycopene, β -Carotene, total phenols and antioxidant activity) were carried out on the fruits of the first harvest.

3.3.1. Determination of SWC

SWC was measured twice daily before and 12 hours after that the irrigation water was applied, on four plants per each genotype and treatment using time domain reflectometry (TDR) and the Tektronix cable tester. The TDR rods (two rods per probe) were installed vertically close to the plants to measure the volumetric water content at 12 cm depth.

3.3.2. Physiological measurements

3.3.2.1. Leaf gas exchange and chlorophyll fluorescence

Leaf gas exchange parameters such as g_s , A and C_i were measured between 11:00 and 13:00 h (local time) by a portable photosynthesis system (model Li-6400; LiCor Inc., Lincoln, Nebraska, USA), operating as an open gas-exchange steady-state system. The measurements were taken on the youngest fully expanded leaves of four different plants from each genotype and treatment.

The CO₂ concentration in the Li-6400 leaf chamber (C_a) was set to 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, the vapour pressure deficit (VPD) and leaf temperature were constant at about the same values as those measured in the surrounding atmosphere of the leaf. Chlorophyll fluorescence was determined at the same time and on the same plants used for leaf gas exchange determinations using a plant efficiency analyzer (PEA, Hansatech, Ltd.). The reading was taken on the upper most fully expanded leaf from the top of the plant. The sample leaf was dark adapted with a lightweight plastic leaf clip for 30 min before the measurement. The measurement consisted of a single strong 1-s light pulse (3000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The fluorescence rise during first second of illumination to the dark-adapted samples was recorded in control unit. Fluorescence values recorded included: initial/minimal fluorescence (F_0), a measure of the stability of the light-harvesting complex; F_v/F_m , which in turn is highly correlated with the quantum yield of net photosynthesis. Where F_m is the maximal fluorescence value and F_v is variable fluorescence ($F_m - F_0$).

3.3.2.2. Plant water status

3.3.2.2.1. Determination of leaf RWC

RWC of fully expanded leaves of four different plants from each genotype was measured in both well-watered and drought stressed plants. To minimize solute leakage and cut surface effects, the entire leaf was used for this purpose. The leaf petiole was carefully cut, leaf fresh weight (FW) was recorded, and it was placed in a water-containing plastic tube in a closed container. The turgid weight (TW) was then recorded after overnight rehydration at 4 °C. For dry weight (DW) determination, samples were oven-dried at 70 °C for 48 h. RWC was calculated according to the following equation:

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

3.3.2.2.2. Determination of ψ_{leaf}

Four leaf from different plants from each genotype and treatment were cut with a sharp blade, wrapped in a plastic bag and put in a Scholander pressure chamber (SKPM 1400; Skye Instruments Ltd., Llandrindod Wells, UK) to measure the leaf water potential (ψ_{leaf}). The pressure was gradually increased until the xylem water became visible through the cut surface of the petiole and the balancing-pressure reading was taken. Measurements were conducted between 11:00 and 13:00 h (local time).

3.3.3. Biochemical measurements

3.3.3.1. ABA determination

Leaves were harvested between 11:00 and 13:00 h (local time). Four leaves from different plants for each genotype-treatment were used. Leaves were immediately put in jars and transferred into liquid nitrogen and stored at -80 °C until ABA extraction. Twenty milligrams of leaf (without midribs) was extracted overnight in 1.5 ml distilled water in the dark at 4 °C on a shaker. The extracts were centrifuged at 10000 x g for 25 min, and the ABA content of the supernatants was quantified in an enzymelinked immunosorbent assay (ELISA) using the Phytodetek-ABA kit (AGDIA, Elkhart, IN, USA), according to the indications of the manufacturer. All assays were made in triplicate. The hormone concentrations were calculated using a standard curve of ABA and the relative optical density, according to the ELISA technique.

3.3.3.2. Proline determination

Proline content was determined according to the method described by Clausen et al. (2005). Briefly, four fully expanded leaves from both treatments of each genotype were collected at 11:00 h (local time). Leaf samples of 1 g FW were ground in a mortar after the addition of liquid nitrogen and of 10 mL of a 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was filtered through two layers of glass-fibre filter (Schleicher & Schull, GF 6, Germany), and the clear filtrate was then used in the assay. 1 mL of glacial acetic acid and 1 mL of ninhydrin reagent (2.5 g ninhydrin/100 mL of a solution containing glacial acetic acid, distilled water and ortho-phosphoric acid 85% at a ratio of 6:3:1) were added to 1 mL of the filtrate. The closed test tubes with the reaction mixture were kept in a boiling water bath for 1 h at 100 °C, and the reaction was terminated at room temperature (21 °C) for 5 min. Readings were taken immediately at a wavelength of 546 nm. The proline concentration was determined from a standard curve and calculated on a fresh weight basis. All assays were made in triplicate.

3.3.4. Growth measurements

Plant height and stem diameter were recorded on six randomly selected plants per genotype and per treatment. Plant height was measured on the surface to the top of plant. Destructive determinations such as root length, LA and plant DW were measured on four plants per genotype and per treatment.

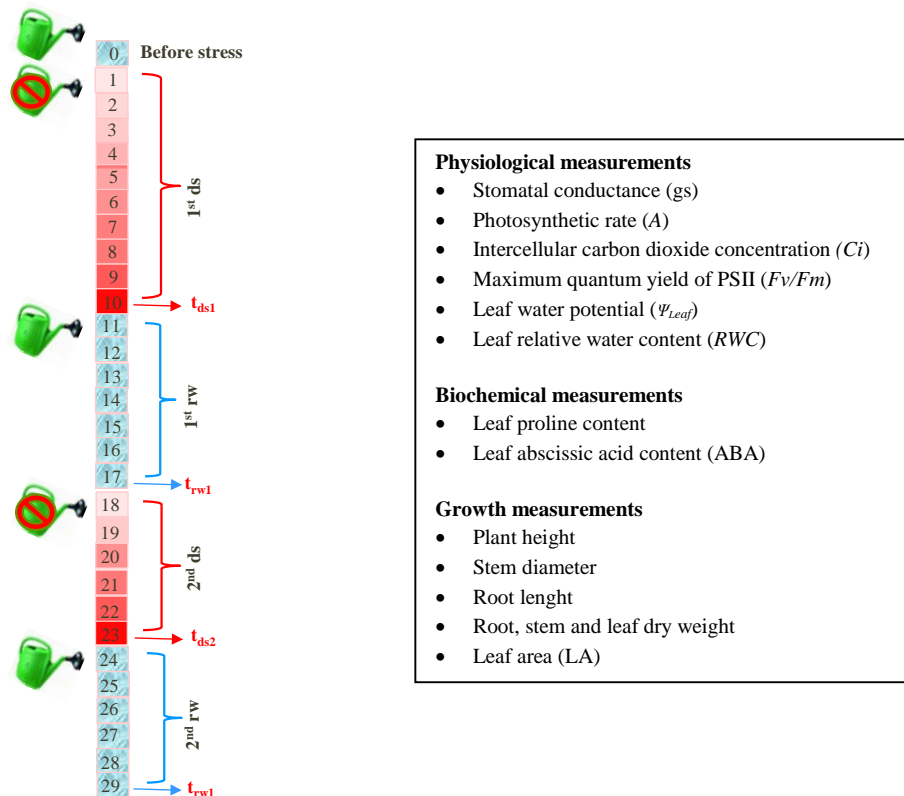
LA was measured immediately for all leaves on a plant using an automatic leaf area meter (ΔT Devices Ltd., Cambridge, UK) after the plant was removed.

Leaf, stem, root and plant DW were obtained after all parts of the plants were separately dried at 70 °C until constant weights were reached. Specific leaf area (SLA) was calculated by dividing LA by leaf DW (Lambrides et al., 2004). Root to shoot (stem+leaves) ratio was obtained by dividing root DW by shoot DW.

3.3.5. Yield determination

Fresh yield (total yield) and fruit number (without fruits affected by physiological disorders) of six plants per genotype in each treatment were measured as cumulative value of the two harvests.

Figure 3 - Schematic diagram of experiment with time indications (days) of drying re-watering cycles. In red color are reported the durations of the first (1° ds – from 1 to 10 day) and second drought stress (2° ds – from 18 to 23 day) imposed, instead in blue color the re-watering phases (1° rw - from 11 to17 day and 2° rw - from 24 to 29 day). The physiological, biochemical and biometric determinations carried out on plants during drying re-watering cycles are also reported.



3.3.6. Quality parameters of tomato fruits

3.3.6.1. Sample preparation

At first harvest, ripe fruits of the first and second trusses, were sampled (10 fruits per plant from the same plants used for yield determination) at commercial maturity (>90% fully coloured fruits, uniform size). Tomatoes were washed with tap water to remove dirt, then washed with distilled water and dried thoroughly with absorbent paper. A part of each whole sample was used for chromatic and biometric parameter measurements as well as firmness. The remaining part of the sample was homogenized for 45 s at 15,000 rpm using a Sterilmixer homogenizer (PBI International, Milano, Italy) and used for all other analyses. Unless specified otherwise, all chemicals used were of analytical grade and supplied by Sigma-Aldrich Chemicals Co. (Milan, Italy). All analyses were carried out in triplicate.

3.3.6.1.1. Chromatic parameters

Color measurements were performed on the surface of the tomatoes using a Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan). According to the CIELab standard, values of the absolute parameters L* (lightness), a* (green/red shade) and b* (blue/yellow shade) were recorded and colour development index (a*/b* ratio), correlated with the colour perceived for fruits (skin colour), was then calculated. The data reported are the average of a set of five determinations (per replicate) carried out on 10 different berries each one consisting of four measurements on opposite points of the tomatoes.

3.3.6.1.2. Biometric parameters

Ten fruits were randomly sampled and the following biometric parameters were measured: fruit fresh weight (g); fruit length and width (cm), which correspond to the polar and equatorial diameters respectively; fruit shape index, calculated by dividing fruit length by fruit width ratio.

3.3.6.1.3. Physicochemical traits

Three fruits were randomly sampled and firmness was measured (five measurements on different points of each tomato) using a texturometer (FT 011, Bertuzzi, Brugherio, Italy). Firmness indicates the force (kg cm^{-2}) offered by the fruit against mechanical pressure and therefore its aptitude for mechanical harvest. The remaining fruits were cut into four pieces, then a 150–200 g tomato sample was homogenised in an Ultraturrax T25 (Janke & Kunkel, Staufen, Germany) for 3 min in an ice bath to prevent oxidation. The homogenate was analysed for the following physicochemical parameters according to AOAC procedures (1984):

- pH, read with a glass electrode pH meter (InLab pH Level 1, WTW, Weilheim, Germany) after standardisation with pH 7 and 4 buffer solutions;
- titratable acidity [g kg^{-1} fresh weight (FW)], as monohydrate citric acid, measured by titration using 0.1 mol L^{-1} NaOH against 20 mL of a filtered 1:1 (v/v) diluted tomato homogenate sample with water;
- TSS ($^{\circ}$ Brix), measured with a hand refractometer (Bertuzzi) at 20°C , the refractometer being washed with distilled water after each use and dried with blotting paper;
- TS (g kg^{-1} FW), determined on 5 g a homogenate sample dried at 70°C in a thermo-ventilated oven until constant weight (~ 72 h).

3.3.6.1.3.1. Reducing sugars

Reducing sugars and total sugars were determined by the methods described by Ranganna (1995) with slight modifications. To a known quantity of sample (10 g), 10 mL of 45% lead acetate solution was added and after 10-20 minutes, 5g potassium oxalate was mixed to remove the excess of lead. The content was filtered through Whatman N^o 1 filter paper and the volume of the filtrate was made up to 100 mL with water. This filtrate was titrated against Fehling's solutions A and B (5 mL each) by using methylene blue as an indicator. The end point was indicated by the appearance of brick red precipitates.

$$\text{Reducing sugars (\%)} = \frac{\text{mg of invert sugar} \times \text{dilution}}{\text{Titre} \times \text{weight of sample (g)} \times 1000} \times 100$$

A standard invert sugar solution was prepared weighing 9.5 mg sucrose (AR) in to a 1.0 L volumetric flask. Added 100 ml of water and 5 ml concentrated HCl. Allowed it to stand for 3 days at room temperature for inversion and then made up to mark by adding water. Factor for Fehling's solution was determined by titrating equal amounts of Fehling's A and B with invert sugar by using methylene blue indicator and the end point was indicated by the complete discoloration of the indicator.

$$\text{Factor for Fehling's solution (mg of invert sugar)} = \text{Titre} \times 2.5$$

3.3.6.1.3.2. Vitamin C

Ascorbic acid (AsA) and dehydroascorbic acid (DHA) contents were determined as reported by Kampfenkel et al. (1995) in samples of homogenate suspension (0.1 g) using 60 g kg⁻¹ metaphosphoric acid and spectrophotometric detection (Cary IE-100 UV-VIS, Varian, Palo Alto, CA, USA) at 525 nm. The linear reading of the standard curve was from 0 to 700 μmol of ascorbic acid. The assay used is based on the reduction of Fe³⁺ to Fe²⁺ by ascorbic acid and the spectrophotometric detection of Fe²⁺ complexed with 2,2'-dipyridyl. Dehydroascorbic acid is reduced to ascorbic acid by preincubation of the sample with dithiothreitol (DTT), and the excess is removed with N-ethylmaleimide. Total ascorbic acid is determined by the 2,2'-dipyridyl method. The concentration of dehydroascorbic acid is then calculated as the difference between total ascorbic acid and ascorbic acid without pretreatment with DTT. Total vitamin C content was calculated as the sum of ascorbic acid and dehydroascorbic acid contents (AsA + DHA). Results were expressed as mg kg⁻¹ fresh weight (FW).

3.3.6.1.3.3. Lycopene and β-carotene

Lycopene and β-carotene fruit contents were evaluated using the extraction method reported by Sharma and Le Maguer (1996) and the HPLC chromatographic determination modified from Arena et al. (2000). The extraction was carried out on 1.0±0.05 g (FW) of cold-milled tomatoes in dark glass vials with 20mL of hexane-ethanol-acetone solution (50 : 25 : 25 v/v/v). To the mixture, stirred for 30 min in a refrigerated shaker, 4mL of distilled water were added, and then a further 2-min agitation was applied. Afterward, the solution mixture was left undisturbed for the polar-apolar layer separation, with decoloured pulp floating between phases. The hexane solution was filtered through a 0.22-mm nylon filter membrane (Albet) and directly injected into the HPLC system. All the extraction procedures were performed in dark conditions to avoid lycopene degradation.

A Beckman HPLC system (Irvine, CA, USA), comprising binary pump module (Programmable Solvent Module 126), injection valve (mod. 210A) with 20-mL biocompatible loop, UV-Vis spectrophotometer set at 472 nm (Programmable Detector Module 166), and column Adsorbosphere C18 250 x 4.6mm 5 mm (Alltech, Columbia, USA) with Adsorbosphere C18 precolumn (45 x 4.5 mm), was used. The following eluent system was used. Eluent 'A' methanol : water (75 : 25 v/v) and eluent 'B' ethyl acetate with starting condition 100% A; 3.5 min 30% A; 8.5 min 20% A; 13.5 min 20% A; 16.5 min 5% A; and 20.5 min 100% A, at 0.7 mL min⁻¹ flow rate. All the adopted solvents were HPLC grade. Carotenoid quantification was achieved by means of calibration curves obtained with standards of lycopene, 90–95% HPLC grade extracted from tomato (Sigma-Aldrich), and β-carotene, HPLC grade 95% from carrots (Sigma-Aldrich), dissolved in hexane. Standard purity level was evaluated by measuring absorbance at 472 and 453 nm, respectively, for lycopene and β-carotene, and adopting the following formulas:

for lycopene

$$\% \text{ Purity} = \frac{\text{Abs} \times 1000}{3450 \times C} \times 100$$

for β-carotene

$$\% \text{ Purity} = \frac{\text{Abs} \times 1000}{2592 \times C} \times 100$$

where *C* is the standard concentration.

The adopted concentration range for calibration curve calculations were 1–50 μg mL⁻¹ and 0.1–5 μg mL⁻¹, respectively, for lycopene and β-carotene. Retention time observed for standards was 14.07±0.05 min and 15.11±0.24 min, for lycopene and β-carotene, respectively.

3.3.6.1.3.4. Total phenols

Total phenols were extracted from quintuplicate samples of tomato tissue with a solution of 10 mL L⁻¹ HCl in methanol and assayed using the Folin–Ciocalteu reagent (Singleton et al., 1999).

Since sugars and other reducing substances (e.g. vitamin C) may interfere with the results of the assay, 20 methanolic extracts were settled on a Strata C-18 end-capped cartridge (Phenomenex, Castel Maggiore, Italy), 1 g for 6 mL, according to the following method. The cartridge was washed with 2 mL of methanol and conditioned with 5 mL of 0.01 mol L⁻¹ H₂SO₄. A 1 mL sample was adsorbed and then 2 mL of 0.01 mol L⁻¹ H₂SO₄ was added. The cartridge was linked to a 20 mL flask and eluted with 2 mL of methanol and 5 mL of deionised water. To the flask content, 1 mL of Folin–Ciocalteu solution was added, followed after 5 min by 4 mL of 200 g L⁻¹ Na₂CO₃, then the flask was made up to volume with deionised water. The mixture was incubated at 20 °C for 2 h. After centrifugation at 2500 × g for 15 min at 4 °C in order to separate the carbonate fraction, the absorbance at 765 nm was measured spectrophotometrically (Cary IE-100 UV-VIS, Varian) against a blank prepared in a flask containing all reagents and 2 mL of methanol without phenols. Results were expressed as mg gallic acid equivalent (GAE) kg⁻¹ FW.

3.3.6.1.3.5. Antioxidant activity determination

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables. The antioxidant properties of the tomato genotypes studied were evaluated in methanolic extracts using the DPPH assay according to Wang et al. (2010), with modifications.

For the DPPH free radical-scavenging assay, 100 μL of tomato extract (1 g mL^{-1} in methanol) was mixed thoroughly with 2 mL of freshly prepared 0.1 mmol L^{-1} DPPH methanolic solution (not buffered). After incubation at room temperature for 30 min in the dark, the absorbance at 517 nm was measured in a spectrometer (Lambda 11, Perkin Elmer, San Jose, CA, USA). Methanol (100 μL) without the extract was used as a blank. The capability to scavenge the DPPH radical was calculated as follows:

$$\text{DPPH – scavenging effect (\%)} = 1 - \frac{A_{517 \text{ sample}}}{A_{517 \text{ blank}}} \times 100$$

Results were expressed as $\mu\text{mol g}^{-1}$ FW.

3.4. Statistical analysis

In order to investigate the effects of the irrigation treatments on morphological, physiological, yield and quality traits of tomato genotypes under study, data were statistically analysed by two-way analysis of variance (ANOVA), considering the interaction treatment \times genotype. Differences between means were evaluated for significance using the least significant differences (LSD) test at $P_{0.05}$ level. All statistical procedures were computed using the statistical package SAS (version 9.1, SAS Institute Inc. Cary, NC, USA).

4. RESULTS AND DISCUSSION

4.1. Effect of drought stress and re-watering on SWC

SWC for control plants oscillated from 33 to 38% during the entirety of the experimental period and the different genotypes did not show differences (Fig. 4). This could be explained because all plants were undergoing the same phenological period (beginning of flowering) so they required a similar amount of water.

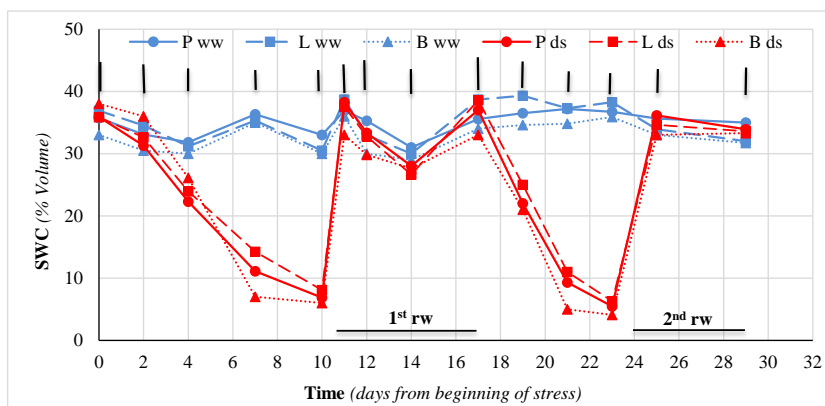


Figure 4 – Volumetric soil water content (SWC) changes during soil drying and re-watering cycle. Blue dots refer to well-watered plants (ww) and red dots to drought plants (ds) of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Vertical bars are LSD values ($P \leq 0.05$) for treatments and genotypes comparisons at a given day of treatments. The duration of re-watering phases (1st and 2nd rw) were indicated by lines below the figure.

Drought affected SWC from 4 d and 2 d of the first and second stress period respectively, when SWC showed significant differences respect to the control for all genotypes. SWC decreased slowly in Pizzutello di Sciacca and Locale di Salina respect to Brigade, in fact at 7 d of the first stressed period SWC of B cv. reached value (7%) significantly lower than the other two genotypes (11.10 for Pizzutello di Sciacca and 14.22% for Locale di Salina). Similar behavior was recorded at 4 d of the second stress period when Brigade reached the value of 5% significantly lower than 9.3% and 11% of Pizzutello di Sciacca and Locale di Salina. SWC decreased at minimum values at 10 d of the first drought-stress cycle (6.85%, 8.08% and 6% for Pizzutello di Sciacca, Locale di Salina and Brigade respectively) and at 6 d of the second drought-stress cycle (5.47%, 6.27% and 4.10%). The SWC decrease was quickly in the second drought-stress cycle respect to the first; in fact it reached the minimum value in four days rather than ten days. This behavior was due to a higher evapotranspiration rate of all plants at second drought-stress cycle than to the first, relating to higher plant growth parameters. The SWC decrease became slower when the W.P. (10%) was reached. Moreover, SWC values reached at the peak of drought stress were not significantly different between genotypes during the two cycles, indicating that all plants underwent similarly intensive constraints. From the beginning of the first re-watering no differences were highlighted between control and stressed plants instead for the second re-watering the differences were not significant only after 2 d.

4.2. Effect of soil water deficit and re-watering on leaf gas exchange

Leaf gas exchange parameters of all genotypes, used in the experiment, showed similar response patterns during progressive water restriction and rewetting. They showed significant differences between the tomato genotypes x watering treatments interaction ($P \leq 0.05$) within each day of treatment.

In general, gas exchange parameters declined significantly with SWC under water restriction.

g_s of control plants remained above $0.30 \text{ mol m}^{-2} \text{ s}^{-1}$ (Fig. 5) during the experiment in all genotypes, it decreased gradually throughout the assay, without a significant difference between genotypes.

At the beginning of the experiment, g_s ranged from 0.53 to $0.63 \text{ mol m}^{-2} \text{ s}^{-1}$ for the different genotypes in the control and stressed treatments.

At the first drought-stress cycle, g_s of Pizzutello di Sciacca and Locale di Salina stressed plants decreased earlier respect to those of Brigade plants. It already declined after 4 d of drought, when SWC was about 25%, reaching a level of 0.40 and $0.35 \text{ mol m}^{-2} \text{ s}^{-1}$ for Pizzutello di Sciacca and Locale di Salina respectively. g_s of Pizzutello di Sciacca and Locale di Salina continued to decline up to 10 d of stress when the minimum value, near zero, was attained. Instead, g_s of Brigade stressed plants retained values not significantly different from control plants after 4 d of stress and rapidly dropped to near zero by 7 d when SWC reached the wilting point. g_s showed similar behavior in the second drought-stress cycle; g_s value of Pizzutello di Sciacca and Locale di Salina declined to a level lower than that Brigade at 2 d of drought when SWC was $\approx 20\%$ but was maintained at higher level during the remaining stress period until the wilting point was reached. g_s of Brigade rapidly dropped to near zero by 4 d. Complete stomatal closure for Locale di Salina and Pizzutello di Sciacca occurred only at 6 d. For all genotypes, during the second dehydration period, g_s reached the minimum value (about zero) earlier than the first drought-stress period.

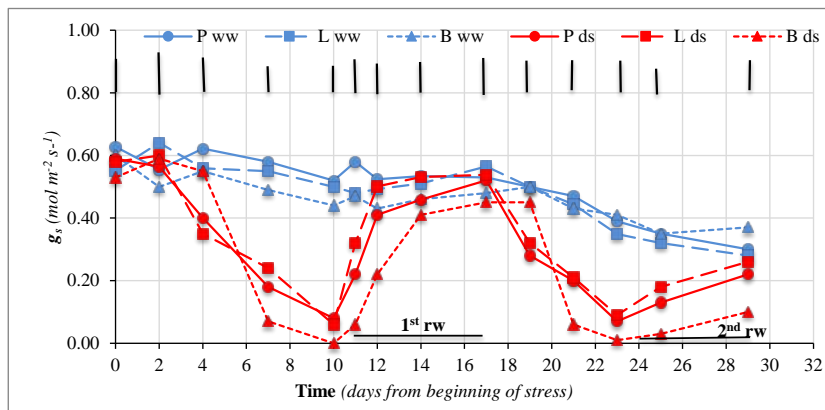


Figure 5 – Responses to stomatal conductance (g_s) to drying-rewetting alternation. Blue dots refer to well-watered plants (ww) and red dots to drought plants (ds) of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Vertical bars are LSD values ($P \leq 0.05$) for treatments and genotypes comparisons at a given day of treatments. The duration of re-watering phases (1st and 2nd rw) were indicated by lines below the figure.

It is thought that rapid stomatal response may act as a drought resistance mechanism, which permits to keep water for later use and thus to maintain higher leaf water potentials (Teare et al., 1973; Jones, 1974). Generally, stomatal closure protects plants against excessive water loss, but also restricts the diffusion of CO_2 into the photosynthetic parenchyma. Thus, the earlier reduction of g_s in Pizzutello di Sciacca and Locale di Salina could be one of the prerequisites for a better performance during drought stress. Quarrie (1982), who connected it with genetic differences in the capacity to produce A, has demonstrated genotypic variation in stomatal response to water deficit.

The first re-watering was followed by recovery of g_s to near control values also if the recovery time was different among the genotypes indicating complete reversibility of the responses to drought stress. g_s of Pizzutello di Sciacca and Locale di Salina after re-watering, reaching nearly as high as that of the control by 2 d, more rapidly respect to Brigade that recovered after 4 d respectively.

In contrast after the second re-watering stomata remained closed for more time; stomatal opening tended to increase slowly in Pizzutello di Sciacca and Locale di Salina recovering a value not significant different from control only at 6 d of re-watering. g_s of Brigade drought-stressed treatments did not reach the level of control treatments at the end of the second re-watering phase.

Pizzutello di Sciacca and Locale di Salina showed a higher average time for the decrease of g_s under dehydration and a rapid recovery after re-watering indicated the greater dynamism of stomata of these genotypes than of those of Brigade.

When plants were re-watered after a period of water stress, stomatal conductance recovers more slowly than leaf water status (Ismaiv and Davies, 2000).

A was non-significantly different among the different genotypes under studied in well-watered plants where it decreased progressively during the experimental period, from 17.27 to 10.00 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Pizzutello di Sciacca, from 19.37 to 11.20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Locale di Salina and from 20.00 to 10.50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Brigade (Fig. 6a).

During the first recovery (100% irrigation), A resulted not different from the control already after 2 d; increased from 3.78 to 13.12 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Pizzutello di Sciacca and from 4.41 to 15.22 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Locale di Salina. At the second re-watering A of stressed plants returned to values not different from control plants only after 6 d. Brigade recovered the stress imposed more slowly respect to the previous genotypes; the recovery of A was completed only after 7 d of the first re-watering period and was not complete in the second re-watering. One of the reasons for this incomplete recovery could be the drought-induced damages of chloroplasts and mitochondria (Vassileva et al., 2011). A of Pizzutello di Sciacca and Locale di Salina stressed plants at 7 d of the first re-watering period showed an ultra-compensating effect, which made the net photosynthesis ratio of the drought treatment greater than that of the control.

Vassileva et al., 2011 reported that drought-tolerant wheat genotype leaves displayed lower g_s and higher A during dehydration. It is noteworthy that the gas-exchange traits of tolerant genotypes recovered quickly to their original levels after rehydration. Rewetting of dry soil led to full recovery of both g_s and A in *Acacia confusa* (Liang et al., 1997).

Genotypic variations in A of several stressed-crop plants were evident from the studies of Gunasekera et al., 1994 and Kicheva et al., 1994. The drought tolerance of Pizzutello di Sciacca and Locale di Salina could be correlated with their capacity to maintain relatively high A values with a less reduction in g_s under drought stress.

In addition, Pizzutello di Sciacca and Locale di Salina stressed plants were able to reduce g_s as much as possible without significantly affecting photosynthesis. A of these genotypes began to decrease significantly only at 7 d and 4 d of the first and second drought period respect to g_s that already decreased at 4d and 2d of the same periods. Early closure of stomata and late decrease in A could be considered an important drought-adapting mechanism of crops (Lu et al., 2002). Sapeta et al. (2010) showed a similar mechanism from *Jatropha curcas* in response to drought.

The low g_s induced by water deficit leads to a decreased CO_2 availability, which has a direct adverse effect on photosynthesis (Chaves et al., 2009). Photosynthesis is among the primary processes affected by drought.

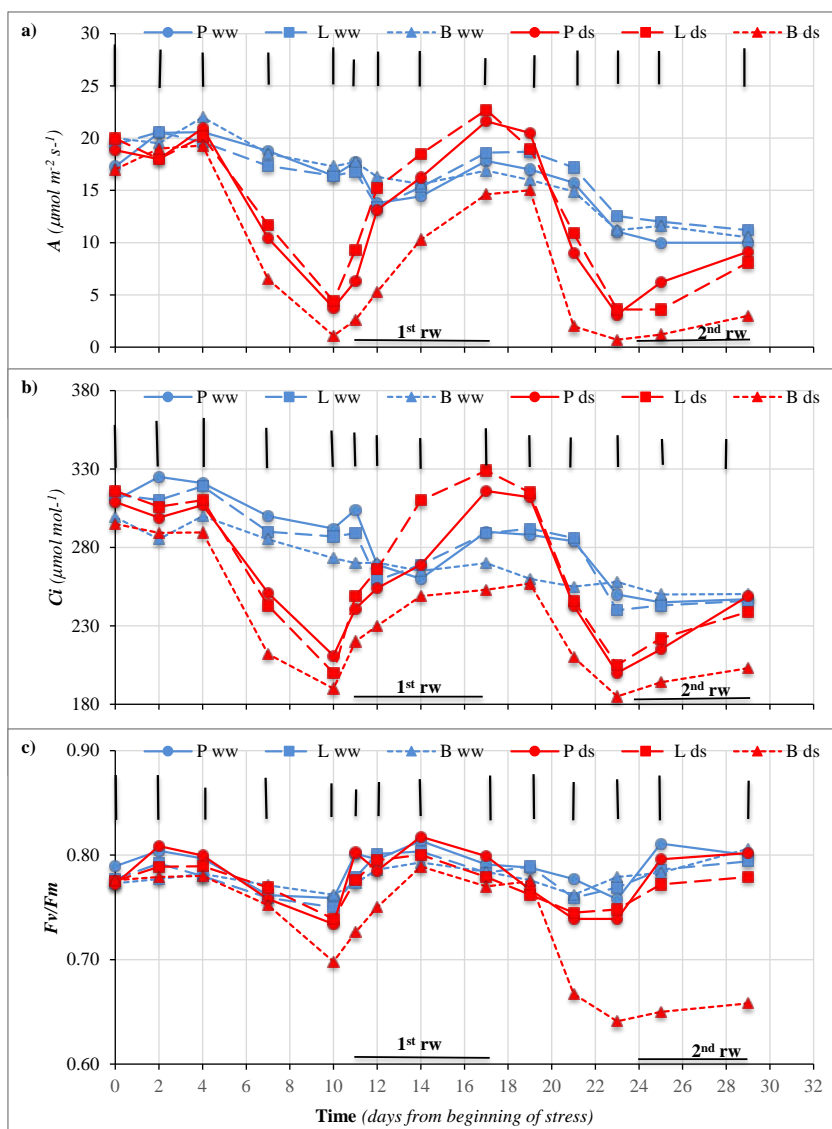


Figure 6 – Responses of **a)** photosynthetic rate (A), **b)** intercellular carbon dioxide concentration (C_i) and **c)** the maximum quantum yield of PSII (F_v/F_m) to drying-rewetting alternation. Blue dots refer to well-watered plants (ww) and red dots to drought plants (ds) of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Vertical bars are LSD values ($P \leq 0.05$) for treatments and genotypes comparisons at a given day of treatments. The duration of re-watering phases (1st and 2nd rw) were indicated by lines below the figures.

For all drought-stressed genotypes, A was highly correlated with g_s (Fig. 7). The response displayed a logarithmic pattern for Pizzutello di Sciacca and Locale di Salina, with an initial phase where decreases in g_s resulted in relatively smaller decreases in A , followed by a second phase, where the relationship became linear and the slopes steeper. Galmes et al. (2011) also reported a non-linear relationship between A and g_s for Mediterranean accessions of *Solanum lycopersicum* in drought conditions. Differently from cv. Brigade A and g_s correlation was linear. This suggests that the impact of initial stomatal closure (high g_s values) on photosynthesis was larger on Brigade than the remaining genotypes.

A strong correlation between g_s and A represents an adjustment of g_s to match the genotype intrinsic photosynthetic capacity under stress (Ramanjulu et al., 1998). Several studies have proposed that stomata closure is the main determining factor in photosynthesis reduction under moderate stress conditions (Sharkey, 1990; Chaves, 1991; Ort et al., 1994; Medrano et al., 2002). The existence of a good correlation between A and g_s does not necessarily mean that A is only under g_s control.

As would be predicted from data presented in Figure 6b, C_i values were reduced substantially at time when photosynthesis was inhibited. The only exception was the stressed plants of Brigade after 4 d of the first re-watering, where C_i was comparable to well-watered plants while photosynthesis remained inhibited and recovered only after 7 d, suggesting some factors other than CO_2 supply (e.g. mesophyll capacity of photosynthesis), which were limiting the CO_2 assimilation.

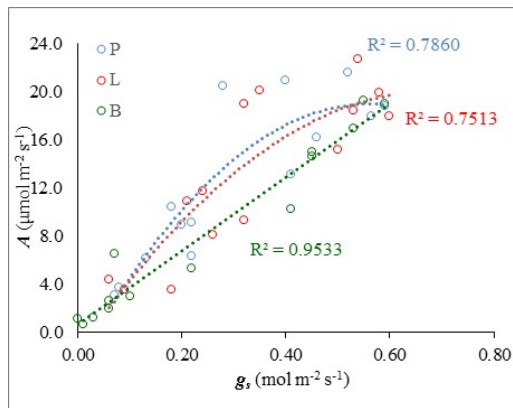


Figure 7 - Relationship between stomatal conductance (g_s) and the photosynthesis rate (A) in tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B) under drought treatment.

The reduced A during water deficits can also be attributed to impairment of the primary photosynthetic machinery. According to He et al. (1995), water stress affects directly the photochemical events largely by affecting PSII activity both by degradation of D1 and D2 proteins of PS2 reaction center and retarded synthesis of these proteins, which leads to lowered electron transport (Kaiser 1987; Chaves 1991; Kicheva et al. 1994; Fedina and Popova 1996; Niinemets, 2002). Chlorophyll fluorescence parameters are direct indicators of the photosynthetic activity (Lichtenthaler et al., 2000) particularly they are indicators of PSII functioning and electron transfer from PSII to PSI and have been used frequently to estimate damage to the photosynthetic system. Photochemical damage is reflected in an increase in F_0 or decreases in F_m or F_v/F_m (Thomas and Turner, 2001). The maximum quantum yield of PSII (F_v/F_m ratio) is an important parameter, which determines the maximum quantum efficiency of PSII. It provides a measure of the rate of linear electron transport, hence, an indication of overall photosynthetic capacity (Tang et al. 2007; Jamil et al. 2007; Balouchi 2010). In healthy leaves, F_v/F_m value is usually close to 0.8 in most plant species, therefore a lower value indicates that a proportion of PSII reaction centers is damaged or inactivated, a phenomenon, termed as photoinhibition, commonly observed in plants under stress (Baker and Rosenqvist 2004; Zlatev 2009; Vaz and Sharma 2011).

Further analyses on the chlorophyll fluorescence of all genotypes of different treatments indicated that the values of F_v/F_m ratio for Pizzutello di Sciacca and locale di Salina were not significantly affected by drought treatments, but whole soil drying reduced F_v/F_m ratio of Brigade (Fig. 6c). At 10 d of the first drought stress period F_v/F_m of Brigade stressed plants dropped to 0.698 respect to 0.762 recorded by control plants; F_v/F_m value did not fully recover only after 4 d of re-watering causing a delay in A recovery respect to g_s and C_i . So the late recovery of A after re-watering in Brigade may be regarded as the rate of internal damage on photosynthesis functions by water stress. The turning point between rapid and slow recovery seen varies by plant species, cultivars and the degree and history of stresses (Miyashita et al., 2005).

Drought stress did not alter the F_v/F_m in bigtooth maples (Bsoul et al., 2006) and papaya (Marler and Mickelbart, 1998). Moseki and Dintwe (2011) also reported a decrease in the dark-adapted F_v/F_m ratio of two sorghum cultivars with increase in duration of withholding water in water stressed plants. In addition Moseki (2000) showed that on re-watering the sorghum cultivar, with higher decrease in F_v/F_m during water stress, took longer to recovery than the cultivar with lower F_v/F_m decrease. Sousa et al. (2004) also observed that an advance phase of water stress, a down regulation of PSII activity occurred as revealed by decreases in the maximum quantum yield of PSII (F_v/F_m) and complete recovery of F_v/F_m occurred 3 days after re-watering. It is therefore logical to suggest that decreases in F_v/F_m ratio exhibited by Brigade during the first drought stress period being followed by a recovery can be ascribed mainly to down-regulation of PSII by the xanthophyll cycle rather than damage to PSII reaction centers. In addition the photoinhibited reaction centers can be also quickly de-assembled and replaced by new ones (Aro et al., 1993).

Differently in the second drought stress period F_v/F_m of Brigade stressed treatments decreased and not recovery after re-watering suggesting the presence of a permanent damage.

Stomatal conductance can be considered the only factor limiting photosynthesis in Pizzutello di Sciacca and Locale di Salina genotypes, whereas in Brigade cv., in addition to stomatal limitation, decreased PSII activity also contributed to decrease and subsequent recovery in photosynthetic performance.

4.3. Effects of soil water deficit and re-watering on leaf ABA accumulation

Under well-watered conditions, leaf ABA content remained a low level (<100 pmol g^{-1} FW) in all genotypes, and no genotypes differences were observed (Fig. 8). Leaf ABA content increased with drought in all genotypes. There were a 2-fold increase in ABA contents in Pizzutello di Sciacca and Locale di Salina after 4d of the first drought period and up to a 4-fold increase and 5-fold increase, for the two genotypes respectively, after 10 d. The differences between Pizzutello di Sciacca and Locale di Salina were evident only at 10 d of stress when Locale di Salina showed ABA content higher than

Pizzutello di Sciacca. ABA content in Brigade stressed plants, also, showed significant increase at 4 d with value not significant different respect to Pizzutello di Sciacca and Locale di Salina but then it increased quickly until to 10 d of stress when recorded a value 13 times higher than control. Brigade had significantly higher ABA content than Pizzutello di Sciacca and Locale di Salina at 10 d of drought, this content was about two times greater. ABA contents for all genotypes showed similar trends during the second drought stress period also if the ABA increases occurred earlier, at 2 d of stress, compared to the first stress period. In Pizzutello di Sciacca and Locale di Salina, ABA increases resulted slower than those of Brigade. Increases of ABA under dehydration have been frequently documented in other species (Ober et al., 1991; Sharp et al., 1994; Alves and Setter, 2000; Cao et al., 2000; Jin et al., 2000; Li et al., 2000; Stoll et al., 2000). Upon re-watering, the leaf ABA contents of Pizzutello di Sciacca and Locale di Salina rapidly decreased, and returned to the control levels within 2d and 6d of the first and second re-watering respectively. Differently in Brigade *cv.*, the ABA increase disappeared only after 7 d of the first re-watering and did not return to pre-stress condition at the second re-watering. These results indicate that the closure of stomata during soil drying could be explained fully by the increase of ABA concentration in leaves as well as the post-stress effects.

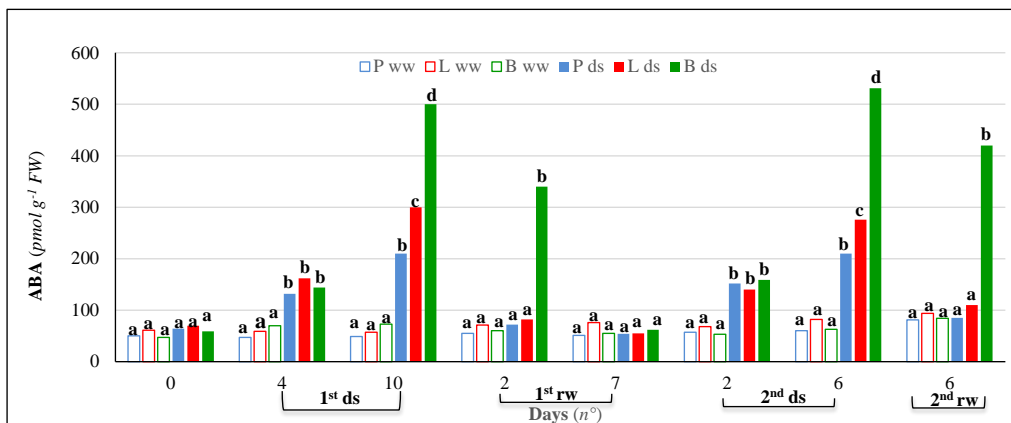


Figure 8 - Leaf ABA accumulation in response to drying-rewetting alternation of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Colored bars refers to well-watered treatments (ww), instead transparent bars to drought treatments (ds). Different letters indicate significant different values for $P \leq 0.05$ within each day of treatment for treatments \times genotypes interaction. Below the figure are reported the indication of the number of drought stress cycles (1st ds and 2nd ds) and re-watering phases (1st rw and 2nd rw).

Figure 9 shows that for all genotypes there were a close relationship between g_s and leaf ABA concentrations suggesting that drying-induced stomatal closure through leaf ABA increases. Many earlier studies have shown that g_s is related to ABA concentration when soil is dried in species such as maize, sycamore and lupine (Zhang and Davies 1987, 1989, 1990; Khalil and Grace 1993; Correia and Pereira 1994, 1995). ABA accumulation has been found to protect plants from damage by drought by inducing stomatal closure, thus reducing water loss via transpiration during early or mild drought stress (Harris and Outlaw, 1991; Wilkinson and Davies, 1997).

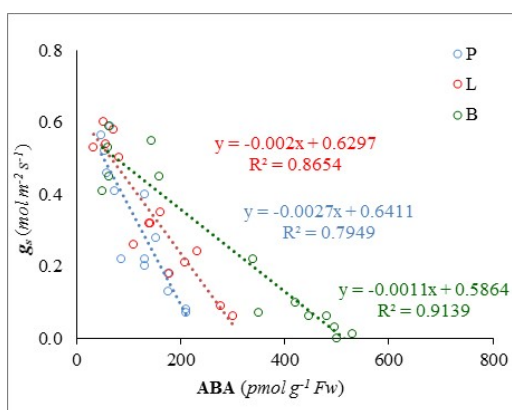


Figure 9 - Relationship between stomatal conductance (g_s) and leaf ABA content in drought-stressed plants of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Lines are fitted linear regressions and correlation coefficient (R^2) are indicated. The differences in the slopes of each genotype was tested by ANOVA ($P=0.05$).

ABA regulation of drought tolerance may involve both the sensitivity of ABA biosynthesis to changes in plant water status and the sensitivity of the stomata to the changes in ABA concentration (Quarrie and Jones, 1979). The rates of change in g_s , with leaf ABA concentration for Pizzutello di Sciacca and Locale di Salina were greater than that for Brigade. So smaller increases in ABA content were necessary to generate a quickly stomata closure. This indicated that stomata in leaves of Pizzutello di Sciacca and Locale di Salina genotypes were more sensitive to ABA accumulation than those of Brigade. The sensitivity of stomata to ABA changes also varied with genotype in other species (Quarrie and Jones, 1979; Cellier et al., 1998). The high ABA sensitivity of Pizzutello di Sciacca and Locale di Salina genotypes under drought treatment may lead to early stomatal closure during drought and limit further water loss, thus lessening damage caused by soil drying. Wang and Huang (2003) showed similar results for Kentucky Bluegrass exposed to drought stress.

Our results on the changes in ABA concentration and stomatal conductance are in agreement with those reported by Fischer et al. (1970) and Correia and Pereira (1994). Fischer et al. (1970) suggested that the lag of stomatal recovery was a result of the persistent effects of ABA produced during the water stress period. Work carried out by Correia and Pereira (1994) on the effects of drought on leaf conductance and on the concentration of ABA in the apoplastic sap of *Lupinus albus* L. leaves, suggested that, an estimated apoplastic ABA concentration did not return to pre-stress values immediately following re-watering. Delay in stomatal reopening upon re-watering could be explained by the ABA in the apoplast.

4.4. Effects of soil water deficit and re-watering on leaf water status and proline accumulation

ψ_{leaf} of well-watered plants ranged from -0.2 MPa to -0.5 MPa during the drying re-watering experiment and in each day of treatment there were not significant differences between the genotypes (Fig. 10).

ψ_{leaf} decreased due to water stress in all genotypes and the decline was more pronounced for Brigade *cv.* than for Pizzutello di Sciacca and Locale di Salina at 10 d and 6 d of the first and second stress period respectively. Differences in ψ_{leaf} values were not significant between control and stressed plants of Pizzutello di Sciacca and Locale di Salina at 4 d and 2 d of the first and second stress period even if the differences were significant in SWC and g_s values. The lower ABA accumulation in Pizzutello di Sciacca and Locale di Salina during drought stress further illustrated that these two genotypes experienced less severe drought stress, which could be due to their superior dehydration avoidance, as demonstrated by their relatively higher ψ_{leaf} under the same soil moisture conditions. Complete stomatal closure for Brigade at 10 and 6 d of the first and second stress period respectively could be resulted from leaf desiccation, which was indicated by its low ψ_{leaf} (below -1.3 MPa) at these times of drought stress. Miyashita et al. (2005) also found that the kidney bean was allowed to maintain its leaf moisture for 5 days even if the soil moisture decreased to about 40%. Hossain et al. (2014) reported that ψ_{leaf} of drought-susceptible soybean genotypes declined sharply throughout the entire water restriction period and remained lower than those of drought-tolerant genotypes.

ψ_{leaf} of stressed plants of Pizzutello di Sciacca and Locale di Salina returned to the control value at 2 d and 6 d of the first and second re-watering phase as soon as g_s recovered. Differently Brigade stressed plants recovered ψ_{leaf} value of control only at 7 d of the first re-watering and did not recover after the second re-watering. Also for stressed plants of Brigade, ψ_{leaf} and g_s had the same behaviour during re-watering phases.

Liang et al. (2002) reported a recovery of ψ_{leaf} 24 h after the addition of water to the treated pots of wheat. Hossain et al. (2014) found that ψ_{leaf} of drought-tolerant soybean genotypes increased faster and reached values nearly equal to that of well-watered plants after 15 d of re-watering whereas in the drought-susceptible genotype the recovery was not complete.

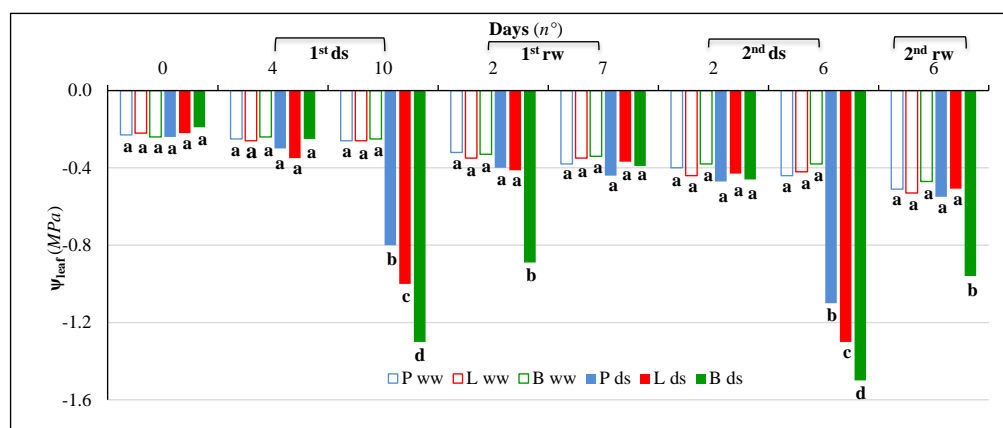


Figure 10 - Response to leaf water potential (ψ_{leaf}) to drying-rewetting alternation of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B). Colored bars refers to well-watered treatments (ww), instead transparent bars to drought treatments (ds). Different letters indicate significant different values for $P \leq 0.05$ within each day of treatment for treatments \times genotypes interaction. Above the figure are reported the indication of the number of drought stress cycles (1st ds and 2nd ds) and re-watering phases (1st rw and 2nd rw).

Similarly, to ψ_{leaf} all genotypes recorded RWC values not significantly different under control conditions and RWC decreasing with the application of drought stress (Fig. 11a). Under drought-stress, the RWC decline such was lower in Pizzutello di Sciacca and Locale di Salina respect to Brigade.

RWC of stressed plants recovered values similar to control plants at the end of the first re-watering (t_{rw1}) in all genotypes, instead the recovery was not complete after the second re-watering (t_{rw2}) for Brigade.

The lower g_s and higher RWC assisted Pizzutello di Sciacca and Locale di Salina to maintain higher leaf water potential under drought conditions and achieve a rapid increase after rewetting.

The rapid recovery of ψ_{leaf} after rewetting accompanied by increased g_s highlights the role of leaf water status in stomatal control (Fuchs and Livingston, 1996).

Hossain et al. (2014) reported that when water stress was intensifying, RWC declined significantly compared to well-watered plants in all soybean genotypes also if this declining tendency was more pronounced in the susceptible genotypes than in the tolerant genotypes. In addition, drought-tolerant genotypes exhibited a more rapid recovery when the soil was rewetted. Siddique et al. (2000) reported that wheat cultivars with less reduction in relative water content could cope-up better under severe stress conditions. He suggested that the high relative water content could help the tolerant genotypes to perform physio-biochemical processes more efficiently under water stress conditions than susceptible genotypes.

Leaf proline increased significantly under drought stress in comparison with control in all genotypes; the highest increase was recorded in Brigade where the proline accumulated in stressed leaves 25 and 22 times the level found in the leaves of well-watered plants in t_{ds1} and t_{ds2} (Fig. 11b).

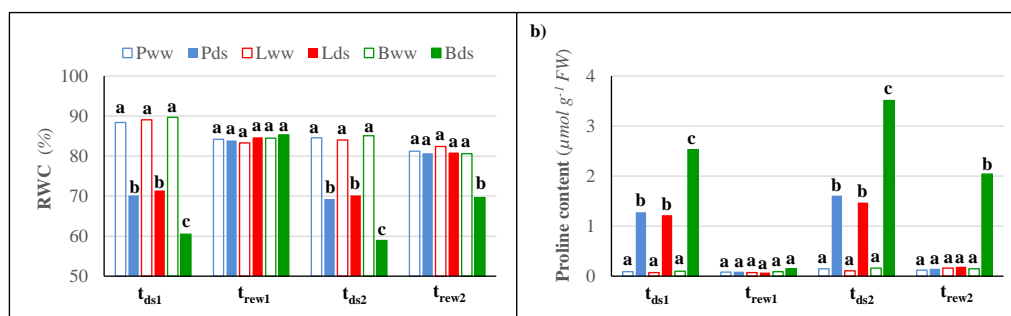


Figure 11 - a) Response to leaf relative water content (RWC) and b) leaf proline content (fresh weight basis – FW) of tomato genotypes Pizzutello di Sciacca (P), Locale di Salina (L) and Brigade (B) at the end of drought stress periods (t_{ds1} and t_{ds2}) and re-watering phases (t_{rew1} and t_{rew2}). Colored bars refers to well-watered treatments (ww), instead transparent bars to drought treatments (ds). Different letters indicate significant different values for $P \leq 0.05$ within each day of treatment for treatments \times genotypes interaction.

There were no significant differences between control plants of different genotypes. Re-waterings reported proline contents to values similar to control in Pizzutello di Sciacca and Locale di Salina, instead in Brigade stressed leaves proline content remained higher than control leaves in t_{rew2} .

Goyal et al. (1985) recorded an 8-fold increase in free proline content in a drought tolerant variety of rice respect to a 3-fold increase of a susceptible variety after 8 days of water stress and on re-watering there was a conspicuous fall in proline content in all varieties.

The tendency of free proline accumulation in plants under any abiotic stress condition is an indicator of a mechanism of avoidance of those stresses. Proline is a reliable indicator of environmental stress imposed on plants (Claussen, 2005).

In our study the lower proline content after water restriction were recorded in Pizzutello di Sciacca and Locale di Salina corresponding with more water retained in the leaves (higher RWC and ψ_{leaf} respect to Brigade).

During drought stress, plants attempt to maintain their turgor through osmotic adjustment by accumulation of compatible solutes like proline, which lower the cellular osmotic potential, facilitate water absorption, and restore intracellular ion concentration. Proline accumulation and improved plant performance under drought stress have already been studied in crop varieties. According to many authors, free proline might be involved in membrane protection during water stress (Kocheva and Georgiev, 2003), or it might be a reserve of readily mobilizable N available upon relief of stress (Hare et al., 1998). Proline acts as an osmolite beside enzymes and other macromolecules, and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs. Proline can also act as an electron receptor preventing photosystems injuries in dealing with ROS function. Proline accumulation facilitates the permanent synthesis of soluble substances in closing stomata. This process is different in various plants (Zhang et al., 2006). Proline accumulation in foliage was seen in olive cultivars (*Olea europaea* L.) in low water condition (Bacelar et al., 2009). Garcia et al. (1997) and Lutts et al. (1999) differently consider proline accumulation to be rather a symptom of damage, than an indicator of plant resistance.

4.5. Growth and morphology

Growth parameters in different genotypes responded differently to drought. Locale di Salina produced higher well-watered plants (124.6 cm) as compared to rest of the genotypes (114.1 cm and 119.8 cm of Pizzutello di Sciacca and Brigade, respectively, Tab. 1).

In drought conditions, plants of all genotypes were able to grow, also if showed a reaction by shortening the length of their heights Results showed that the maximum reduction in plant height was caused in the Brigade cv. in t_{ds1} and t_{ds2} .

The reduction in height may be associated with a decline in cell enlargement under water stress. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure (Munns et al., 2000; Ogbonnaya et al., 2003; Jaleel et al., 2008). A reduction in soil moisture may reduce the availability of nutrients to the plant and consequently reduce plant height, growth and yield (Hale et al., 2005; Razmjoo et al., 2008). Drought stress reduces plant growth, so the carbon fixed during photosynthesis could be used to form secondary metabolites (Hale et al., 2005).

Results from this study are similar to those found by Kirnak et al. (2001) where plant height of water stressed plants were smaller than the equivalent component in the well-watered plants. Bradford and Hsiao (1982) and other investigators have shown that stem and plant growth may be inhibited at low water potential despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment. Nyabundi and Hsiao (1989) reported that when tomato plants are subjected to different levels of water stress under field conditions, vegetative growth is inhibited.

Re-watering the plants, in t_{rw1} , the effects of water stress on plants were eliminated for Pizzutello di Sciacca and Locale di Salina but not for Brigade. In t_{rw2} the height of drought-stressed plants did not recovery the values of the control for all genotypes.

How crops adapt to soil water conditions is an extremely complex issue. Many experiments have shown that crops respond to the drought stress followed by re-watering, by exhibiting a fast growth once the stress disappears (Xu and Zhou, 2007; Liu et al., 2001). This is considered an adaptation to environmental changes.

Table 1 – Growth parameters of tomato genotypes (plant height, stem diameter, root length, root dry weight - Root DW, shoot dry weight - Shoot DW, root to shoot ratio – Root/Shoot) at the end of drought stress periods (t_{ds1} and t_{ds2}) and re-watering phases (t_{rw1} and t_{rw2}). Different letters indicate significant different values for $P \leq 0.05$ within each time of sampling (t_{ds1} , t_{ds2} , t_{rw1} , t_{rw2}) for treatments \times genotypes interaction.

Parameters	t_{ds1}		t_{rw1}		t_{ds2}		t_{rw2}	
	ww	ds	ww	ds	ww	ds	ww	ds
Plant height (cm)								
Pizzutello di Sciacca	62.3 b	53.2 d	90.6 bc	88.3 c	102.1 c	93.2 e	114.1 c	100.7 e
Locale di Salina	69.2 a	58.4 c	98.2 a	95.8 a	112.4 a	99.5 d	124.6 a	105.1 d
Brigade	66.1 a	52.4 d	91.5 b	85.9 c	106.3 b	91.7 e	119.8 b	102.4 de
Stem diameter (mm)								
Pizzutello di Sciacca	5.9 c	5.6 c	7.1 c	7.3 c	7.8 cd	7.6 d	8.2 c	8.1 c
Locale di Salina	6.8 b	6.5 b	7.9 b	7.6 b	8.4 b	8.1 bc	8.8 b	8.5 bc
Brigade	7.5 a	7.9 a	8.6 a	8.5 a	9.1 a	9.3 a	9.6 a	9.8 a
Root lenght (cm)								
Pizzutello di Sciacca	18.2 c	22.6 b	26.2 c	30.6 b	30.3 c	35.2 b	33.6 c	38.5 b
Locale di Salina	25.3 a	25.9 a	36.3 a	36.9 a	41.9 a	41.8 a	43.3 a	43.5 a
Brigade	22.8 b	17.32 c	33.1 b	28.3 c	36.2 b	31.2 c	37.9 b	32.9 c
Root DW (g)								
Pizzutello di Sciacca	1.27 a	1.30 a	1.89 c	2.02 c	2.26 b	2.22 b	2.52 c	2.66 c
Locale di Salina	1.56 a	1.21 b	2.69 a	2.5 ab	2.88 a	2.60 b	3.35 a	3.01 b
Brigade	1.41 a	0.95 c	2.38 b	1.95 c	3.00 a	2.10 d	3.06 b	2.31 c
Shoot DW (g)								
Pizzutello di Sciacca	23.58 bc	19.11 d	33.23 c	32.55 c	41.74 d	33.56 f	44.59 d	39.95 d
Locale di Salina	31.25 a	25.34 b	44.38 a	44.20 a	51.47 a	44.20 b	56.63 a	52.07 b
Brigade	26.71 b	22.11 c	36.81 b	29.90 d	45.13 bc	37.08 e	49.11 c	42.29 d
Root/Shoot ($g\ g^{-1}$)								
Pizzutello di Sciacca	0.054 b	0.068 a	0.057 d	0.062 bc	0.054 b	0.066 a	0.057 a	0.067 b
Locale di Salina	0.050 b	0.047 bc	0.060 cd	0.056 d	0.056 b	0.059 b	0.059 a	0.059 a
Brigade	0.053 b	0.043 c	0.065 a	0.065 a	0.066 a	0.056 b	0.062 a	0.055 a

The increase of stem diameter was not affected by drought (Tab. 1). Brigade control plants presented larger stems compared to the plants of Pizzutello di Sciacca and Locale di Salina.

Morphological components such as root and shoot DW of each genotypes were found to be influenced by drought stress (Tab. 1). All genotypes recorded higher shoot DW under well-watered condition respect to drought, in both t_{ds1} and t_{ds2} . In t_{ds1} all genotypes recorded a similar reduction in terms of shoot DW (about 21-23% respect to control); in t_{ds2} Locale di Salina showed a lower shoot DW reduction (17% respect to control) as compared with the other genotypes (24% of Pizzutello di Sciacca and 22% of Brigade).

In t_{rw1} there was a recovery of shoot DW to near control values for Pizzutello di Sciacca and Locale di Salina; instead, in the second re-watering the recovery was not complete for all genotypes.

With regard to root DW, a reduction of this parameter was found in Locale di Salina and Brigade drought stressed plants respect to control in t_{ds1} and t_{ds2} . Root DW reduction was higher in Brigade (48% and 43% respect to control in t_{ds1} and t_{ds2}) than Locale di Salina (30% and 11% respect to control in t_{ds1} and t_{ds2}). Nahar and Gretzmacher (2011) also reported root

and shoot dry matter production of two tomato cultivars BR-2 and Marglobe decreased gradually with increasing water stress.

Kameli and Losel (1996) reported that drought stress might increase root weight and decrease shoot weight. The absorption of nutrients from the soil depends on the availability of water to roots. It is, also, reported that soil water deficit may reduce root growth and limit nutrient uptake by roots (Arndt et al., 2001).

Locale di Salina stressed plants recovered in t_{rw1} the root DW value of control; instead, they did not recovery in t_{rw2} . The recovery of root DW from stress conditions was not complete for Brigade after both re-waterings.

No significant differences were found in root DW between control and stressed plants of Pizzutello di Sciacca in all sampling times. Under drought stress, the absence of root DW differences was probably due to higher root length of stressed plants than control that compensate the root mass reduction.

Root length behaviour in response to drought was very different among the genotypes. Pizzutello di Sciacca plants had a longer root length in drought condition than in control (18.2 cm vs. 22.6 cm in t_{ds1} and 30.3 cm vs. 35.2 cm in t_{ds2}). These plants increased their roots to maximize water up take from the soil.

Root characteristics, especially root length is important for a plant to have comparatively well-established above-grounded parts by exploiting the available water (Ekanayake et al. 1985). The longer roots measured for Pizzutello di Sciacca in drought stress conditions may help the plant to extract water from the deep soil. Comparable results were reported on wheat cultivars (Almaghrabi, 2012) and pearl millet (Leila, 2007). Macar et al. (2009) in their study also explained that drought stress induced by PEG prevented radical extension in Chickpea. Kulkarni and Deshpande (2007) explained early and rapid elongation of roots is main indication of drought tolerance.

Differently by Pizzutello di Sciacca, Locale di Salina recorded root length values in drought plants not significantly different from well-watered plants in both t_{ds1} and t_{ds2} . Brigade could be considered the most susceptible if we take in account that drought stress induced an arrest in root growth; so root length of stressed plants was lower than those of control plants (17.32 cm vs. 22.8 cm in t_{ds1} and 31.2 cm vs. 36.2 cm in t_{ds2}). Nahar and Gretzmacher (2011) also found that drought stress caused a reduction in root length of BR-1 and BR-2 tomato cultivars and did not have any influence on BR-4 and BR-5 cultivars.

Drought and control plants of all genotypes preserved, regards to root length, in the re-watering phases the same differences visible at the end of drought periods.

Data on root/shoot (Tab. 1) revealed that stress plants had differential response to drought condition. Control plants of Brigade had a root/shoot ratio higher than that of stressed ones (0.053 vs. 0.043 in t_{ds1} and 0.066 vs. 0.057 in t_{ds2}). Differently from Brigade, drought caused an increase of root/shoot in Pizzutello di Sciacca. The differences in root/shoot between stressed and control plants were not significant in Locale di Salina.

The results indicate that drought affected the shoot growth but not the root growth of Pizzutello di Sciacca, therefore the root/shoot increased under water deficit conditions. The root/shoot increase under water stress is due mainly to decreased shoot weight rather than increased root weight. Root mass rarely increases under stress, whereas root length and root volume often increase in response to stress. Generally, when water availability is limited, the root/shoot of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000). Similar results were obtained in chickpea plants (Kalefetoglu Macar and Ekmekci, 2009). Fernández et al. (1996) also found that drought affected shoot growth before the root growth in young cotton plants grown in pots.

The increased root/shoot implies the development of larger ratio of root length density to leaf area, which translates into a better capacity for sustaining drought stress (Blum, 1996). Li and Wang (2003), Gazal and Kubiske (2004), Bargali and Tewari (2004) consider a high root/shoot as a drought tolerant parameter. The emphasized that the first and most common feature of plant growth is increasing of root/shoot ratio in arid regions.

Water stress significantly reduced LA and leaf DW in all genotypes (Tab. 2). The highest reduction in both parameters was noticed in Brigade with values of 35% and 44% in t_{ds1} and 37% and 54% in t_{ds2} for LA and leaf DW, respectively. Both LA and leaf DW were reduced of about 22-28% in Pizzutello di Sciacca and Locale di Salina in t_{ds1} and t_{ds2} .

Drought-induced reduction in LA is ascribed to suppression of leaf expansion through reduction in photosynthesis (Rucker et al., 1995). Khakwani et al. (2012) also found a minor reduction of leaf DW under water stress in rainfed varieties as compared to irrigated varieties. The possible reason for minimum reduction was attributed to the fact that the accumulated solutes were used as substances for rapid recovery growth when stress was relieved. Christopher et al. (2004) reported similar results, where differences between drought resistant and susceptible varieties under stressed condition were due to the portioning of resources during development. The drought susceptible lines had thinner leaves, which reduced total dry weight. It has been reported that smaller leaf area may be ascribed to acceleration of leaf senescence and abscission (Constable and Hearn, 1978) or to the sensitivity of leaf expansion to water stress (Boyer, 1970; Whiteman and Wilson, 1965).

In stressed plants of Pizzutello di Sciacca and Locale di Salina, the first re-watering allowed the recovery of LA and leaf DW to values not significantly different from those of control plants. Instead, after the second re-watering LA and leaf DW values of control plants remained higher than of stressed plants.

Drought treatment substantially inhibited the SLA of Pizzutello di Sciacca leading to a reduction in SLA of 7% and 10% in t_{ds1} and t_{ds2} , as compared to the well-watered treatment (Tab. 2). On the contrary, in Brigade stressed plants, SLA had values lower than of well-watered plants (270.3 vs. 288.8 in t_{ds1} and 261.8 vs. 294.5 in t_{ds2}).

SLA decreases because of increases in leaf thickness or density, or both (Niinemets, 2001). A lower SLA is usually a consequence of an increase in the density or thickness of foliar tissue and normally occurs when the costs of the assimilatory apparatus are increased (Centritto, 2002), such as during long periods of drought. The morphological features, like SLA, are associated with plant drought adaptation strategies (Liu and Stutzel, 2004) and their plasticity may improve drought adaptation. Morphological traits from Mediterranean cultivars, such as the ability to alter SLA, may be used for drought-tolerant cultivar improvement. Hayatu and Mukhtar (2010) reported in their study a reduction in SLA of cowpea under severe water stress as adaptive mechanism that helps the plant to reduce water loss from the evaporative surfaces.

Table 2 – Leaf morphological features of tomato genotypes (leaf area - LA, leaf dry weight - Leaf DW, specific leaf area - SLA) at the end of drought stress periods (t_{ds1} and t_{ds2}) and re-watering phases (t_{rw1} and t_{rw2}). Different letters indicate significant different values for $P \leq 0.05$ within each time of sampling (t_{ds1} , t_{ds2} , t_{rw1} , t_{rw2}) for treatments \times genotypes interaction.

Parameters	t_{ds1}		t_{rw1}		t_{ds2}		t_{rw2}	
	ww	ds	ww	ds	ww	ds	ww	ds
LA (cm^2)								
Pizzutello di Sciacca	2813 b	2257 c	4039 c	4014 c	5370 b	4377 c	5936 b	5137 c
Locale di Salina	3435 a	2765 b	4930 a	4688 ab	6018 a	4695 c	6301 a	4917 c
Brigade	3160 a	2348 c	4475 b	3358 d	5483 b	3994 d	5829 b	4103 d
Leaf DW (g)								
Pizzutello di Sciacca	10.64 b	9.17 cd	14.43 c	13.95 c	18.89 b	16.93 c	20.97 b	18.04 c
Locale di Salina	13.03 a	10.41 c	18.23 a	17.52 ab	22.46 a	18.34 bc	23.92 a	19.04 c
Brigade	11.69 a	8.13 d	16.04 b	12.79 c	20.94 a	13.56 d	21.85 b	14.88 d
SLA ($cm^2 g^{-1}$)								
Pizzutello di Sciacca	264.4 c	246.1 d	279.9 a	287.7 a	284.3 a	258.5 c	283.1 a	284.8 a
Locale di Salina	263.6 c	265.6 c	270.4 b	267.6 b	267.9 b	255.0 c	263.4 b	258.2 b
Brigade	270.3 b	288.8 a	279.0 a	262.5 b	261.8 c	294.5 a	266.8 b	275.7 c

4.6. Effect of drought on yield characteristics

Differences in total yield were shown from different genotypes under study (Tab. 3). Brigade recorded the highest yield under well-watered conditions ($1012.35 g pt^{-1}$). In general the stressful treatments negatively affected the total yield in all genotypes; Brigade stressed plants recorded a fruit production which was less than half ($460.15 g pt^{-1}$), than that produced under full irrigation ($1012.35 g pt^{-1}$). Yield reduction of Pizzutello di Sciacca and Locale di Salina under drought treatments was 27% and 32% respectively if compared with control plants.

Table 3 - Effect of drought stress on yield and some biometric traits of fruits of long-storage tomatoes Pizzutello di Sciacca and Locale di Salina and processing tomato cv. Brigade (fresh weight basis). The differences within each parameter were analysed by ANOVA ($P=0.05$) for genotype \times treatment interaction and different letters indicate significant different values for $P \leq 0.05$.

Treatments	Total yield	Fruits number	Fruit weight	Fruit length	Fruit width	Fruit shape
	$g pt^{-1}$	$n^{\circ} pt^{-1}$	g	cm	cm	(length/width ratio) $cm cm^{-1}$
Pizzutello di Sciacca						
ww	772.74 c	64.02 a	12.07 dc	2.81 b	2.36 c	1.19
ds	563.38 d	45.71 c	11.45 d	2.74 b	2.41 c	1.14
Locale di Salina						
ww	823.65 b	52.32 b	15.74 c	2.66 b	2.84 b	0.94
ds	477.28 e	36.07 d	13.23 c	2.55 b	2.69 b	0.95
Brigade						
ww	1012.35 a	17.40 e	58.16 a	5.62 a	4.61 a	1.22
ds	460.15 e	11.44 f	40.22 b	5.16 a	4.29 a	1.20

The same severe depressive effects of water stress upon yield are widely reported in the literature on processing tomato open-field cultivated (Patanè and Cosentino, 2010; Ozbahce et al., 2010; Pernice et al., 2010; Favati et al., 2009). The soil water deficit during yield formation and ripening caused blossom-end rot. This condition restricted the movement of calcium from the soil to the fruit (Saure, 2001; Nonami et al., 1995), a result consistent with previous findings (Marouelli et al., 2004; Cho et al., 1998). In good agreement with our findings, Zegbe et al. (2006) also reported that partial root zone drying (PRD) from the first truss to fruit-set caused a significant yield decrease.

Yield reduction of Brigade *cv.* was due to a double effect: the reduction of the number of fruits per plant and the reduction of fruit weight. Instead, in Pizzutello di Sciacca and Locale di Salina there were not significant differences in fruit weight between drought and control plants.

Birhanu and Tilahun (2010) reported a decreased number and sizes of tomato fruits from plants subjected to moisture stress. Zotarelli et al. (2009) also reported the same observation of water stress on tomato yield parameters.

A lower number of fruits is the result of flower abortion as tomato is very sensitive to water stress during flower and fruit set (Pulupol et al. 1996).

The reason for the low fruit weight in Brigade stressed plants is that the soil is dry for a long time, resulting in a low fruit water content. This prolonged dryness decreases the accumulation of moisture in the fruit. This result is consistent with findings by Ramalan and Nwokeocha (2000), who showed that fruit weight was affected by soil moisture levels.

The fruits produced in drought condition, on average smaller than those of control, mainly contributed to the low yields obtained under water stress conditions. These results are in agreement with the findings of Marouelli and Silva (2007), who observed that the number of fruits per plant was reduced as soil water tension during fruit development and maturation growth stages increased, mainly since many small green fruits aborted or did not enlarge under drier conditions.

The reduction of number of fruit per plant, fruits size with an increased amount of water stress level was also evident in previous work conducted on tomato (Candido et al., 2001). Water stress consistently reduced production of the most valuable extra-large sized fruit. A study conducted to evaluate the effect of irrigation cutback on yield showed that total marketable yields doubled and while yields of high value extra-large fruit tripled with irrigation (Smajstrla et al., 1994).

The differences among the genotypes were significant with Pizzutello di Sciacca consistently having more number of fruits per plant and lower fruit weight. The low fruit weight of Pizzutello di Sciacca and Locale di Salina (< 20 g) was a parameter that characterized long-storage tomato.

The fruit shape index is vital in evaluating consumer preference and meeting quality standards (Gerhard et al., 2001). Fruit shape is affected by especially genotypic variability in addition to environmental growing conditions (White et al., 2000). Fruit length decreased proportionally to fruit width in plants of all genotypes exposed to water stress, so the fruit shape remained constant (Tab. 3). Tomato genotypes differed in fruit shape; Pizzutello di Sciacca and Brigade had an elongate fruit (fruit shape > 1) respect to Locale di Salina characterized by round fruit. Stikic et al. (2003) also reported that PRD caused a significant reduction in fruit numbers, but this effect was not significant for fruit diameter. However, Madrid et al. (2009) observed that the values of physical properties including the equatorial diameter, longitudinal diameter, and geometric mean diameter of processing tomato fruits increased with increasing irrigation.

4.7. Effect of drought on physicochemical traits of tomato fruits

For all genotypes, the value of L* (lightness) was not affected by water shortage. In Brigade significantly higher values of a* (red color) and b* (yellow color) were recorded for fruits from well-irrigated plants, indicating greater red/yellow colour in comparison with water-stressed fruits (data not shown). Both a* and b* components significantly decreased in drought conditions, but the decrease of a* was more pronounced than the relative decrease of b*. As a consequence a*/b* ratio, usually considered as a reopening index, recorded significantly higher value in control fruits of Brigade (Tab. 4), indicating greater colour development than in those of stressed fruits. Color is principally associated with the lycopene content of tomato and is generally considered the most important attribute determining the product quality (Garcia and Barret, 2006). In our study, higher a* values of control fruits of Brigade may be attributed to higher lycopene content respect to that of stressed fruits in accord to data reported by Barbagallo et al.(2013). In Pizzutello di Sciacca and Locale di Salina there were not significant differences in a*/b* ratio between control and stressed plants.

Table 4 - Effect of drought stress on physicochemical traits of fruits of long-storage tomatoes Pizzutello di Sciacca and Locale di Salina and processing tomato *cv.* Brigade (fresh weight basis). The differences within each parameter were analysed by ANOVA (P=0.05) for genotype \times treatment interaction and different letters indicate significant different values for $P \leq 0.05$.

Treatments	Firmness	Skin color	pH	Tritatable acidity	Total soluble solids (TSS)	Total solids (TS)	Reducing sugars
	$Kg\ cm^{-2}$	a^*/b^*		% <i>ac. citrico</i>	$^{\circ}Brix$	$g\ Kg^{-1}$	$g\ Kg^{-1}$
Pizzutello di Sciacca							
ww	2.71 c	1.46 b	4.20 a	0.25 b	5.75 d	72.85 c	35.21 b
ds	3.85 a	1.58 b	4.18 a	0.26 b	7.77 a	102.74 a	53.98 a
Locale di Salina							
ww	2.79 c	1.82 a	4.23 a	0.24 b	6.00 e	67.48 d	36.12 b
ds	3.82 a	1.94 a	4.24 a	0.26 b	7.42 b	90.77 b	52.74 a
Brigade							
ww	2.42 d	1.59 b	4.22 a	0.32 a	5.65 d	56.21 e	35.34 b
ds	3.02 b	1.31 c	4.28 a	0.31 a	6.41 c	64.63 d	39.93 c

The effects of drought on physicochemical traits were generally the converse of those on fruit yield and fruit weight. Fruit firmness was significantly improved in the absence of irrigation in all genotypes. Pizzutello di Sciacca and Locale di Salina

stressed fruits recorded an increase of about 40% of fruit firmness respect to values of well-watered fruits (Tab. 4). In Brigade the increase of fruit firmness, under drought treatment, was only 25%. Pizzutello di Sciacca and Locale di Salina showed higher values of fruit firmness respect to Brigade for both stressed and control fruits. According to Guichard et al. (2011) the positive effect of water stress may be associated with a decrease in internal firmness of the fruit, which could lead to lower pressure on the cell walls and thus to greater epidermal elasticity, possibly compensated by strengthening of the cell walls. These results are in agreement with those previously reported for tomato by other authors (Sofa et al., 2004). No favorable effect of drought stress on pH was observed (Tab. 4). This is an important characteristic in terms of processing requirements; values of pH in both water regimes did not exceed the minimum (≤ 4.30) allowed to define the product as 'good' according to the reference scale of analytical parameters for processing tomato pulp (Silviero, 1998). Conversely, titratable acidity did not change with water regime (Tab. 4). Hanson et al. (2006) and Tuzel et al. (1994) also observed no effect of water regime on tomato acidity.

Table 4 shows that TSS such as TS was significantly affected by soil water deficit in all genotypes. Pizzutello di Sciacca stressed fruits showed a TSS increase of 35% respect to control fruits, the increase was more limited in Locale di Salina (24%) and Brigade (13%). As expected, the very early cut-off of irrigation induced higher fruit quality in terms of total solids (TS) and total soluble solids (TSS) in comparison with the fully irrigated treatment. In this study, water supply increased the total yield in all genotypes but significantly reduced the TSS.

TSS content in the fruit is an important quality factor for tomatoes grown for processing. TSS is the principal parameter affecting paste yield (Patanè and Cosentino, 2010; Johnstone et al., 2005). It is desirable to have high values of TSS in the fruit because it improves the quality of the processed product (De Pascale et al., 2001). Cemeroglu et al. (2003) reported that average TSS content in industrial tomatoes must be at least 5°Brix. TSS content of stressed fruits was also significantly different between the tomato genotypes. Pizzutello di Sciacca drought stressed fruits recorded the highest value of TSS (7.77 ° Brix). Tomatoes higher in TSS content may require less energy to evaporate water from the fruit with important implications for industrial purposes (for paste or concentrated juice) (Favati et al., 2009). This aspect justify the use of the long-storage tomato in programs of genetic improvement to obtain better performances by processing tomato cultivars. Marouelli and Silva (2007) and Garcia and Barrett (2006), also observed, a negative correlation between yield and TSS. Many other authors found that imposing soil water stress increases the TSS content to values higher than those found in fruits under full irrigation (Helyes et al., 2012; Patanè and Cosentino, 2010; Ozbahce and Tari, 2010; Favati et al., 2009; Hanson and May, 2004; Zegbe-Dominguez et al., 2003). Ho et al. (1987) proposed that this phenomenon was due to an increase in the phloem sap concentration and a decrease in its flux resulting in reduced water availability in the plant. The phloem flux is largely responsible for the increase in tomato size (Guichard et al., 2011). Therefore, a decrease in this flux accompanied by an increase in its sugar concentration results in fruits of smaller size but with higher dry matter content. In contrast, Hanson et al. (2006) stated that the TSS values of well-irrigated tomatoes did not differ significantly from the TSS values of tomatoes grown under water stress.

Tomato fruit taste was improved by drought in all genotypes because of the increased reducing sugars (Tab. 4). The increase of reducing sugars in tomato fruit under drought was higher for Pizzutello di Sciacca (53%) and Locale di Salina (46%) respect to Brigade (13%). Reducing sugars content followed similar behavior of TSS. Tomato fruit flavor is a function of both taste and aroma components (Malundo et al., 1995), and tomato taste is largely determined by the contents of soluble sugars and organic acids and their ratio (Kader, 2008; Dorais et al., 2001).

4.8. Effect of drought on some antioxidant contents of tomato fruits

Vitamin C (the sum of AsA and DHA) is an important constituent of tomato fruits since it prevents oxidative damage that might take place during fruit ripening, thus maintaining the shelf life of the fruit (Slimestad and Verheul, 2009). The vitamin C content was significantly greater in tomato fruits under limited soil water conditions (Tab. 5). The highest increase in vitamin C content under drought conditions was recorded in Brigade (18.5%) but Pizzutello di Sciacca and Locale di Salina showed higher value of vitamin C respect to Brigade both in control (25% and 16% more than Brigade) and stressed fruits (15% and 12% more than Brigade). Other studies have highlighted that the tomato nutrient content, such as vitamin C, increased as a result of the soil water depletion, even if it seems to be cultivar dependent (Mahajan and Singh, 2006; Dumas et al., 2003; Mitchell et al., 1991).

Tomatoes contain various carotenoids, with lycopene accounting for more than 80% of the total carotenoids present in fully red-ripe fruits (Lenucci et al., 2006).

The lycopene content of Brigade fruits exposed to drought stress (98.6 mg kg^{-1}) was lower than that of fruits grown under favourable water conditions (129.4 mg kg^{-1}) (Tab. 5). This result is in agreement with data reported by Barbagallo et al. (2013) about the reduction of lycopene content in fruits of Brigade, grown in open-field conditions, under water stress. Naphade (1993) in a study on the effects of four irrigation regimes on three tomato cultivars reported that the fruit lycopene content decreased in response to moisture stress. In red and pink cherry tomato cultivars, the total carotene of the fully ripe fruits and the amount of lycopene both increased when there were soil water deficits (Matsuzoe et al., 1998). Irrigation probably indirectly affected lycopene concentration in Brigade by inducing more and larger fruits, and thus had a dilution effect on ingredients.

In Pizzutello di Sciacca and Locale di Salina lycopene content had higher values respect to those of Brigade and did not showed significant differences between stressed and control fruits. Barbagallo et al. (2008) also reported the absence of

significant differences in lycopene content under 50% of water regime. Lycopene content in control fruits was not significant different between the two genotypes.

Differently by lycopene content, β -carotene increased with drought (Tab. 5) in Pizzutello di Sciacca (31%) and Locale di Salina (27%). Pizzutello di Sciacca had a higher β -carotene content in control (9.8 mg Kg⁻¹) and stressed fruits (12.4 mg Kg⁻¹) respect to those of Locale di Salina (8.1 mg Kg⁻¹ and 10.3 mg Kg⁻¹ in control and stressed fruits) and Brigade (8.4 mg Kg⁻¹ and 7.9 mg Kg⁻¹). β -carotene content did not showed significant differences in Brigade stressed fruits respect to control. In red and pink large-fruited tomatoes, soil water deficits also tended to increase the amount of lycopene per fresh mass of the fruit in the region of the outer pericarp, but it had no effect on the amount or distribution of β -carotene (Matsuzoe et al., 1998). Riggi et al. (2008) reported a higher β -carotene/lycopene ratio in fruits of Brigade developed under soil water deficit, suggesting a negative influence of water stress on lycopene accumulation. Srivastava and Handa (2005) reported that the effects of SWC on lycopene and β -carotene accumulation could be explained by the evaluation of their role in the biosynthesis of some plant water-stress related hormones, such as ABA. This hormone is involved in plant stress responses (drought, salinity, cold); in particular, water stress causes a rapid increase in its content (Mitchell et al., 1991). Indeed, under water stress, the carotenoid biosynthetic pathway is more oriented towards β -carotene than towards lycopene, and β -carotene is the precursor for ABA formation under water stress conditions (Riggi et al., 2008). The lower lycopene content measured in the present study in water-stressed fruits of Brigade could have accounted for the lower values of the colour a*/b* ratio reported above.

Results concerning the effect of water stress on carotenoids in processing tomato are rather contradictory (Pernice et al., 2010; Dumas et al., 2003). In this study lycopene content decreased significantly in response to water stress in *cv.* Brigade and β -carotene increased in Pizzutello di Sciacca and Locale di Salina. This is in accordance with Dumas et al. (2003) who reported that water shortage reduces lycopene content in some varieties of processing tomato whereas it stimulates β -carotene production in others.

Table 5 - Effect of drought stress on some antioxidants in fruits of long-storage tomatoes Pizzutello di Sciacca and Locale di Salina and processing tomato *cv.* Brigade (fresh weight basis). The differences within each parameter were analysed by ANOVA (P=0.05) for genotype x treatment interaction and different letters indicate significant different values for P \leq 0.05.

Treatments	Vitamin C mg Kg ⁻¹	Lycopene mg Kg ⁻¹	β -Carotene mg Kg ⁻¹	Total phenols mg GAE Kg ⁻¹	DPPH % reduction
Pizzutello di Sciacca					
ww	435.2 c	149.2 a	9.8 c	890.8 c	77.6 c
ds	474.6 a	157.9 a	12.4 a	1773.3a	99.4 a
Locale di Salina					
ww	403.1 d	155.3 a	8.1 d	878.2 d	72.5 d
ds	462.4 b	166.7 a	10.3 b	1597.2 b	89.2 b
Brigade					
ww	347.2 e	129.4 b	8.4 d	721.4.e	59.7 f
ds	411.7 d	98..6 c	7.9 d	794.8 c	65.8 e

A great variation in total phenolic content was recorded for the genotypes under study. Total phenolics ranged from 890.8 mg GAE Kg⁻¹ of Pizzutello di Sciacca to 721.4 mg GAE Kg⁻¹ of Brigade in fruits obtained under control conditions (Tab. 5). The results are in agreement with Fu et al. (2011), who found 735 mg GAE Kg⁻¹, in cherry tomatoes grown hydroponically in the greenhouse. Values for total phenolic content were between the low levels reported by Hernandez-Suarez et al. (2008) and Ahmed et al. (2011) (50–167 mg GAE kg⁻¹) and the high levels measured by Zhou and Yu (2006) under open field conditions (3000–5000 mg GAE kg⁻¹) and by Luthria et al. (2006) under tunnel cultivation (3500–4500 mg GAE kg⁻¹). The obtained values were also lower than those found by Barbagallo et al. (2008) in tomatoes under glasshouse cultivation in a Mediterranean climate, highlighting the great variation in total phenolic content depending on cultivar, growing stage and crop conditions.

Total phenolic content differed significantly according to the irrigation regime. Water stress caused an increase in total phenolic content in all genotypes with values that were up to 2.3-fold higher in Pizzutello di Sciacca (1773.3 mg GAE Kg⁻¹) than that of the Brigade (794.8 mg GAE Kg⁻¹). Pek et al. (2014) also showed that total phenolic concentrations in tomato fruits were lower with optimum water supply in comparison with deficit irrigation.

The literature contains limited studies on factors affecting the levels of phenolic compounds in tomato, in particular on the effect of water stress on total phenols of tomato fruit.

Antioxidant activity represents a measure of the capacity of food extracts to inhibit or delay oxidative processes. It derives from distinct bioactive antioxidant compounds present in such extracts (Martinez-Valverde, 2002). Tomato is recognized as one of the most important sources of antioxidants, namely lycopene, ascorbic acid, flavonoids and phenolic acids, chlorogenic acids, etc. Radical extinguishment is considered one of the major mechanisms of antioxidants that inhibit oxidative processes. The DPPH radical is a relatively stable organic radical that is widely used as a substrate to assess antioxidant activity in vegetables (Ahmed et al., 2011; Martinez-Valverde, 2002).

Antioxidant activity measured by the DPPH assay recorded higher values in Pizzutello di Sciacca and Locale di Salina (77.6% and 72.5% respectively) compared with Brigade (65.8%) already in the control fruits (Tab. 5).

DPPH was positively affected by water shortage, the difference in radical-scavenging percentage between fruits produced in drought and well-watered conditions were highly significant for all genotypes. The increase in DPPH value in the stressed respect to control fruits was 28%, 23% and 12% for Pizzutello di Sciacca, Locale di Salina and Brigade, respectively. Barbagallo et al. (2013), also, recorded an increase of about 10% of DPPH percentage reduction in the stressed fruits of Brigade *cv.* respect to the control.

Toor and Savage, (2005) found that the antioxidant activity in tomato was due to ascorbic acid (28–38%) and phenolics (60–70%). Ahmed et al. (2011) also reported a significant correlation between DPPH radical scavenging activity and phenolic content. The higher phenolic content of tomatoes grown under water stress and their higher antioxidant activity determined in the present study confirm these findings, highlighting the important contribution of phenolic compounds to the antioxidant pattern of tomato.

5. CONCLUSIONS

Drought is a worldwide problem, constraining global crop production and quality seriously, and recent global climate change has made this situation more serious. The research of more drought-resistant crops is necessary to alleviate future threats to food availability in the world.

This research was carried out to compare the response to drought stress of two long-storage tomato genotypes respect to a well-known drought sensitive processed tomato genotype.

Based on the results of this study, it can be concluded that different physiological, biochemical and morphological adaptive mechanisms were involved to confer drought tolerance in tomato genotypes Pizzutello di Sciacca and Locale di Salina respect to Brigade.

Genotypes differences in drought response varied in stomatal sensitivity to changes in ABA level during drought. Drought tolerance of long-storage tomato genotypes could be related to higher sensitivity of stomata to ABA accumulation under drought stress conditions that allowed them an earlier closure of the stomata, reducing drought damages.

Pizzutello di Sciacca and Locale di Salina were able to maintain a higher tissue water status (RWC and ψ_{leaf}) and photosynthetic activity by regulating stomatal opening in response to soil water deficit.

Under drought stress, stomatal conductance was the only factor limiting photosynthesis in Pizzutello di Sciacca and Locale di Salina, whereas in Brigade, in addition to stomatal limitation, a decreased PSII activity as well as a reduced efficiency of electron transport also contributed to decrease in photosynthetic performance. Damage to PSII activity, was responsible of a late or not complete recovery of photosynthesis after re-watering in Brigade.

In addition to physiological parameters, morpho-anatomical adaptations to water deficit could explain the difference in drought resistance among the studied genotypes. In particular, Pizzutello di Sciacca was able to reduce water losses through the leaf surface by reducing LA and increasing leaf thickness (SLA) and at the same time, it invested in root growth (higher roots length) to improve the plant water uptake.

Water is an important factor influencing tomato yield and fruit quality. Drought negatively influenced tomato yield with reduction of the number of fruits per plant and mean fruit weights; Pizzutello di Sciacca and Locale di Salina was found to be the genotypes with lower yield reduction.

This study also revealed that water deficit exerted beneficial effects upon fruit quality, mostly in terms of TSS and TS with interesting implications for industrial purposes. In fact, a high total solids content of the fruit improves the efficiency throughout the industrial process (for paste or concentrated juice).

Together with TS, a raise in antioxidant contents (vitamin C, phenols) of the fruit under drought regime was also observed, with human health benefits. Furthermore, drought stress produced a quality fruit improvement in Pizzutello di Sciacca and Locale di Salina higher than Brigade.

From this investigation, it can be concluded that the two long-storage genotypes, thanks to their drought tolerant traits, can be considered a possible alternative to common commercial genotype for cultivation in semiarid areas.

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