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# "Role of vegetable grafting in the control of abiotic stresses and effects on yield and quality"

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## Abstract

The large-scale spread of vegetable grafting was initially promoted to increase tolerance to biotic stresses, however, grafting onto appropriate rootstocks showed up to improve scion performances under many abiotic stress conditions and often resulting in higher yield levels. Low temperature stress is one of the main issues in pepper cultivation. Genetic variability for abiotic stress tolerance is low inside the species and the number of rootstocks available for pepper is limited. Aiming to increase germplasm availability for pepper grafting and to find a rootstock that could alleviate chilling stress, grafting compatibility of *Capsicum pubescens* and *Capsicum baccatum* accessions originated from high altitudes with a widespread pepper cv was assessed and grafted transplants were used in chilling stress experiments. Two of these accessions were able to increase biomass accumulation under low temperatures compared to a commercial rootstock and showed no yield decrease or quality worsening when cultivated under optimal temperature conditions. Tomato is both a widespread crop and a model plant and it is usually grafted in specialized agricultural systems. In soilless cultivation, tomato plants can experience root hypoxia as a consequence of both misjudgments in water requirements and the progressive decrease of the air capacity of the substrate over the cultivation cycles. Commercial rootstocks with a different genetic origin were tested for their effectiveness to increase tolerance to roots hypoxia and it resulted in better physiological performances of grafted plants compared to self-grafted ones under stress conditions.

The effects of grafting on yield and quality traits of tomato often depend on the specific rootstock-scion combinations. Through an experiment in which seven cherry tomato cultivars were grafted onto eight rootstocks, including both interspecific and intraspecific hybrids and compared to un-grafted plants, it was possible to identify some variables in which the role of the scion prevailed and others in which rootstock contribution was predominant. The analysis of the coefficients of variation, compared to the absolute values observed in each grafting combination, provided information regarding the ability of rootstock and scion to influence the different examined variables and on its potential benefits.

## Sommario

La diffusione dell'innesto erbaceo è stata inizialmente promossa dalla possibilità di conferire alla pianta una maggiore tolleranza agli stress biotici. Successivamente, però, l'impiego di portinnesti appropriati si è rivelato utile anche per migliorare la vigoria della pianta e le performance del nesto in presenza di stress di natura abiotica, con ripercussioni positive sulla resa. Le basse temperature rappresentano uno dei principali stress abiotici per il peperone. La variabilità genetica per la tolleranza alle basse temperature è limitata, come anche il numero di portinnesti disponibili. Per cercare di ovviare a questo problema, una volta verificata la compatibilità d'innesto di accessioni di *Capsicum pubescens* e *Capsicum baccatum* prelevate ad elevate altitudini con una cultivar comune di peperone, è stata valutata la tolleranza alle basse temperature da parte delle piante innestate. Due accessioni tra i nuovi portinnesti testati hanno incrementato la biomassa prodotta in condizioni di stress rispetto a un portinnesto commerciale usato come controllo, senza compromettere la resa e la qualità in condizioni di temperatura ottimali.

Il pomodoro è sia una specie ortiva di grande importanza che una pianta modello e l'innesto erbaceo è una pratica abituale nella coltivazione specializzata. Nelle coltivazioni fuori suolo, le piante di pomodoro possono essere esposte a ipossia radicale in seguito a errori nella programmazione dei turni irrigui e dei volumi di adacquamento nonché a causa della progressiva diminuzione della capacità per l'aria dei substrati impiegati per più cicli di coltivazione. Al fine di analizzare la possibilità di migliorare la tolleranza all'ipossia radicale del pomodoro tramite l'innesto è stato predisposto un esperimento con piante auto-innestate e innestate su portinnesti commerciali ibridi di diversa provenienza genetica. Le piante innestate hanno mostrato performance migliori dal punto di vista fisiologico rispetto alle auto-innestate in condizioni di ipossia.

Gli effetti dell'innesto su resa e qualità del pomodoro dipendono spesso dalla specifica combinazione portinnesto-nesto. Analizzando la risposta di sette cultivar di pomodoro ciliegino innestate su otto portinnesti ibridi interspecifici e intraspecifici, e non innestate, è stato possibile identificare alcune variabili influenzate prevalentemente dal nesto o dal portinnesto. Inoltre, mediante l'analisi e la contestualizzazione dei coefficienti di variabilità di ogni nesto, è stato possibile fare emergere la capacità del nesto e del portinnesto di influenzare le diverse variabili esaminate e di identificarne i potenziali benefici.

## Foreword

Agriculture plays the essential role to supply the demand for food commodities for the increasing world population and, in this view, vegetable consumption is extremely important to assure the sufficient nutrients and antioxidants intake in the human diet. In the last decades, the productivity of specialized agricultural systems for vegetable production continued to increase but to achieve a sustainable intensification of cultivation, the large-scale adoption of an integrated pest management (IPM) approach had to be adopted (Bottrell 1979; Pretty and Bharucha, 2015; Shlevin et al., 2018). Grafting plays a central role in IPM and its use spread in the last three decades (Kubota et al., 2008; Lee et al., 2010). Vegetable grafting was initially promoted as a tool to increase crops tolerance to biotic stresses, but several additional advantages started to show up, including the ability of certain rootstocks to alleviate the effects of abiotic stresses (Schwarz et al., 2010). Grafting onto rootstocks selected for their higher tolerance to abiotic stresses provides, in this way, a rapid and efficient solution to the slower breeding program aiming to introduce these desired traits in high-quality cultivars.

Due to their economic importance and their nutritional and nutraceutical properties tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) are among the most widely grown fruit vegetables all over the world (Giuffrida and Leonardi, 2012; Li et al., 2017; Vitale et al., 2014). Tomato has been also largely used as a model plant for research purposes, and grafting is now considered as a standard practice in specialized tomato cultivation (Ezura, 2009; Keatinge et al., 2014).

A huge number of rootstocks have been developed for tomato, but several topics concerning tomato grafting still need to be better investigated, like the effectiveness of grafting in alleviating stress conditions of roots hypoxia or the possible effects of the rootstocks to modify scion characteristics. In pepper, the number of available rootstocks, as well as the intra-specific genetic variability for abiotic stress tolerance, are really limited and to expand rootstocks germplasm availability is essential to take full advantage of the grafting-induced increase in abiotic stress tolerance. Considered the high thermal requirements of pepper, the higher tolerance to low root-zone temperatures should be one of the objectives of pepper rootstocks development.

## Introduction

## Vegetable grafting: origin and diffusion

Although the grafting of herbaceous species has been successfully performed and described in ancient manuscripts in China and in Korea around the 5$^{th}$ and the 17$^{th}$ century respectively, it never took on relevance until the 20$^{th}$ century (Bie et al., 2017; Kubota et al., 2008; Lee and Oda, 2010). Since the higher tolerance to some soilborne of watermelon (*Citrullus lanatus*) grafted onto some gourd rootstocks (*Cucurbita moschata*) had been proved in the late 1920s, vegetable grafting started gaining attention in Japan and Korea. Immediately after, some studies showed the effectiveness of wax gourd (*Benincasa hispida*) as a watermelon rootstock due to their high graft compatibility and to its high tolerance to fusarium wilt and that in particular encouraged watermelon grafting spreading in Japan and Korea already in the 1930s.

In that period this innovation was almost only applied for watermelon cultivation even if grafting of other vegetable crops, like melon (*Cucumis melo*) had also been successfully tested; in the case of melon, this was probably due to its relatively higher tolerance to soilborne diseases (Davis et al., 2008). Cucumber (*Cucumis sativus*) grafting compatibility with various rootstocks had also been demonstrated, but it was not used on a commercial scale until 1960s, when Japanese growers started to graft this species for enhancing fusarium wilt and sub-optimal

temperature tolerance. In the same period, vegetable grafting started to gain attention in Europe; researches on this topic had been conducted in France in the 1950s and during the following decade melon grafting onto *Benincasa spp.* rootstocks became common for increase fusarium wilt tolerance and mitigate the detrimental effects of low soil temperatures in early grown melon under the un-heated greenhouse environment (Davis et al., 2008). Between the 1950s and 1960s also the commercial grafting of Solanaceae was started, at first for eggplant (*Solanum melongena*) grafted onto *Solanum integrifolium*, and later for tomato (*Solanum lycopersicum*).
Almost in the same period, with the rapid development and spread of intensive agricultural systems and protected cultivation technologies that prevented growers from crop rotations, the management of soilborne diseases and pests acquired much more relevance. However, the higher cost per plant related in part to the necessity to buy and grow also the rootstock seeds, but especially to the high labor cost of the grafting process itself, still represented the main obstacle to vegetable grafting spread (Barrett et al., 2012; Kubota et al., 2008). The methyl-bromide phase out and the restrictions in the use of other fumigants and pesticides, especially in the European Union, however, spotlighted once again the crucial issue of the soilborne and pest management and this forced the large-scale adoption of a holistic approach known as integrated pest management (IPM) (Bottrell, 1979; Shlevin et al., 2018). IPM definition has been recently summarized as a careful consideration of all available plant protection methods and their subsequent integration (Lamichhane et al., 2016). Vegetable grafting plays a central role in IPM so, despite the relatively high cost, this technique finally spread all over Europe as well as in the Middle East and in many countries of Asia during the last three decades (Kubota et al., 2008; Lee et al., 2010).
As grafting was reaching the status of standard practice in vegetable intensive cultivation for increasing crops tolerance to soilborne diseases and pests, several additional advantages started to show up. The right choice of rootstock-scion combination results, in fact, in enhanced plant vigour, extended harvesting period, increase of yield and sometimes in a better fruit quality and prolonged postharvest shelf-life (Bie et al., 2017). Moreover, grafting onto rootstocks selected for their higher tolerance to abiotic stresses provides a rapid and efficient solution to the slower breeding program aiming to introduce these desired traits in high-quality cultivars. The use of an appropriate grafting combination can, in fact, increase salt tolerance and mitigate or eliminate yield reduction caused by nutrient toxicity or heavy metals pollution of the soil; fertilizer use efficiency can also be enhanced making possible the cultivation of marginally fertile soils through a better nutrient uptake (Colla et al., 2010, 2012; Savvas et al., 2010). Grating onto tolerant rootstocks can also alleviate thermal and water stresses, that are among the most important environmental factors affecting crops growth and productivity worldwide (Schwarz et al., 2010).

## Vegetable grafting: principles and practices

Fruit trees grafting is a common practice that has been known and used for thousands of years whereas the grafting of herbaceous plants, known as “vegetable grafting”, is more recent and started to be used for commercial purposes only in the early 20$^{th}$ century (Bie et al., 2017; Lee and Oda, 2010). However, the principles behind the successful union between rootstock and scion are the same both in herbaceous and in woody plants. An essential requirement is the contact between the cambial regions of rootstock and scion; undifferentiated cells of callus proliferation develop both from rootstock and scion and lead to callus bridge formations and, finally, to the differentiation of new vascular tissues with the production of secondary xylem and phloem connections (Davis et al., 2008; Kawaguchi et al., 2008). Since in vegetable grafting the rootstocks and scion should have the same stem diameter to allow cambial tissues to be in close contact, it is essential to determine with preliminary trials the exact delay that should be adopted

between their sowings in the different periods of the years. For example, tomato rootstocks are usually sown around 5-10 days earlier than scion and grafting is usually performed around 20-25 days after scion sowing (Davis et al., 2008; Lee et al., 2010). Moreover, the number of seeds used is calculated considering not only rootstock and scion germinability but also the expected percentage of grafting success. Vegetable grafting is almost always performed manually and many grafting methods do exist. Among that, splice grafting, also called tube grafting, is the most widely used for both cucurbits and solanaceous crops. In splice grafting, plants with 2-4 true leaves and a stem diameter of 1.5 – 2.5 mm are used and they are prepared for grafting by 24 hours under shaded conditions and being not watered. Both rootstock and scion are cut with the same angle (35-45°) and joined together through grafting clips **(Figure 1)** (Bie et al., 2017; Rosskopf et al., 2017). Independently of the plant kind, dehydration of tissues must be avoided after grafting and it became a crucial issue in vegetable grafting; for that reason, the nurseries are equipped with special healing chambers in which plants are maintained under controlled conditions immediately after the grafting process in order to limit transpiration until good xylematic and phloematic connections are established. Usually grafted transplants are left inside the healing chamber for around one week; at the beginning of this period plants are kept in dark conditions at a relative humidity around 85-95 %, whereas both shading and relative humidity are gradually adjusted to make the following transfer to greenhouse conditions less traumatic (Rosskopf et al., 2017).

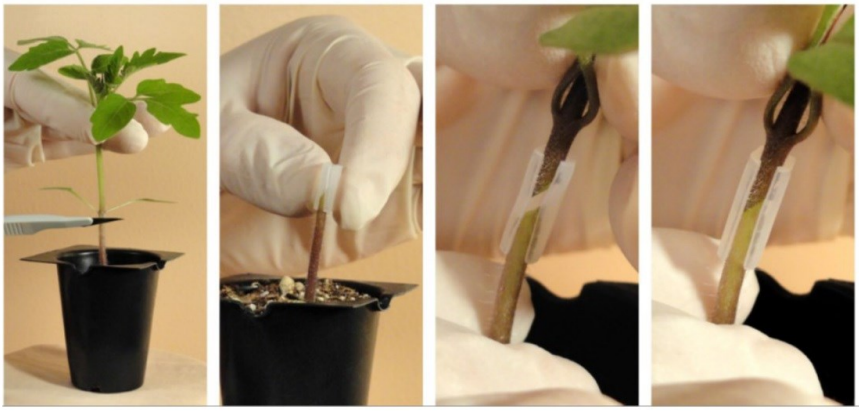


**Figure 1** - Splice grafting process in tomato (Rosskopf et al., 2017).

## Abiotic stresses

The difference between the potential yield in optimal conditions and the actual yield level observed in operative conditions has been named “yield gap”; this difference can get to incredibly high values in developing countries, where it can easily reach the 70 % of potential yield. Anyway, this “yield gap” represents a huge loss of production worldwide due both to biotic and abiotic factors. It has been estimated that biotic factors contribute for around one third to total “yield gap”, whereas abiotic factors such as salinity, heavy metal contamination, nutrient deficiencies and toxicities, drought, flooding and suboptimal temperatures determine the remaining part (Rouphael et al., 2018; Schwarz et al., 2010).

## Low temperatures effects on plants

Low-temperatures stress is among the main abiotic factors affecting crops growth and productivity worldwide (Shi et al., 2015). “Freezing temperatures” refers to thermal levels below

0 °C whereas “chilling temperatures” can be defined as temperatures above 0 °C but clearly below the optimal thermal window for a certain plant and is often used to indicate temperatures between 0 and 15 °C (Zhu et al., 2007). Sometimes authors use “chilling temperatures” to indicate a different thermal range (e.g. 0-12 °C; 0-20 °C), in this case, they usually specify that (Allen and Ort, 2001; Somerville, 1995).
Greaves (1996) defined “sub-optimal temperatures stress” as any reduction in plant growth or induced metabolic, cellular or tissue injury that results in limitations of its yield potential as a consequence of the exposure to temperatures below the thermal thresholds for optimal biochemical and physiological activity or morphological development. As a result of the induced alteration of many physiological processes, chilling temperatures strongly affect seeds germination and determine stunted seedlings, leaves yellowing and severe damages of shoot apexes. During the reproductive stage, cold stress often affects pollen sterility and determines abnormal ovary development named ovary swelling which, in turn, leads to deformed fruits (Cruz-Huerta, 2010; Cruz-Huerta et al., 2011; Yadav, 2010). The root system is also greatly affected, both physiologically and morphometrically, by sub-optimal temperatures, leading to a reduction in roots growth rate and roots size as well as modifications in roots architecture and functioning. Roots architecture modifications under chilling stress show both interspecific and intraspecific variations and also inside the same species they can change a lot between different varieties or accessions (Lee et al., 2009; Mcmichael et al., 1993).
However, the optimal temperature window can be very different for the diverse plant species and varieties; so, the same temperature could be part of the optimal range for one plant and represent a stressful condition for another one. Moreover, the optimal range of temperature changes with the growth phase, resulting in more devasting effects of cold stress when it occurs at certain stages. The time needed for the chilling stress symptoms to appear is also species- and varieties-dependent and the recovery ability depends on the relationship between the stress intensity and its duration. Anyway, plants originated from warm areas usually have a higher optimum temperature window and often undergo many physiological disorders even when exposed to chilling temperatures being not always able to recover completely (Allen and Ort, 2001; Yadav, 2010).
Although some putative sensors for low temperature have been proposed, no specific low-temperature receptor has still been identified in plants. Cold stress, in fact, seems to be perceived by multiple primary sensors through a series of signals in which membranes rigidification, calcium, reactive oxygen species, protein kinase, protein phosphatase and lipid signalling cascades seem to be involved generating signals transductions that lead to the activation of transcription factors (Chinnusamy et al., 2007; Miura and Furumoto, 2013; Yadav, 2010; Zhu, 2016). Transcription factors are proteins which control the rate of transcription of the genetic information of a DNA trait to the relative mRNA by binding a determined DNA sequence, so they act as regulators of gene expression and ultimately determine cold-responsive genes expression in this case (Latchman, 1993).
The lowering of the temperature induces membranes rigidification finally resulting in the disturbance of all membrane processes, including ion channels opening and membrane-associated electron transfer reactions (Ruelland et al., 2009). The “transition temperature” is the thermal level at which lipid membranes properties change e.g. from a semi-fluid phase to a semi-crystalline phase; that temperature range also depends on saturated/unsaturated fatty acids ratio. The phase behaviour of double layer lipid membranes, in fact, is also related to membrane composition as long as it determines how strong Van der Waals interaction will be; in particular, the longer the lipids tails are, the lower their mobility will become at the same temperature. Even more influence on membrane fluidity is determined by the saturated/unsaturated fatty acids ratio since it determines how well lipid can pack together. In unsaturated fatty acids, in fact, one or

more carbon-carbon double bonds are present, so the higher the content of unsaturated fatty acids the more free spaces will remain between them making the membrane more permeable to water and small molecules and increasing membrane fluidity (Rawicz et al., 2000; Yadav, 2010). Cold-sensitive plants usually have a higher saturated fatty acids percentage in plasma membranes so that solidification of membranes and the above-mentioned alterations occur faster (Allen and Ort, 2001).
The exposition to low temperatures is almost always associated with an increase in reactive oxygen species (ROS). Among the reasons of that ROS increase there is the reduced scavenging activity of antioxidant enzymes, both directly (since like other proteins they have an optimal range of temperature) and through the formation of secondary RNA structures, thus affecting genes expression and protein synthesis. In normal conditions some of the triose-phosphates produced during Calvin cycle at the chloroplastic level, are transferred to the cytosol, where sucrose synthesis gives back inorganic phosphate, that can return inside the chloroplast. Low temperatures, however, strongly affect sucrose synthesis leading to a gradual triose phosphates accumulation in the cytosol. The resulting lack of inorganic phosphate in the stroma prevents the ATP synthesis and the following regeneration of ribulose-1,5-biphosphate, finally resulting in impairments between the energy trapped by the antenna complexes and the actual capacity to biochemically process it. That results in high ROS production inside the chloroplast, especially if chilling stress occurs under light conditions (Allen and Ort, 2001; Hatfield and Prueger, 2015; Ruelland et al., 2009). Among them, hydrogen peroxide and superoxide showed to have a particularly detrimental effect on PSII, so chlorophyll fluorescence parameters related to its functionality, like Fv/Fm usually show a decrease following chilling stress, especially under light (Kalaji et al., 2017; Tjus et al., 2001). So, while scavenging activities decrease with temperature, ROS production due to disturbances in electron transfer reactions at mitochondrial and chloroplastic level rapidly increases, and the unbalances between their formation and scavenging often leads to membranes leakage **(Figure 2)** (Ruelland et al., 2009).

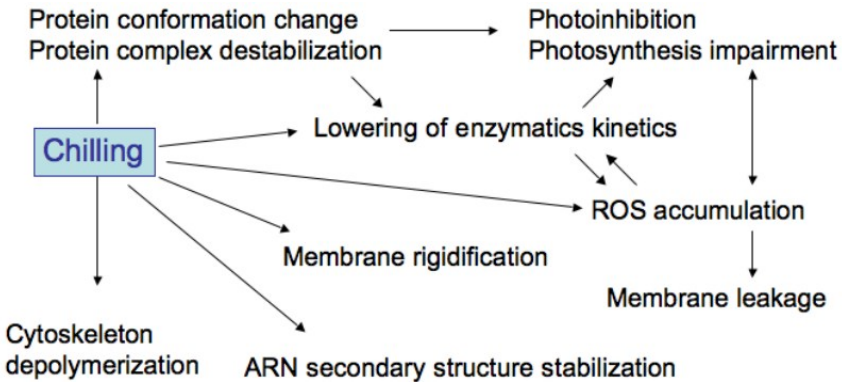



**Figure 2** - Chilling effects on plant cellular processes (Ruelland et al., 2009).

## Flooding effects on plants

Plants are aerobic organisms and, in their respiratory metabolism in mitochondria, they need oxygen as electron acceptor (Dresbøll et al., 2013).
Flooding causes the water saturation of soil pores dramatically reducing the rate of gas diffusions in the root environment. In a similar situation, the aerobic activity of both roots and soil microorganisms rapidly decreases soil oxygen concentration directly affecting roots growth rate

and development, with indirect effects on aerial part growth and finally on crop productivity (Visser et al., 2003).
Flooding induces inhibition in roots, stem and leaves development as well as epinasty, leaves abscission and fruit drop have been associated with the accumulation of ethylene. This hormone seems also involved in adaptative responses including aerenchyma formation and adventitious roots development (Fiebig and Dodd, 2016). Under partial waterlogging of the root system in tomato, an increased elongation rate have been observed in above layer roots indicating that plants try to realize a compensation for the strongly reduced elongation rate of submerged roots (Dresbøll et al., 2013).
The mechanism by which plants perceive oxygen concentration in the surrounding environment remained unknown until recent years. The main reason was that previous researches especially focused on genes that were expressed in response to hypoxia while the transcription factor *RAP2.12* is constitutively expressed instead (Perata, personal communication, November 8, 2017). Its encoded protein RAP2.12 is so always present in plant cells under optimal conditions, however, its activity is prevented since it is normally kept fixed to plasma membranes by acyl-CoA-binding proteins (ACBP1 and ACBP2); this also protects it from proteasomal degradation. Under low oxygen, RAP2.12 is released from plasma membranes and moves into the nucleus activating hypoxia-response genes expression. Then, when low oxygen stress ceases, RAP2.12 degradation occurs through proteasome-mediated proteolysis and via N-end rule pathway, resulting in a progressive reduction in hypoxic response (Licausi et al., 2011).
Like almost every abiotic stress, flooding can rapidly cause unbalances in redox state of the cells since it is almost always associated with a sharp increase in the formation of reactive oxygen species (ROS) including superoxide ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$); hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide ($H_2O_2$). In this process, NADPH oxidases activity seems to be among the primary source of ROS formation. This enzyme induces electron movements from NADPH to oxygen ($O_2$), so generating superoxide ($O_2^{\cdot -}$) that is then usually converted to hydrogen peroxide ($H_2O_2$) (Pucciariello and Perata, 2017). The extremely high reactive nature of these compounds leads to severe damage of proteins and nucleic acids, as well as to membranes leakage through lipids peroxidation. In plants that show a higher tolerance to roots hypoxia, this has been at least in part attributed to a higher ability in $H_2O_2$ detoxification. Although other compounds like carotenoids and ascorbate are contributory, antioxidant enzymes play a central role as ROS scavengers, and among them, catalase (CAT) and ascorbate peroxidase (APX) is the most important enzymatic regulators of $H_2O_2$ (Tang et al., 2010).
Plants early responses to oxygen starvation in roots environment include reductions in stomatal conductance, photosynthesis, leaf elongation and leaf water potential. These alterations have been proved to be regulated by chemical communications and not simply by hydraulic signals (Else et al., 1995).

## Can grafting improve abiotic stress tolerance?

## Low temperatures

Grafting usefulness under low temperatures depends on rootstock tolerance to temperatures below the optimum windows of the scion, on rootstock-scion interaction and on the severity and duration of stress (Schwarz et al., 2010). Under chilling temperatures plants also suffer water stress since inhibition in water uptake from soil is not always accompanied by a similar reduction in evaporative demand (Aroca et al., 2001), and this justifies the fact that in plants crosstalk between cold- and dehydration-signalling pathways do exist (Shinozaki and Yamaguchi-Shinozaki, 2000; Shinozaki et al., 2003). Usually, at a certain absolute humidity a reduction of the air temperature will decrease evaporative demand from leaves, however, the soil specific

capacity is much higher than the air one so it is really common in the field, especially in unheated protected environments, that air and leaves warms faster than soil in the morning. Under these conditions, the increase in evaporative demand will not always be supported by roots water uptake and transport since soil temperature affects root hydraulic conductance (Venema et al., 2005).
Comparing one chilling-tolerant maize cultivar with a sensitive one, at two temperatures (25 °C and 5 °C) the higher root hydraulic conductance of the tolerant genotype promoted the plant acclimation to the stress conditions. Authors proposed a possible increase in the aquaporins contribution to root hydraulic conductance throughout the chilling treatment since their experiment with $HgCl_2$, that inhibits aquaporins activity, resulted in a higher inhibition of root conductance over time (Aroca et al., 2001). In a similar way rootstocks aquaporins can play a key role in the maintenance of a good hydraulic conductance through grafting; according to this, cucumber aquaporins were reported to be more sensitive to low temperatures compared to the figleaf gourd ones (Lee et al., 2005).
Moreover, plants that are more tolerant to low temperatures often show a higher root to shoot ratio and use this adjustment during cold acclimation; in this case the plant perceives the gradual changes in environmental conditions and actively modifies assimilates partitioning in such a way to overcome the reduced water and nutrient uptake under sub-optimal temperature (Janská et al., 2009; Schwarz et al., 2010). The use of a rootstock that is more tolerant to thermal ranges below the optimum of the scion has been proved to maintain a higher root growth rate under chilling stress conditions in many vegetables including tomato and cucumber, and this results in a higher root to shoot ratio that also contributes to hydraulic conductance adjustment and nutrient uptake under low temperatures (Tachibana, 1987; Venema et al., 2008).
Low temperatures also induce a rapid increase in ROS formation, that leads to lipids peroxidation and membrane leakage and grafting onto tolerant rootstocks proved to induce an up-regulation of ROS scavenging system under stress, resulting in a higher control of their detrimental effects. Long-distance hormonal communications and transport of genetic material, such as mRNAs and small RNAs, seem to be involved in this process and in coordinate growth and development between rootstock and scion (Bie et al., 2017; Gao et al., 2009; Ntatsi et al., 2014; Schwarz et al., 2010).

## Flooding

Flooding induced oxygen starvation results in rapid ROS formation and in a general reduction in stomatal conductance, photosynthesis, leaf elongation and leaf water potential. Under prolonged hypoxia inhibition in roots, stem and leaves development as well as epinasty, leaves abscission, fruit drop and also plant death can occur (Else et al., 1995; Fiebig and Dodd, 2016; Tang et al., 2010). Although compared to other abiotic stresses little information is known about vegetable-graft induced tolerance, available data proved that flood-tolerant rootstocks can be effective in alleviating physiological disturbances caused by hypoxia (Schwarz et al., 2010).
For example, grafting of bitter melon (*Momordia charantia*) showed a higher flood-tolerance when grafted onto a *Luffa cylindrica* rootstock, probably thanks to a mitigation of the detrimental effects of hypoxia on net photosynthesis, stomatal conductance, transpiration and RuBisCO activity (Liao and Lin, 1996). Similarly, good results were obtained grafting watermelon (*Citrullus lanatus*) onto *Lagenaria siceraria*; additionally, in this case, aerenchyma formation together with adventitious roots have been observed in grafted plants but not in un-grafted ones (Yetisir et al., 2006).
Eggplant (*Solanum melongena*) rootstocks have been reported to partially alleviate detrimental effects of flooding in tomato; that has been sometimes attributed to the containing of secondary bacterial diseases or to a higher APX activity leading to a better $H_2O_2$ scavenging (AVRDC,

2002; Lin et al., 2004). Anyway, no efforts have been made so far for the breeding of commercial eggplant rootstock for tomato. One of the reasons relies on the lower tolerance of this species to many soilborne and pests compared to *S. lycopersicum* × *S. habrochaites* hybrids, since it still remains one of the main goals of breeding companies (Fullana-Pericàs et al., 2018; King et al., 2010).
Until few years ago, almost all the rootstocks for tomato commercially available were represented by interspecific hybrids of *S. lycopersicum* × *S. habrochaites*, however, many commercial rootstocks of different genetic origin are today available for tomato, representing a pool of genetic variability to test for flooding and hypoxia tolerance.

## Aim of the thesis

Due to their economic importance and nutritional and nutraceutical properties pepper and tomato are among the most widely grown fruit vegetables all over the world (Giuffrida and Leonardi, 2012; Li et al., 2017; Vitale et al., 2014). Abiotic stresses, including sub-optimal temperatures and flooding, limit crops productivity worldwide; however, the grafting of high-quality traits cultivars onto tolerant rootstocks is a rapid and effective method to reduce their detrimental effects (Lee and Oda, 2010; Schwarz et al., 2010). In the present work three lines of research have been followed:

**Line 1 – increase of germplasm availability in pepper rootstocks for low-temperature tolerance**

Pepper is a thermophilic species coming from tropical areas of Central and South America, with an optimal thermal window for growth around 23-25 °C during the day and 17-18 °C in the night. Pepper growth stops around 12°C and leaves can die and flower abortion will start if the temperature falls below 6 °C **(Table 1)** (Starke Ayares, 2014).

**Table 1** - Pepper thermal requirements at different growth stages (Starke Ayares, 2014)

| Stage of development | Minimum threshold | Optimum | Maximum threshold |
|---|---|---|---|
| Germination | 23 °C | 26 - 28 °C | 30 °C |
| Vegetative growth | 21 °C | 23 - 25 °C | 28 °C |
| Fruit set (day) | 20 °C | 23 - 25 °C | 28 °C |
| Fruit set (night) | 15 °C | 17 - 18 °C | 20 °C |
| Fruit ripening | 18 °C | 20 - 24 °C | 30 °C |

In the Mediterranean region, pepper is mostly cultivated in unheated greenhouses during winter, with transplant usually between the second half of October and the first week of November. During these periods of the year, the exposure to sub-optimal temperatures are common, especially during the night and early in the morning.
The domestication and the selective pressure of breeding companies aimed to obtain high qualitative traits strongly reduced genetic variability for cold stress tolerance among the available germplasm in many species including pepper (Schwarz et al., 2010).
Even though in the last decades grafting became a widespread technique in intensive agricultural systems for many fruit vegetables such as tomato, eggplant, melon and watermelon, it never spread in a similar way for pepper, so a really limited number of rootstocks is available for

pepper compared to other Solanaceae crops (King et al., 2010). In the few regions in which vegetable grafting became the standard for pepper cultivation, like Korean peninsula, it was promoted for the control of *Phytophthora capsici*, however, in recent years the interest in pepper grafting as a useful and sustainable practice is rising in other regions including Europe (Saporta and Gisbert, 2013). The main goal of breeding companies in the selection of new breeding lines to use as rootstocks, in fact, remained for a long time limited to the tolerance to biotic stresses including *Fusarium, Verticillium, Phytophthora,* nematodes and other soilborne and pests (Penella and Calatayud, 2018). The grafting of elite cultivars onto robust rootstocks was, however, proved to reduce or prevent yield losses due both to biotic and abiotic stresses also in pepper (Doñas-Uclés et al., 2015; López-Marín et al., 2013; Oka et al., 2004; Penella et al., 2013). *Capsicum* spp. are generally cold sensitive, however, they originate from a wide range of South and Central American regions even very different for elevation and average day temperature (7 – 29 °C) (De Swart et al., 2006). Recognising that to expand parental sources is the most important problem to solve the bottleneck in pepper breeding, researches aimed to increase pepper tolerance to low temperatures looking for other species in the same genre, suggested *C. pubescens* and *C. baccatum* as possible candidates to introduce in pepper breeding programs (Ou et al., 2015; De Swart et al., 2006). Although in pepper grafting incompatibilities are frequent, the graft compatibility with *C. pubescens* and *C. baccatum* has been sometimes reported (Palada and Wu, 2008; Penella et al., 2013; Saporta and Gisbert, 2013). Some authors have found that, in tomato, the inhibition of roots development plays a central role in vegetative growth reduction under chilling temperatures and that grafting onto high-altitude accession LA1777 (*Solanum habrochaites*) was able to maintain a higher roots development and grafted plants growth under stress conditions (Venema et al., 2008). However, the good results concerning vegetative growth study were followed by a significant reduction of yield under greenhouse conditions regardless temperature level, probably because of roots signals involvement in fruit set and the following reduction of fruit number per plant (Ntatsi et al., 2014). As a consequence, high-altitude accessions able to improve low-temperature tolerance when used as rootstocks should be also tested under optimal conditions to assure that yield and fruits quality is, at least, maintained.

Taking it all into account, the aim of the first line of research of the present work was to increase the germplasm availability of pepper rootstocks for low-temperature tolerance. For that purpose, four experiments were articulate in order to:

1- test *C. pubescens* and *C. baccatum* accessions originated from high altitudes for their tolerance to thermal ranges below the pepper optimal window, compared with a widespread pepper rootstock;
2- test grafting compatibility of selected *C. pubescens* and *C. baccatum* lines with a widespread bell pepper (*C. annuum*) cultivar and evaluate the quality of grafted transplants;
3- test the tolerance to chilling stress of *C. annuum* grafted onto the selected accessions and onto a widespread pepper rootstock compared to un-grafted plants;
4- evaluate long-term grafting compatibility, as well as yield and fruit quality of *C. annuum* grafted onto the selected accessions and onto a widespread pepper rootstock compared to un-grafted plants under optimal greenhouse conditions.

**Line 2 – Tomato grafting as a tool to alleviate physiological disturbances under root-zone oxygen starvation**

Although flooding is not a usual condition in tomato cultivation under a protected environment at all, there are a series of circumstances that can lead tomato plants grown in soilless systems to experience root-zone oxygen starvation. When irrigation dose and frequency are not rigorously determined basing on objective calculations, it almost always results in an inappropriate irrigation scheduling that leads to over-irrigation (Pardossi et al., 2011). Chronic over-irrigation can affect both soil properties (including oxygen availability) and plant physiology finally resulting in growth limitation (Fiebig and Dodd, 2016). The negative effects of the misjudgments in water requirements become particularly important in soilless cultivation, especially when the substrate has already been used for other cultivation cycles; the air capacity of the substrates, in fact, progressively decreases with cultivation cycles (Giuffrida et al., 2008).
For these reasons, tomato plants in soilless cultivation can more likely experience roots hypoxia as long as the substrate is reused. On the other hand, the possibility to extend the use of the same substrate to an additional growing cycle has a positive impact on the environment and strongly reduces the cost of growing media per year. Although a rigorous determination of the correct irrigation scheduling plays an essential role, a deeper knowledge about specific flood tolerance of well-established tomato rootstocks can provide useful guidelines for growers to extend substrate life without risking to induce severe stress in case of erroneous over-irrigation.
For that purpose, the tolerance to hypoxia of a cherry tomato cultivar grafted onto widespread commercial rootstocks was tested compared to self-grafted plants. Special attention was focused on the maintenance of gas exchange and photosystem II efficiency as well as on the ability to alleviate ROS induced membrane leakage.

**Line 3 - The effects of grafting combination on cherry tomatoes yield and quality**

Tomato is one of the most important vegetable crops worldwide and has been largely used as a model plant for research purposes; moreover, grafting is now considered as a standard practice in specialized tomato cultivation (Ezura, 2009; Keatinge et al., 2014).
Throughout the years, breeding companies put many efforts in the selection of robust rootstocks to improve tomato productivity; anyway, productivity and fruit quality usually show contradictory trends and grafting-induced enhancement of yield is often accompanied by a loss in terms of product quality (Kyriacou et al., 2017). Even if some specific quality trait was reported to be enhanced in some grafting combination, the more realistic goal is not about using grafting for improving tomato quality, but to find a solution in order to maximize crop productivity, also under limiting conditions, at least without affecting fruit quality.
Due to the extremely high importance of this topic, the effects of grafting on tomato yield and quality have been studied during recent years and are still under investigation (Flores et al., 2010; Savvas et al., 2011). However, many of the results reported are conflicting, probably due to differences in environmental conditions, rootstocks genetic origins and the use of different scion cultivars or even different types of tomato. Moreover, it seems that, in many cases, grafting effects on fruit quality are not simply determined by the rootstock or the scion used, but are related with the specific grafting combination. In the past, the most popular approach was to examine the effects of different rootstocks on one or few scions. Although giving useful information, this may not consider the typical characteristics of the scion, which actively supports the rootstock with photosynthesis products and is part of an intricate network of hormonal communications with the root system. To observe the rootstocks-induced changes in

tomato yield and fruit quality without missing the scion active role, large experimental designs including many rootstocks-scion combinations are needed.
In this context, an experiment was conducted focusing on cherry tomato type, including seven cultivars grafted onto eight rootstocks that were examined for yield and fruit quality and compared with un-grafted plants.
From previous information, productive vigour data of un-grafted plants under comparable growing conditions for a number of cherry tomato cultivars were known. Moreover, that was always strongly correlated with the typical fruit weight and calibre of each cultivar under similar (non-stressed) growing conditions. The cultivars with an intermediate calibre and productive vigour are the most common since they usually represent a good compromise between productivity and appealing for the consumers. However, to be more representative of the available cultivar scenery, among the seven scions used in the experiment, two with an average fruit diameter around 26-29 mm, four with 31-34 mm and one with 34-37 mm were included, showing low, intermediate and high productive vigour respectively. Interspecific hybrids between tomato and *S. habrochaites*, *S. peruvianum* and *S. pimpinellifolium*, as well as an intraspecific tomato hybrid were used as rootstocks.
Besides the classic results discussion in relation to the specific grafting combinations, a new approach was adopted to analyse the large amount of data obtained in order to highline:

(1) to what extend each examined variable was actually affected from rootstock and scion respectively, both in an overall analysis of the scions and focusing on the most common group of productive vigour (intermediate)

(2) the active role of the scion in control yield and quality traits changes in grafted plants, quantifying the ability of each scion to impose its characteristics for the different examined variables.
This will also answer if potential cultivar able to impose their characteristics, would do that in a variable-dependent or -independent way.

# 1. Grafting pepper onto high-altitude accessions of *Capsicum pubescens* and *Capsicum baccatum* can enhance vegetative growth and roots development under chilling stress without affecting fruit yield and quality under optimal temperature conditions

## Introduction

Low-temperatures stress is a major environmental factor affecting crops growth and productivity worldwide (Shi et al., 2015). Suboptimal temperatures stress has been defined as any reduction in plant growth or induced metabolic, cellular or tissue injury that results in limitations to its yield potential as a result of the exposure to temperatures below the thermal thresholds for optimal biochemical and physiological activity or morphological development (Greaves, 1996). When exposed to sub-optimal temperatures plants undergo many morphological and physiochemical disturbances at both vegetative and reproductive level (Yadav, 2010). Chilling temperatures determines a remarkable reduction in seeds germination, stunted seedlings, leaves yellowing, severe shoot apexes damage, impairment of photosynthetic apparatus and reduction in photosynthetic rate and leaves initiation and expansion. Regarding reproductive stage, cold stress often determines pollen sterility and alteration in ovary development (ovary swelling) resulting in deformed fruits (Cruz-Huerta, 2010; Cruz-Huerta et al., 2011; Yadav, 2010).

Exposition to sub-optimal temperatures also affects root system both morphometrically and physiologically, leading to a reduction in roots growth rate and roots size as well as modifying roots architecture and functioning (Schwarz et al., 2010). Plants damage following exposure to low temperatures are species-specific and their recovery ability also depends on the relationship between the stress intensity and its duration; however, plants originated from warm-climate regions usually undergo many physiological disorders even when exposed to sub-optimal non freezing temperatures (chilling temperatures) and are not always able to recover completely (Allen and Ort, 2001).

Pepper is a thermophilic species coming from tropical areas of Central and South America, with optimal temperatures around 25-28 °C during the day and 16-18 °C during the night. Pepper stops growing at 10-12°C and leaves can die and flower abortion will start if the temperature falls below 6 °C (Starke Ayares, 2014).

Covering more than 480,000 ha pepper is one of the most widely grown fruit vegetables all over the world due to its economic importance and its nutritional and nutraceutical properties (Giuffrida and Leonardi, 2012; Li et al., 2017). In the Mediterranean region, pepper is mostly cultivated in unheated greenhouses during winter, with transplant usually between the second half of October and the first week of November. During these periods of the year, the exposure to sub-optimal temperatures are common, especially during the night and early in the morning.

The domestication and the selective pressure of breeding companies aimed to obtain high qualitative traits strongly reduced genetic variability for cold stress tolerance among the available germplasm in many species (Schwarz et al., 2010). *Capsicum* spp. are generally cold sensitive, however, they originate from a wide range of South and Central American regions even very different for elevation and average day temperature (7 – 29 °C) (De Swart et al., 2006). Recognising that to expand parental sources is the most important problem to solve the bottleneck in pepper breeding, researches aimed to increase pepper tolerance to low temperatures looking for other species in the same genre, suggested *C. pubescens* and *C. baccatum* as possible candidates to introduce in pepper breeding programs (Ou et al., 2015; De Swart et al., 2006). The constant effort of breeding companies in the development of cultivars with higher tolerance to biotic and abiotic stresses requires a lot of time and do not always gives good results especially for abiotic stresses tolerances which are usually regulated by several genes and complex nets of

metabolites interactions. Moreover, high-quality traits are often shown by cultivars that do not present high tolerance to biotic and abiotic stresses. In this context vegetable grafting of elite cultivars onto rootstocks with a higher tolerance to environmental stresses can often represent an effective alternative to the relatively slow breeding methodology (Flores et al., 2010).
There is a really limited number of rootstocks available for pepper compared to other Solanaceae crops (King et al., 2010) and, although in pepper grafting incompatibilities are frequent, the graft compatibility with *C. pubescens* and *C. baccatum* has been sometimes reported (Palada and Wu, 2008; Penella et al., 2013; Saporta and Gisbert, 2013). The low-temperature tolerance in related wild species can much especially larger in accessions collected at high-altitudes with large thermal excursion during the day (Venema et al., 2005). Some authors have found that, in tomato (*Solanum lycopersicum*) vegetative growth reduction under chilling temperatures, the inhibition of roots development plays a central role and that grafting onto high-altitude accession LA1777 (*Solanum habrochaites*) was able to maintain a higher roots development and grafted plants growth under stress conditions (Venema et al., 2008). However, the good results concerning vegetative growth study were followed by a significant reduction of yield under greenhouse conditions regardless temperature level, probably because of roots signals involvement in fruit set and the following reduction of fruit number per plant (Ntatsi et al., 2014). For that reasons, merely identify high-altitude accessions of related species as putative rootstocks for improving low-temperature tolerance basing on controlled stress experiments loses almost every importance if vegetative growth is improved but yield and fruits quality is not, at least, maintained also when stress conditions will cease.
In this study five accessions of *C. pubescens* and *C. baccatum* var. *baccatum* originated from high altitude regions of South America (or selecting started from high altitude accessions) were compared with a widespread pepper rootstock for chilling tolerance. Then, grafting compatibility with bell pepper (*C. annuum*) was assessed through an experiment of grafted transplant quality evaluation using *'Tiberio'* $F_1$ as scion and the chilling tolerance of grafted plants was evaluated using un-grafted *'Tiberio'* $F_1$ as a control. Finally, the same grafting combinations were used in a greenhouse experiment under optimal temperature conditions in order to verify if yield and fruit quality would be affected by grafting onto the putative rootstocks compared to the commercial rootstock and to un-grafted and self-grafted *'Tiberio'* $F_1$.

## Material and methods

***Plant material***
Three *Capsicum pubescens* and two *Capsicum baccatum* var. *baccatum* accessions have been selected among 12 genotypes tested for germination rate and uniformity of germination (data not shown) giving priority to the ones originated from higher altitude areas **(Table 1)**. *C. pubescens* accessions PI585267, PI585273 and PI355812 (CP 2; CP 3 and CP 4) and *C. baccatum* var. *baccatum* accession PI238061 (CB 5) were collected, 2000 m above the sea level and are maintained from the United States Department of Agriculture through the U.S. National Plant Germplasm System (USDA – NPGS). *C. baccatum* var *baccatum* 'Bacclaudio' (CB 9) is a local ecotype selected in the north of Italy starting from high altitude accessions provided from USDA – NPGS and showed the higher germination rate among the 12 genotypes tested. The widespread pepper rootstock 'Capsifort' $F_1$ (Capsifort) (Monsanto, the Netherlands) was used as a control.
The *C. annuum* cv 'Tiberio' $F_1$ was used as scion in the grafted transplant quality experiment, in chilling tolerance evaluation of grafted plants and in the greenhouse experiment.

***Experiment 1 – High-altitude accessions growth under two thermal regimes compared to a widespread pepper rootstock***

The experiment was carried out in separate growth chambers (15 $m^3$ each one) in which the light was provided through high pressure sodium lamps (PAR was 150 µmol $m^{-2}$ $s^{-1}$). A close hydroponic system including a covered black tank filled with 16 L of nutrient solution and one aeration pump for each accession has been installed inside the growth chamber. The top of each tank was prepared to hold nine plants in such a way as the plant to plant distance would be the same both within and among the tanks. To assure the homogeneity of radiation levels among the tanks PAR measurements were performed before the beginning of the experiment; moreover, each tank position was changed once a week to minimize potential differences in other microclimatic conditions.
Two sets of temperature were used, the first with optimal conditions, in which a day temperature of 25 °C and a night temperature of 15 °C (25/15 °C) were maintained, and the second of chilling stress, with an average day temperature of 15 °C and 5 °C of minimum night temperature (15/5 °C). In the chilling stress set of temperatures, data collected through data loggers during previous greenhouse experiments concerning average, minimum and maximum values of temperature per hour were used to set growth chamber parameters in order to recreate microclimate conditions of a typical unheated greenhouse during winter months.
For each accession, nine homogeneous plants were used for the experiment and at least 15 plants were sampled for day 0 biometrical measurements. Since different species were used, for the variables in which significant differences between means were found on day 0, the data recorded at the end of the experiment (day 28) were expressed both as absolute values and in terms of relative increase. Biometric measurements are reported for both the set of temperature; for chilling conditions, gas exchanges changes during the experiment, electrolyte leakage and Chlorophyll a (Chl. a) fluorescence are also reported.

***Experiment 2 - Grafting compatibility and grafted plants quality***

Basing on mean germination time and early seedling growth data previously obtained, seeds of *C. baccatum* and *C. pubescens* accessions were sown 7 and 14 days before Capsifort respectively in order to reach the same stem diameter (2.0 mm) on the grafting day. Grafted transplant production was performed at a specialized vegetable nursery (Centro Seia s.r.l.; Italy); sweet pepper (*C. annuum*) 'Tiberio' $F_1$ was sowed 3 days after Capsifort and used as scion. 30 days later, splice grafting of the different accessions was performed manually by the same worker. Plants were then transferred in a hardening chamber with a 26 °C/24 °C day/night temperature and 98 % of relative humidity for 6 days, then the final stage was reached in a controlled greenhouse to prevent infections. When plants had six true leaves (at least 1 cm long) a transplant quality analysis was carried out in the laboratory of the University of Catania. SPAD (SPAD-502 meter), stem diameter 1 cm under and 1 cm above the grafting point, plant height, number of leaves (length of 1 cm at least), leaf area and dry matter of leaves, stem and roots were analysed. Xylem sap exudation was also analysed; for that purpose, the stem was cut 1 cm above grafting point while maintaining roots inside a baker containing nutrient solution and xylem sap was collected through a glass pipette for 30 minutes and weighted.

***Experiment 3 – Chilling stress tolerance of C. annuum cv 'Tiberio' $F_1$ grafted onto high altitude accessions of C. pubescens and C. baccatum and onto Capsifort***

Sweet pepper 'Tiberio' $F_1$ grafted onto the above-mentioned *C. pubescens* (T/CP2; T/CP3; T/CP4) and *C. baccatum* (T/CB5; T/CB9) accessions and onto Capsifort (T/Capsifort) together with un-grafted plants used as a control (T) were grown in a hydroponic system for 28 days under chilling stress (15/5 °C set of temperatures) as previously described for un-grafted plants in experiment 1. Gas exchanges and Chl. a fluorescence were measured every 7 days and electrolyte leakage every 14 days. No significant difference among means was observed on day 0

biometric measurements so they were expressed as absolute values recorded at the end of the experiment.

***Experiment 4 – Yield and fruit quality of C. annuum cv 'Tiberio' $F_1$ grafted onto high altitude accessions of C. pubescens and C. baccatum and onto Capsifort***

Sweet pepper 'Tiberio' $F_1$ grafted onto Capsifort (T/Capsifort), *C. pubescens* (T/CP2; T/CP3; T/CP4) and *C. baccatum* (T/CB5; T/CB9) accessions, together with un-grafted (T) and self-grafted (T/T) plants used as a control was cultivated for 150 days in an unheated greenhouse in Sicily, with a transplant on February 26$^{th}$, 2018. 2.5 plants m$^{-2}$ were used; the greenhouse was covered with a polyethylene-EVA film (150 µm) and white/black mulching film (30 µm) was used. Conventional agricultural practices were adopted and the fruits were harvested when the commercial ripening was reached (around 1/3 of the fruit surface). Gas exchanges at the end of the experiment, commercial yield and un-marketable fruits production are reported. For the quality analysis, fruits of the second and third bifurcation were analysed for average weight, dry matter, total soluble solids (TSS), titratable acidity and antioxidant activity (DPPH).

***Gas exchanges and chlorophyll fluorescence measurements***

Gas exchanges analysis were performed weekly on the second fully expanded leaf through an LCi Portable Photosynthesis System (ADC BioScientific Ltd.). Net photosynthesis (A), stomatal conductance (gs), and internal $CO_2$ concentration (Ci) were determined and expressed as µmol $CO_2$ m$^{-2}$ s$^{-2}$, mol $H_2O$ m$^{-2}$ s$^{-1}$ and µmol $CO_2$ mol$^{-1}$ air, respectively.

PSII efficiency was determined through Chlorophyll a fluorescence analysis using an OS1-FL fluorometer (Opti-Sciences Corporation, Tyngsboro, MA); chlorophyll fluorescence excitation was performed by a 660 nm solid-state light source coupled with filters able to block λ above 690 nm; the modulated light intensity was adjusted from 0 to 1 µE. Fluorescence detection was performed between 700 and 750 nm using a PIN silicon photodiode coupled with appropriate filtering to remove extraneous light. Saturation of the photosystem II was provided by a filtered 35W halogen lamp (350-690 nm). All the measurements were performed after a 20 minutes leaf dark-adaptation through OS cuvettes (Opti-Sciences Corporation, Tyngsboro, MA) (Liu et al., 2005). The ratio between the variable and $F_V/F_M$ is a useful ratio which has been shown to be proportional to the quantum yield of photosystem II (PSII) photochemistry and exhibits a high degree of correlation with the quantum yield of net photosynthesis (Kitajima and Butler, 1975).

***Electrolyte leakage***

Leaves electrolyte leakage was measured at day 0, 14 and 28. For each measurement 20 leaf discs (1 cm$^2$) were collected and placed on a 50 ml tube containing 20 ml of ultra-pure water, the tubes were then placed in a shaker at 100 r.p.m. for 24 hours at 25 °C. After a first EC reading (EC1) at the end of the 24 hours shaking, the test tubes were placed in an autoclave at 120 °C for 20 minutes in order to destroy cellular structures and left to cool again at 25 °C for the second EC reading (EC2). Electrolyte leakage was calculated as:

$$Electrolyte\ leakage\ (EL) = \frac{EC1}{EC2} \times 100 \quad (1)$$

***Biometric measurements***

Since they can be performed as non-destructive analysis, plant height, number of leaves (with a lamina length of 1 cm at least) and stem diameter were measured both at day 0 and at day 28 in the plants used for the experiment. Day 0 leaf area was calculated through a multiple regression obtained for each genotype by the analysis of a large number of homogeneous transplants, using the height and the number of leaves as predictors. Day 28 leaf area was determined with a leaf

area meter (Delta T device). Plant height, number of leaves, stem diameter and leaf area were expressed as a percentage of increase from day 0 to day 28. At the end of the experiment root, stem and leaf dry weights were determined using a thermo-ventilated oven at 70 °C until constant weight was reached. No significant differences in dry weights were observed at day 0 among the different genotypes transplants, so their absolute value at day 28 was reported. Specific leaf area at day 28 was calculated as:

$$Specific\ leaf\ area\ (SLA) = \frac{leaf\ area\ (cm^2)}{leaves\ dry\ weight\ (g)} \quad (2)$$

Leaf mass ratio (LMR), also called leaf mass fraction, represents the fraction of a plant's total leaf mass to the entire biomass of the plant and it was calculated as:

$$Leaf\ mass\ fraction\ (LMR) = \frac{leaves\ dry\ weight\ (g)}{plant\ dry\ weight\ (g)} \quad (3)$$

Leaf area ratio represents the product of SLA and LMR and can be defined as:

$$Leaf\ area\ ratio\ (LAR) = \frac{leaf\ area\ (cm^2)}{plant\ dry\ weight\ (g)} \quad (4)$$

SLA and LAR were expressed in $cm^2\ g^{-1}$, whereas LMR was expressed as a percentage.

***Quality of fruits***

Only homogeneous fruits (same ripening stage) were used for quality analysis. Each fruit analysed was cut longitudinally along the ripened area; one half was grounded for titratable acidity and total soluble solids determinations, whereas the other one was cut in smaller pieces for the production of the freeze-dried material necessary for antioxidant analysis (DPPH). Titratable acidity was determined by neutralization of the free acids with a titration solution of 0.1 N NaOH up to the changing colour of phenolphthalein. Results were expressed as mg/L of citric acid equivalents. The total soluble solids content was determined by a digital refractometer DBX-55° (Atago CO, Ltd, Japan) provided with an automatic temperature compensation system. The samples used for antioxidant activity analysis were both weighted before and after freeze-drying in order to express the obtained results in a fresh weight base. Antioxidant activity was determined using 1,1-diphenyl-2-piricridrazile (DPPH); in detail, 0.05 g of freeze-dried sample finely grounded was mixed with 1.5 mL of a methanol solution (80%), sonicated for 30 minutes and centrifuged for 10 minutes at 5 °C and 5000 × g. 0.01 mL of supernatant was added to 1.4 mL of a daily prepared DPPH solution 150 µM in methanol:water (95:5), vortexed and incubated in the dark at room temperature (20 °C) for 30 minutes, then, the absorbance at 517 nm was spectrophotometrically measured. A calibration curve was created measuring the percentage of inhibition of the absorbance at 517 nm of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97%) and antioxidant activity was expressed as micromoles of Trolox per g of fresh weight (µmol Trolox/kg FW).

***Statistical analysis***

Bartlett's test was used as a homoscedasticity test and differences among treatments were determined by two-way or one-way ANOVA performed through CoStat 6.451 (CoHort). The multiple mean comparisons were performed through the Student-Newman-Keuls test (at least for P = 0.05). Among data reported as percentage root to shoot ratio and leaf mass ratio have been arcsine transformed before statistical analysis (percentage data are reported discussed). Pearson's correlation analysis was performed through Minitab 16 statistical software (Minitab Inc.).

# Results

## *Experiment 1 - High-altitude accessions growth under two thermal regimes compared to a widespread pepper rootstock*

### *Electrolyte leakage*

Membrane leakage under severe chilling stress changed in a species dependent way throughout the experiment, as reported in **Figure 1**. At day 0 electrolyte leakage was around 14 % and 16 % in all the genotypes and, during the experiment, it increased the most in Capsifort rising from 16.2 % to 29.3 % from day 0 to 14, and reaching 37.5 % on day 28. *C. pubescens* accessions had a similar trend with a higher increase in the first two weeks of the experiment, but with significantly lower values. *C. baccatum* accessions showed the lowest values on days 14 and 28 and different trends compared to the other genotypes. CB5 electrolyte leakage didn't change during first two weeks (average value of 16.2 %) but reached 20.4 % at the end of the experiment whereas in CB9 it slightly increased from 15.2 to 18.4 % in the first two weeks and had the lowest value at the end of the experiment (17.7 %).

### *Chlorophyll fluorescence*

Weekly chlorophyll fluorescence changes under low temperatures are reported in **Figure 2**. During the first week of exposition to chilling stress, all the accessions except CP3 showed a significant reduction on Fv/Fm ratio. In Capsifort the decrease in this ratio was the most pronounced reaching a value of 0.71 on day 7, then it started to recover showing no more differences with CP4 and CB5 from day 14 to the end of the experiment. CB9 Fv/Fm slowly decreased together with CP4 and CB5 until day 14 to an average value of 0.75 and then started to rise again reaching the same value of CP2 and CP3 on day 28 (0.80 in the average). CP2 and CP3 Fv/Fm ratios were almost unaffected by the severe chilling stress used.

### *Gas exchanges*

Net photosynthesis (A) (µmol $CO_2$ $m^{-2}$ $s^{-2}$), stomatal conductance (gs) (mol $H_2O$ $m^{-2}$ $s^{-1}$) and internal $CO_2$ concentration (Ci) (µmol $CO_2$ $mol^{-1}$ air) of the high-altitude accessions under study and Capsifort weekly recorded from day 0 to day 28 of chilling stress (15/5 °C) are reported in **Figure 3**. Net photosynthesis was 9.60 µmol $CO_2$ $m^{-2}$ $s^{-2}$ on day 0 and decreased to 4.93 µmol $CO_2$ $m^{-2}$ $s^{-2}$ on day 7 in the average of the genotypes, except for CP3 in which it never decreased throughout the experiment; however, the relative decrease was stronger in Capsifort (-66 %). Its recovery was also affected by genotype, with a more efficient response in CP2 and CB9, that reached an average of 10.96 µmol $CO_2$ $m^{-2}$ $s^{-2}$ in the average with CP3 on day 14, whereas didn't increase in Capsifort during the same time (4.87 µmol $CO_2$ $m^{-2}$ $s^{-2}$ on day 14). On day 21 a complete recovery was observed in *C. pubescens* accessions and in CB9 (11.52 µmol $CO_2$ $m^{-2}$ $s^{-2}$ in the average), whereas Capsifort showed the lowest value (7.12 µmol $CO_2$ $m^{-2}$ $s^{-2}$). On day 28 the only difference was between CP3 (12.2 µmol $CO_2$ $m^{-2}$ $s^{-2}$) and the rest of the genotypes (8.45 µmol $CO_2$ $m^{-2}$ $s^{-2}$). During the first week a gs reduction from 0.18 to 0.09 mol $H_2O$ $m^{-2}$ $s^{-1}$. Starting from the day 14, *C. pubescens* accessions showed a higher stomatal conductance with a final value of 0.19 mol $H_2O$ $m^{-2}$ $s^{-1}$, whereas Capsifort and *C. baccatum* it was 0.09 mol $H_2O$ $m^{-2}$ $s^{-1}$ on day 28. Between the genotypes, the same decrease was observed in the first week for Ci (-65 % from day 0 to day 7), with a gradual species-dependent separation during the rest of the

experiment, with the highest values in *C. baccatum* accessions, intermediate in *C. pubescens* accessions and minimum in Capsifort.

***Biometric measurements***

For all the variables examined, significant interactions between genotype and temperature regime were observed **(Tables 2; 3 and 4)**.

At 25/15 °C, stem diameter at the end of the experiment was higher in the tested accessions than in the commercial rootstock Capsifort. Anyway, cold stress affected stem diameter in all the genotypes and no one of the high altitude accessions exceeded Capsifort absolute value at 15/5 °C (5.1 mm). However, CP2 and CB9 showed a higher relative increase (+100 %) compared to the commercial rootstock (+56 %). Capsifort stem growth was not different between the two thermal levels analyzed, suggesting an inhibition effect already at 15°C of night temperature **(Figure 4)**.

Plant height and its relative increase throughout the experiment were severely affected by temperature. Plant height at the end of the experiment was maximum in CB5 (73 cm) and minimum in Capsifort (39 cm) at 25/15 °C whereas at 15/5 °C CP3 and CB5 showed the highest (35 cm) and the lowest (16 cm) value. In the 25/15 °C treatment, the highest relative stem elongation was reached by CB5 (+410 %) whereas at 15/5 °C day/night temperature the highest value was the one of CP2 (+183 %). Capsifort showed the lowest value of height increase both at 25/15 °C (+176 %) and, together with CB9, at 15/5 °C (+59 % in the average) **(Figure 5)**.

Severe chilling stress had a tremendous effect on the number of leaves developed during the experiment and on leaf area. Leaves number on day 28 was the highest in CP2 and CB5 (115.5 in the average) at 25/15 °C, whereas it was the lowest in Capsifort and CB9 (51 in the average), but no significant differences were observed at 15/5 °C. In CB2 the number of leaves on day 28 was more than 10 times the one on day 0 (+956 %), showing the highest rate of new leaves development. Anyway, at 15/5 °C, no differences were observed among the tested genotypes also in terms of relative number of leaves increase with an average value at the end of the experiment of +132 % compared to day 0. **(Figure 6)**.

At 25/15 °C leaf area was maximum in CB5 (3122 $cm^2$) and minimum in Capsifort (1220 $cm^2$), whereas no significant differences were observed between genotypes under severe low-temperature stress conditions (413 $cm^2$). The relative leaf area increase at 25/15 °C was maximum in CB5, with a surface on day 28 around 15 times the one on day 0 (+1434 %) and minimum in Capsifort (+603 %). Under low-temperatures stress, *C. pubescens* accession CP2 had the highest relative leaf area increase (+694 %) whereas the average leaf area increase for all the other genotypes was +416 % **(Figure 7)**.

Chilling stress (15/5 °C) generally affected dry matter accumulation in leaves, stem and roots. Leaves dry weight on day 28 was maximum in CP2 and CB5 at 25/15 °C, with an average of 4.7 g per plant, and minimum in Capsifort (2.1 g). At 15/5 °C, CP2 and CB5 were the only accessions that exceeded Capsifort leaves dry weight; the reduction in leaves dry weight passing from 25/15 °C to 15/5 °C was, statistically significant in all the genotypes except Capsifort and CB9 **(Figure 8a)**. Stem dry weight followed the same trend of leaves at 25/15 °C with a maximum of 2.8 g in the average of CP2, CP3, CP4 and CB5. However, at 15/5 °C stem dry weight was significantly higher in CP3 (1.5 g) compared to Capsifort and CB5 (0.5 g) **(Figure 8b)**. Temperature regimes affected roots dry weight in a genotype-specific way and its reduction under severe chilling stress was significant in CB5 only. At 25/15 °C roots dry weight was higher in CP2, CP3, CP4 and CB5 (1.41 g in the average) and lower in Capsifort and CB9 (0.67 g in the average). The *C. pubescens* accessions CP2 and CP3 roots dry weight was not affected by chilling stress and showed a significantly higher values (1.34 g in the average) compared to the other genotypes (0.62 g in the average) at 15/5 °C **(Figure 8c)**.

All the *C. pubescens* accessions tested and CB5 showed a higher biomass production (8.59 g in the average) at 25/15 °C compared Capsifort. At 15/5 °C, total dry weight was significantly higher in CP2 and CP3 (4.89 g in the average) compared to Capsifort and CB5 (2.17 g in the average) **(Figure 8d)**. No significant differences were observed in roots to shoot ratio among the genotypes at 25/15 °C (19 % in the average), however, passing from 25/15 °C to 15/5 °C, a significant increase was observed in Capsifort, CP2 and CP3, that reached 35.3 % in the average **(Figure 8e)**.
Specific leaf area (SLA) significantly decreased in all the genotypes under stress conditions. SLA was maximum in CB5 at 25/15 °C (669 $cm^2$ $g^{-1}$); this accession, together with Capsifort, also showed a higher SLA (317 $cm^2$ $g^{-1}$ in the average) compared to the other accessions (193 $cm^2$ $g^{-1}$ in the average) **(Figure 9a)**. Leaf mass ratio (LMR) didn't change passing from 25/15 °C to 15/5 °C in CP2, CP4 and CB9, whereas it increased in CB5 and decreased in Capsifort and CP3 **(Figure 9b)**. A significant reduction in leaf area ratio (LAR) was observed passing from 25/15 °C to 15/5 °C in all the genotypes; anyway, in absolute terms, Capsifort and CB5 showed the highest LAR at 25/15 °C (3.44 $cm^2$ $g^{-1}$) and CB5 also reached the highest value at 15/5 °C (1.96 $cm^2$ $g^{-1}$) **(Figure 9c)**.

***Experiment 2 - Grafting compatibility and grafted plants quality***

***Biometric measurements***
The biometric measurements of transplant at six true leaves (at least 1 cm long) are reported in **Table 5**. The stem diameter under grafting point was species-dependent, with higher values in Capsifort and *C. pubescens* used as rootstocks (3.2 mm in the average) compared to *C. baccatum* ones (2.8 mm in the average). The scion diameter one cm above grafting point was significantly larger in Capsifort (3.9 mm) compared to the other rootstocks (3.5 mm in the average). Capsifort also showed the maximum height of 12.3 cm, whereas the minimum was observed in CB5, even if not different from CP3 (7.65 cm in the average). Capsifort reached the highest leaf area (84 $cm^2$) and leaves fresh weight (2.92 g). However, leaves dry weight was the highest in CP3 and CB5 (0.41 g in the average) and Capsifort showed the lowest dry biomass accumulation in leaves (0.32 g). The only significant difference among stem dry weight was between CP2 and CB5 (0.159 vs 0.139 g respectively). Roots dry weight was higher in CP2 (0.28 g) compared to the other new rootstocks (0.23 g in the average).

***SPAD and stem xylem sap exudation***
SPAD was higher in CB9 compared to Capsifort, CP2 and CP4 (55.2 vs 52.0 in the average). The xylem sap exudation was significantly higher in Capsifort (0.08 g) compared to CP3, CB5 and CB9 (0.02 g in the average) **(Table 5)**.

***Correlations among variables***
Significant Pearson's correlations among variables are reported in **Table 6**. Stem diameter under grafting point was positively correlated with the stem diameter above grafting point (0.359 ***), height (0.275 ***), xylem sap exudation (0.328 ***), leaves fresh weight (0.299 ***), stem dry weight (0.273 ***) and roots dry weight (0.292 ***), and negatively correlated with SPAD (-0.211 **). Stem diameter above grafting point was positively correlated with height (0.479 ***), leaf area (0.524 ***), xylem sap exudation (0.273 ***), leaves fresh weight (0.577 ***) and roots dry weight (0.0.335 ***). Height was positively correlated with leaf area (0.763 ***), xylem sap exudation (0.363 ***), leaves fresh weight (0.776 ***) and stem dry weight (0.353 ***) and negative correlated with leaves dry weight (-0.426 ***). Number of leaves was positively correlated with leaf area (0.227 **), leaves fresh weight (0.275 ***), leaves dry

weight (0.275 ***), stem dry weight (0.435 ***) and roots dry weight (0.232 ***). Leaf area was positively correlated with xylem sap exudation (0.378 ***), leaves fresh weight (0.907 ***) and stem dry weight (0.321 ***) whereas xylem sap exudation was correlated in a positive way with leaves fresh weight (0.470 ***) and in a negative way with leaves dry weight (-0.256 **). Leaves fresh weight was positively correlated with stem dry weight (0.411 ***) and roots dry weight (0.356 ***). A positive correlation was also observed between leaves dry weight and stem dry weight (0.240 **) and between stem dry weight and roots dry weight (0.446 ***).

***Experiment 3 - C. annuum grafted onto high altitude accessions under chilling stress***

***Electrolyte leakage***

Electrolyte leakage was higher on day 0 in T/Capsifort (20.64 %), and lower in all the other grafting combinations and in un-grafted plants (15.77 % in the average). However, once subjected to chilling stress, T/Capsifort, T/CP3, T/CP4 and T/CB5 showed the same trend of ungrafted plants increasing up to 22.98 % and 26.02 % respectively on day 14 and 28. On the contrary, in T/CP2 and T/CB9, no significant increase was observed compared to day 0, with an average value of 16 % on day 14 and values of 16.8 % and 18.8 % respectively at the end of the experiment **(Figure 10)**.

***Chlorophyll fluorescence***

Chlorophyll fluorescence of '*Tiberio'* $F_1$ un-grafted and grafted onto Capsifort and onto the high-altitude accessions under study weekly recorded from day 0 to day 28 of chilling stress is reported in **Figure 11**. Grafted and un-grafted plants showed a different trend in Fv/Fm ratio; after a general decrease during the first week, on day 7 Fv/Fm decrease ceased in grafted plants (except for T/CP4). During the second half of the experiment Fv/Fm recovery was observed in grafted plants, with a maximum in T/CB9 on day 28 (0.80); on the other hand, in control plants Fv/Fm decreased until day 21 (0.76) and only a partial recovery occurred in the last week (0.77).

***Gas exchanges***

Net photosynthesis was not statistically different between grafting combinations on day 0, with an average of 8.78 µmol $CO_2$ $m^{-2}$ $s^{-2}$ and decreased in day 7 reaching an average of 6.09 µmol $CO_2$ $m^{-2}$ $s^{-2}$ for grafted plants, and 3.45 µmol $CO_2$ $m^{-2}$ $s^{-2}$ in control plants (**Figure 12**). Un-grafted plants photosynthesis stayed lower for almost all the experiment (3.8 and 5.7 µmol $CO_2$ $m^{-2}$ $s^{-2}$ on day 14 and 21 respectively). Grafted plants recovered their photosynthetic ability from day 14. It continued to increase until day 21 in T/CP3 (10.7 µmol $CO_2$ $m^{-2}$ $s^{-2}$) and didn't change until the end of the experiment. Net photosynthesis was 9.5 µmol $CO_2$ $m^{-2}$ $s^{-2}$ in T/CB9 and 7.3 µmol $CO_2$ $m^{-2}$ $s^{-2}$ in the average of the remaining grafting combinations in day 28.

Stomatal conductance (gs) strongly decreased in the initial week of exposure to low temperatures, reaching 0.12 mol $H_2O$ $m^{-2}$ $s^{-1}$ in the average of T/Capsifort, T/CP3, T/CB5, T/CB9, and 0.07 mol $H_2O$ $m^{-2}$ $s^{-1}$ in T, T/CP2 and T/CP4 on day 7. '*Tiberio'* $F_1$ grafted onto CP3 showed an overall higher gs maintenance with a maximum of 0.20 mol $H_2O$ $m^{-2}$ $s^{-1}$ and a final value of 0.12 mol $H_2O$ $m^{-2}$ $s^{-1}$ together with T/CP4, whereas all the other grafting combinations, together with un-grafted plants had 0.06 mol $H_2O$ $m^{-2}$ $s^{-1}$ on the same day.

$CO_2$ internal concentration showed the same trend both in un-grafted and in grafted plants during the first two weeks of the experiment, whereas, at day 21 and 28 it changed in a genotype dependent way, with values higher then Capsifort in CP3 and CP4, but lower in T, T/CB5 and T/CB9.

***Biometric measurements***

Plant height, number of leaves, leaf area, the biomass of the different plant organs and their ratios showed highly significant differences and are reported in **Table 7**. As compared to T/Capsifort, no differences were found in the plants grafted onto the new rootstocks in terms of number of leaves and leaf area, except for T/CP2, that showed the same leaves number but a lower leaf area (-36.2 %). Low temperatures strongly affected the development of un-grafted plants, which showed a lower height and a generally reduced biomass accumulation compared to grafted plants. Plants grafted onto CP3 and CB9 showed a higher biomass production compared to the commercial rootstock (+25 %); that was related to a higher development of the root system (+50 %) and also to a higher biomass accumulation in the stem (+50 %) in T/CB9. Leaves dry weight, in fact, was not different from T/Capsifort in plants grafted onto the new *C. pubescens* and *C. baccatum* rootstocks. Among the plants grafted onto these high altitude accessions, T/CP3, T/CP4 and T/CB9 showed higher roots:shoot ratio (28.9 % in the average) than in T/CP2 and T/CB5 (21 % in the average). SLA was significantly higher in T/CP4 and T/CB5 than in un-grafted Tiberio and T/Capsifort, whereas LMR was higher in T/Capsifort (55 %) compared to T/CP3 and T/CB9 (46 % in the average), although no significant differences were found with ungrafted plants for this variable. T/CB5 was the only grafting combination that showed a higher LAR compared to Capsifort (+21.8 %).

***Experiment 4 – Yield and fruit quality of C. annuum cv 'Tiberio' $F_1$ grafted onto high altitude accessions of Capsicum pubescens and Capsicum baccatum and onto Capsifort***

***Gas exchanges***

Gas exchanges of '*Tiberio'* $F_1$ un-grafted, self-grafted and grafted onto Capsifort and onto the new rootstocks under study were collected at the end of the experiment and are reported in **Table 8**. Among control plants, self-grafted (T/T) and un-grafted (T) *Tiberio'* $F_1$ showed no difference regarding net photosynthesis, stomatal conductance and internal $CO_2$ concentration. On the other hand, grafting onto Capsifort as well onto the above mentioned *C. baccatum* rootstocks significantly increased net photosynthesis compared to un-grafted and self-grafted plants (+16.6 % in the average).

The stomatal conductance of T/CP2 was significantly lower than all other treatments except T/CP4, and the internal $CO_2$ concentration was higher in T/CP3, T/CB5 and T/CB9 compared to control (+19 %) and didn't differ compared to Capsifort.

***Yield***

Yield and its components were affected by grafting combination and are reported in **Table 9**. Like for gas exchanges analysis, self-grafted (T/T) and un-grafted (T) '*Tiberio'* $F_1$ showed no differences, so their average value for each variable will be used for the following comparison. Marketable yield was higher in T/Capsifort, T/CP3, T/CB5 and T/CB9 compared to un-grafted and self-grafted plants (+47.4 %). This was mainly related to the higher fruit weight of T/Capsifort, T/CP3 and T/CB9 (+16.1 %). On the other hand, the number of marketable fruits per plant was reduced by grafting onto *C. pubescens* accessions CP2 and CP4 compared to T/Capsifort (-37.5 %). No differences were observed neither in the number nor in the total fresh weight of un-marketable fruits per plant, with an average value of 2.53 fruits plant$^{-1}$ and 214.0 g plant$^{-1}$ respectively. Due to the intrinsic variability of un-marketable fruits size, their average weight was only significantly different between T/Capsifort and T/CB5 compared to T/CP2 (99.45 g fruit$^{-1}$ vs 65.8 g fruit$^{-1}$). The total fresh weight of fruits was higher in T/Capsifort, T/CP3, T/CB/5 and T/CB9 compared to control (+47.3 %), even if total fruits dry weight was not different between T/CP3 and T.

***Fruits quality***

No significant differences were observed in TSS, with an average value of 5.45 % between grafting combinations **(Table 10)**. Titratable acidity was only different between T and T/CP2 with a value of 1.58 and 1.22 mg/L of citric acid respectively. Fruits of '*Tiberio'* $F_1$ grafted onto *C. baccatum* accessions showed a higher antioxidant activity (536.5 µmol Trolox $kg^{-1}$ FW in the average) than T/T, T/Capsifort and T/CP4 (416.3 µmol Trolox $kg^{-1}$ FW in the average).

## Discussion

***Experiment 1***

At 15/5 °C other authors reported a tremendous reduction in photosynthetic rate compared to 35 °C in *C. annuum*, *C. baccatum* and *C. pubescens* of unknown origin, down to 2.3 – 4.7 µmol $CO_2$ $m^{-2}$ $s^{-2}$; however they referred to plants grown under 12 h/12 h photoperiod at steady day/night temperatures, so the exposition to 5 °C was not limited to the late night and dawn period but lasted 12 h/day (Ou et al., 2015). Although this approach seemed justified for a similar physiological research, it wouldn't be useful for the aim of our study. In the chilling stress treatment, in fact, we recreated the environmental condition of an unheated greenhouse during winter months in Mediterranean regions, in which plants use to undergo sub-optimal temperatures mainly during the last hours of the night and during dawn.

Although a rapid and severe PSII chronic damage is common in warm-climate species when sub-optimal temperatures occur under light conditions, it is not usually observed immediately when the exposure to low temperatures take place on the night (Allen and Ort, 2001). In our experiment, after one week of exposition to severe chilling stress, all the accessions except CP 3 showed a significant reduction on Fv/Fm ratio. The optimum value for this index of photosystem II efficiency is, in fact, around 0.80 (Kalaji et al., 2017). In Capsifort the decrease in this ratio was the more pronounced. On the other hand, Capsifort, as well as the *C. baccatum* accession CB9, showed no significant reduction in biomass accumulation both in the roots and in the aerial part passing from 25/15 °C to 15/5 °C treatment; however, this result should be considered bearing in mind that at 25/15 °C their biomass production was significantly lower compared to the other genotypes.

Focusing on *C. baccatum* biomass production under low-temperatures, no difference with Capsifort was showed by CB5, and in CB9 differences resulted to be significant only for leaves dry weight. However, the results obtained both from gas exchanges and the stress-related physiological variables give a deeper understanding of their actual low-temperature tolerance. Unlike Capsifort, both that accessions were able to contain membrane leakage levels in leaves, but the Fv/Fm ratio recovery during the experiment was incomplete in CB5 and, in the last week, it was significantly lower compared to CB9. Moreover, although net photosynthesis kinetics trend was similar in CB5 and CB9, with a decrease in the first week of exposure to chilling stress and a gradual recovery that lead to a comparable value at the end, during almost all the experiment (at least from the day 7 to the day 21) net photosynthesis was significantly higher in CB9 (up to +40 % on day 14).

Even if Capsifort and *C. baccatum* accessions showed a lower stomatal conductance compared to *C. pubescens* ones, the CB5 lower photosynthetic rate at the end of the experiment seems unlikely to have stomatal conditions origin since it was not associated with a reduced $C_i$, so other mechanisms were probably involved. On the contrary, the reduced gs seems the most probable cause of the first-week inhibition of A almost independently from the genotype and, considering the significantly lower $C_i$ levels compared to the high altitude accessions under study, likely lasted in Capsifort also in the second half of the experiment. For that reason, it seems reasonable

to conclude that, as a general rule, also accessions that showed an overall higher tolerance to the set of temperature used underwent stressful conditions at the beginning of the exposure to chilling stress. It took some days, but the restoring of a new physiological equilibrium for these accessions was more effective than in Capsifort. Many factors, like detoxification of reactive oxygen species, damage control and repair, restructure of the plasma membrane and acceleration in osmolytes synthesis were probably involved in the effectiveness of their long-term response (Yadav, 2010).
Anyway, although physiological results seemed to indicate CB9, among the two *C. baccatum* accessions under study, as more tolerant to chilling stress compared to Capsifort, CB9 showed only an higher leaves biomass production.
Among the *C. pubescens* accessions, CP2 and even more CP3 appaired to be the more tolerant to low-temperature stress, since their electrolyte leakage was lower compared to Capsifort one and their Fv/Fm ratio seemed basically unaffected by the thermal regime adopted. Additionally, as opposed to *C. baccatum* accessions, their high net photosynthesis, as well as the maintaining of a good level of stomatal conductance, allowed a significantly higher biomass production (total dry weight) under chilling stress compared to Capsifort. CP2 and CP3 produced also higher roots biomass under stress conditions, suggesting that roots growth and activity were maintained and that they supported the aerial part through a reorganization of sink-source equilibrium and a significant increase in roots to shoot ratio.

***Experiment 2***
Surprisingly, the grafting compatibility with the *C. annuum* cv 'Tiberio' $F_1$ was really high for all the high altitude accessions used as rootstock, considering that no significant differences were observed in the grafting success percentage with the commercial rootstock 'Capsifort' $F_1$ (data not shown). In this experiment, the higher roots dry weight of T/CP2 compared to the other accessions tested could simply be related with a different roots growth during the pre-grafting period, anyway, Capsifort showed an intermediate roots dry weight with no differences compared to all the other genotypes.
An overall analysis of the one way ANOVA suggests that in T/Capsifort the variables related with water uptake and cellular expansion indicate an optimal water status inside of the plant, that could have contributed to the higher leaf lamina expansion and stem elongation compared to the other grafting combinations. The positive correlation of stem elongation (height), leaf area and leaves FW with xylem sap exudation indicates that limitation in hydraulic conductance could be involved.
Leaves initiation and development, follows a flexible program, which is actively adjusted even inside the same variety on the basis of the growth stage and the environmental circumstances (Bar and Ori, 2014). The fact that no differences in the number of leaves were observed indicates that the leaf initiation seemed not to be compromised by grafting onto the high-altitude accessions tested. Moreover, leaves dry weight was significantly higher in the plants grafted almost onto every high-altitude accession than in T/Capsifort.
SPAD measurements are extensively used in agricultural applications since they provide values proportional to the chlorophyll content of the leaves (Ling et al., 2011); in the present experiment plants grafted onto the *C. baccatum* accession CB9 showed significantly higher SPAD values.
The results obtained demonstrated that the high-altitude accessions of *C. pubescens* and *C. baccatum* tested can be used as rootstocks for bell pepper, since a high grafting compatibility with *C. annuum* 'Tiberio' $F_1$ was obtained, together with a good overall transplants quality. Neither leaves initiation nor stem and roots dry weight were affected, whereas leaves dry weight accumulation and, sometimes, chlorophyll content was also improved. Anyway, the concern about their effectiveness as rootstocks was related to the reduced xylem sap exudation, leaf

expansion and stem elongation, compared to *'Capsifort'* $F_1$. Anyway, it was not possible to predict if that would have last or actually become a serious issue without a long-term grafting experiment.

***Experiment 3***

As it was predictable, the clear separation of membrane leakage levels among almost all the genotypes under study that was obtained during the experiment 1 was not confirmed for pepper grafted onto that genotypes under similar stress conditions. In this case two main groups emerged; in the first group, including T/Capsifort, T/CP3,T/CP4 and T/CB5 electrolyte leakage reflected the same trend of un-grafted 'Tiberio' $F_1$, whereas in the other one, including T/CP2 and T/CB9 it was completely unaffected suggesting that a rootstock mediated enhancement of membrane protections mechanisms could have occurred, probably via osmolytes accumulation. The accumulation of osmolytes, in fact, have been demonstrated to contribute to stabilizing enzymes, membranes and other cellular components. Under chilling stress, they also play an important role in the retailoring of membrane lipid composition to optimize the liquid/crystalline structure necessary for proper membranes functioning (Yadav, 2010). Regarding the set of temperature used, both electrolyte leakage and chlorophyll fluorescence results highlighted the lower tolerance of un-grafted 'Tiberio' $F_1$ compared to grafted plants. That is in agreement with the effective role of tolerant rootstocks in alleviating scion stress conditions under sub-optimal temperature previously reported (Schwarz et al., 2010). In this process, a reduction of lipid peroxidation induced by impairment in ROS production and scavenging equilibrium under stressful condition is essential, and rootstocks that are more tolerant to low temperatures are often reported to promote regulation in antioxidant enzyme production and activity in the scion, finally resulting in better growth performance (Li et al., 2015).

Usually, total leaf area is thought to play a major role in plant growth and productivity compared to instantaneous photosynthetic rate since large differences in the photosynthetic area available for the plant have a stronger impact on the actual biomass production potential. On the other hand, the photosynthesis inhibition caused by chilling stress can have a crucial effect when it takes place early in the growing season and it becomes more detrimental as the low-temperature exposure lasts in time (Allen and Ort, 2001).

In our case, when CP2 was used as rootstock, exactly the same trend of T/CB9 was observed, both for net photosynthetic rate maintenance and membrane leakage inhibition; however, in T/CP2 biomass production (total DW) was significantly lower, and it seems clearly related to the fact that CP2 was not able to limit the chilling-induced reduction of leaf area compared to un-grafted plants, as confirmed by their lowest SLA.

No differences in leaves biomass were observed between T/Capsifort, T/CP2, T/CP3 and T/CB9, but only T/CP3 and T/CB9 showed the highest total dry weight accumulation and it was related to their higher roots biomass. At least in T/Capsifort, this could be explained by significant sink-source differences with 'Tiberio' $F_1$ grafted onto CP3 and onto CB9, as demonstrated by its higher LMR value. For T/CP2, however, LMR was not statistically different from T/CP3 and T/CB9; moreover, roots dry weight was in contrast with the highest value recorded in un-grafted CP2 during experiment 1 (at 15/5 °C), and it suggests that other mechanism, like scion to rootstock hormonal communications, could be involved.

An overall analysis of the results obtained in the present experiment identifies CP3 and CB9 as the rootstocks that better promoted the vegetative growth, with a higher maintenance of photosynthetic rate, leaves initiation, leaf area expansion and roots and total biomass production.

The larger root system that they developed, could alleviate stress conditions under winter months in a unheated greenhouse environment. Usually, at a certain absolute humidity a reduction of the air temperature will decrease evaporative demand from leaves, however, the soil specific

capacity is much higher than the air one; for this reason, it is common that air and leaves warms faster than soil in the morning, especially in unheated protected environments. Under these conditions, the increase in evaporative demand will not always be supported by roots water uptake and transport since soil temperature severely affects root hydraulic conductance in a genotype-specific way, in this context, the maintaining of roots growth and activity, possibly accompanied by an increase in roots to shoot ratio can be determinant (Venema et al., 2005).

***Experiment 4***

Only little information are available about the yield of pepper grafted onto *C. baccatum* and *C. pubescens* (Palada and Wu, 2008; Penella et al., 2013; Saporta and Gisbert, 2013), although not using accessions from high altitudes; moreover some of that researches refer to *C. baccatum* var. *pendulum* and not to *C. baccatum* var. *baccatum* like in our case, or do not provide the complete name at all.

Among the *C. pubescens* rootstocks under study, the only one that showed a marketable yield comparable with the one of the commercial rootstock '*Capsifort'* $F_1$ was CP3, that was among the two *C. pubescens* accessions that better performed under chilling stress during the experiment 1, as un-grafted plant, and the only one that improved scion performances under the same conditions during the experiment 2, even more than '*Capsifort'* $F_1$.

Both the *C. baccatum* rootstocks gave yield results similar to Capsifort grafted plants, even if CB5 fruit weight was lower than Capsifort. Anyway, among *C. baccatum* rootstocks, the only one that enhanced grafted plant tolerance to low temperature was CB9.

No data are available about fruit quality of pepper grafted onto *C. baccatum* and *C. pubescens* concerning TSS, titratable acidity and antioxidant activity. In the present experiment, we found that fruit quality was almost completely unaffected by the grafting combination for total soluble content and titratable acidity. Anyway, the aim of the study was about test if the yield and quality of fruits would be compromised or not by grafting sweet pepper onto the high-altitude accessions under study like previously reported, for example, in tomato grafted onto a high-altitude accession of *Solanum habrochaites* (LA 1777) (Ntatsi et al., 2014). The most interesting result concerning fruit quality was the higher antioxidant activity in the fruits of plants grafted onto *C. baccatum*, especially CB9. In recent years, in fact, an increasing interest in the relationships between grafting combination and fruit quality and nutraceutical properties occurred (Davis et al., 2008; Flores et al., 2010; Krumbein and Schwarz, 2013; López-Marín et al., 2017).

## Conclusions

No one of the *C. pubescens* and *C. baccatum* accessions used as rootstocks showed long-term grafting incompatibility with the *C. annuum* scion both in vegetative growth under chilling stress and in greenhouse cultivation under no termal stressing conditions. In the accessions that showed a higher low-temperature tolerance, it was not automatically transferred to scion once grafted. The *C. baccatum* CB9 and the *C. pubescens* CP3 were able to alleviate chilling stress in '*Tiberio'* $F_1$ better than the commercial rootstock '*Capsifort'* $F_1$ when used as rootstocks and showed not to affect yield levels and fruit quality characteristics comparing to '*Capsifort'* $F_1$.

**Table 1** - *Capsicum spp*. accessions used in the preliminary seeds germination test; accessions individuated for the present study are reported in bold (where indicated, information about the site of collection and its elevation were provided from the United States Department of Agriculture – USDA, through U.S. National Plant Germplasm System).

| **Code** | **Name** | **Species** | **Origin** | **Elevation** |
|---|---|---|---|---|
| CP CC 1 | Rocopica | *C. pubescens* × *C. cardenasii* | | |
| **CP 2** | **PI585267** | ***C. pubescens*** | **Ecuador, Azuay** | **2372 m a.s.l.** |
| **CP 3** | **PI585273** | ***C. pubescens*** | **Ecuador, Carchi** | **2740 m a.s.l.** |
| **CP 4** | **PI355812** | ***C. pubescens*** | **Ecuador, Azuay, San Fernando** | **2800 m a.s.l.** |
| **CB 5** | **PI238061** | ***C. baccatum* var. *baccatum*** | **Bolivia, Cochabamba** | **2560 m a.s.l.** |
| CB 6 | PI441659 | *C. baccatum* var. *baccatum* | Brasil, Bahia, Vitoria de Conquista | 900 m a.s.l. |
| CB 7 | CAP 215 | *C. baccatum* var. *baccatum* | | |
| CB 8 | Pimenta do passarinho | *C. baccatum* var. *baccatum* | | |
| **CB 9** | **Bacclaudio** | ***C. baccatum* var. *baccatum*** | | |
| CB 10 | PI596056 | *C. baccatum* var. *pendulum* | Bolivia, Chuquisaca | 1900 m a.s.l. |
| CB 11 | PI596057 | *C. baccatum* var. *pendulum* | Bolivia, Chuquisaca | 1870 m a.s.l. |
| CB 12 | PI596058 | *C. baccatum* var. *pendulum* | Bolivia, Chuquisaca | 2150 m a.s.l. |

## ***Experiment 1 – Tables and figures***

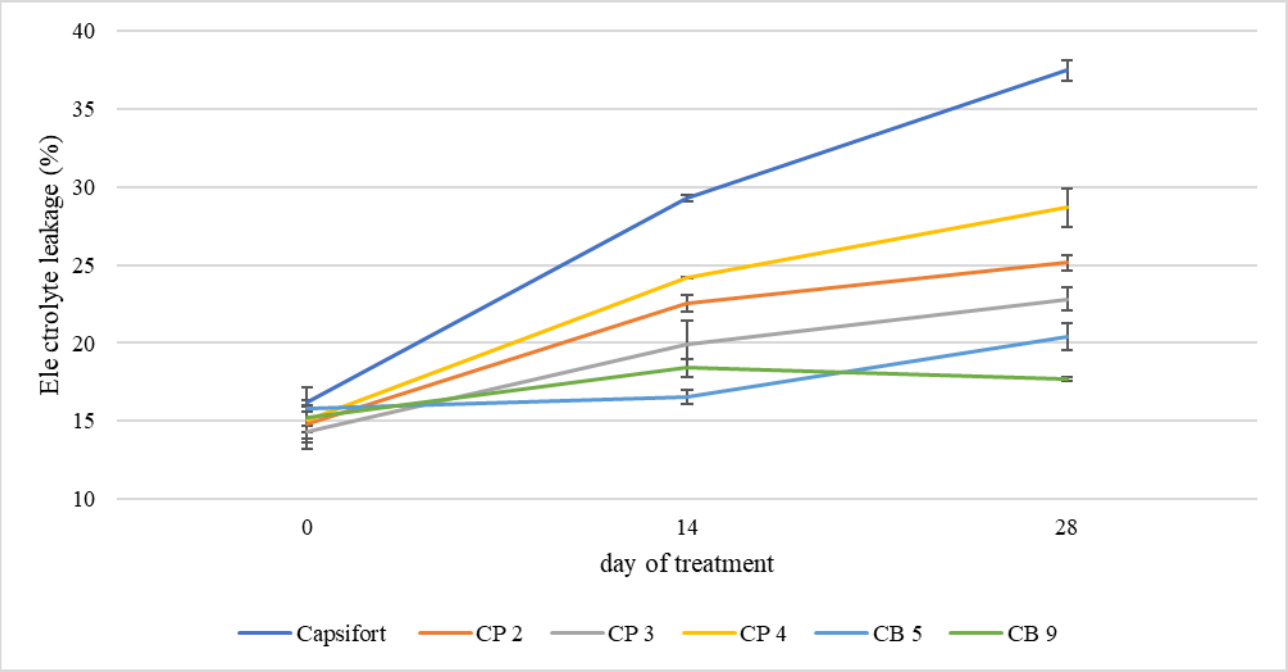



**Figure 1** - Electrolyte leakage on day 0, 14 and 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

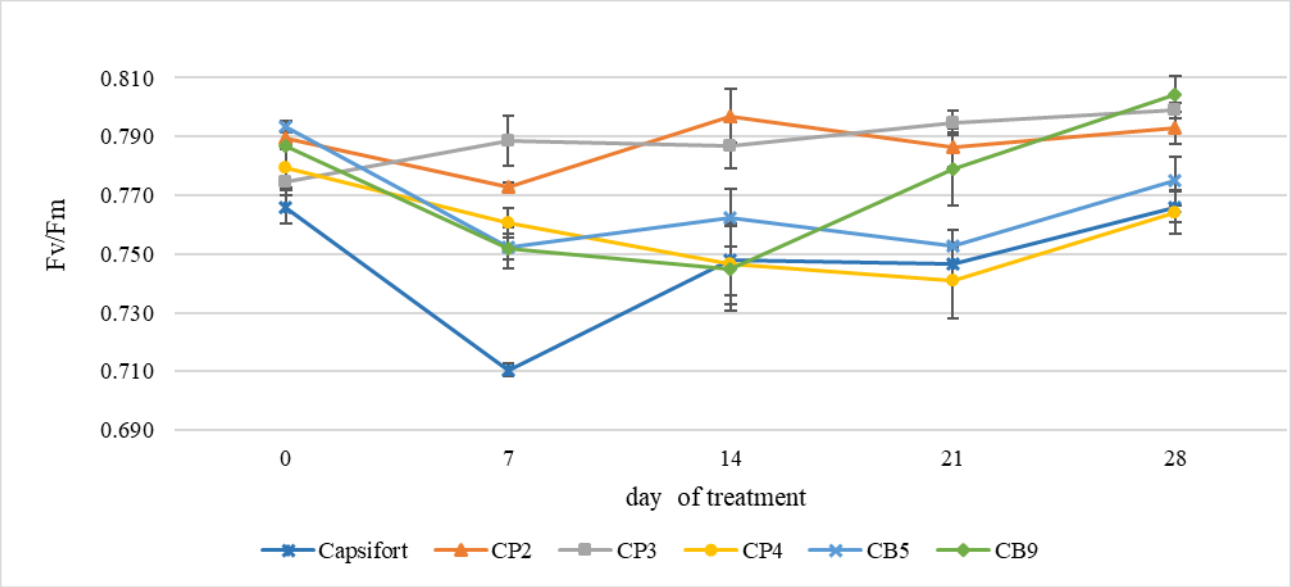



**Figure 2** - Fv/Fm values weekly recorded from day 0 to day 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

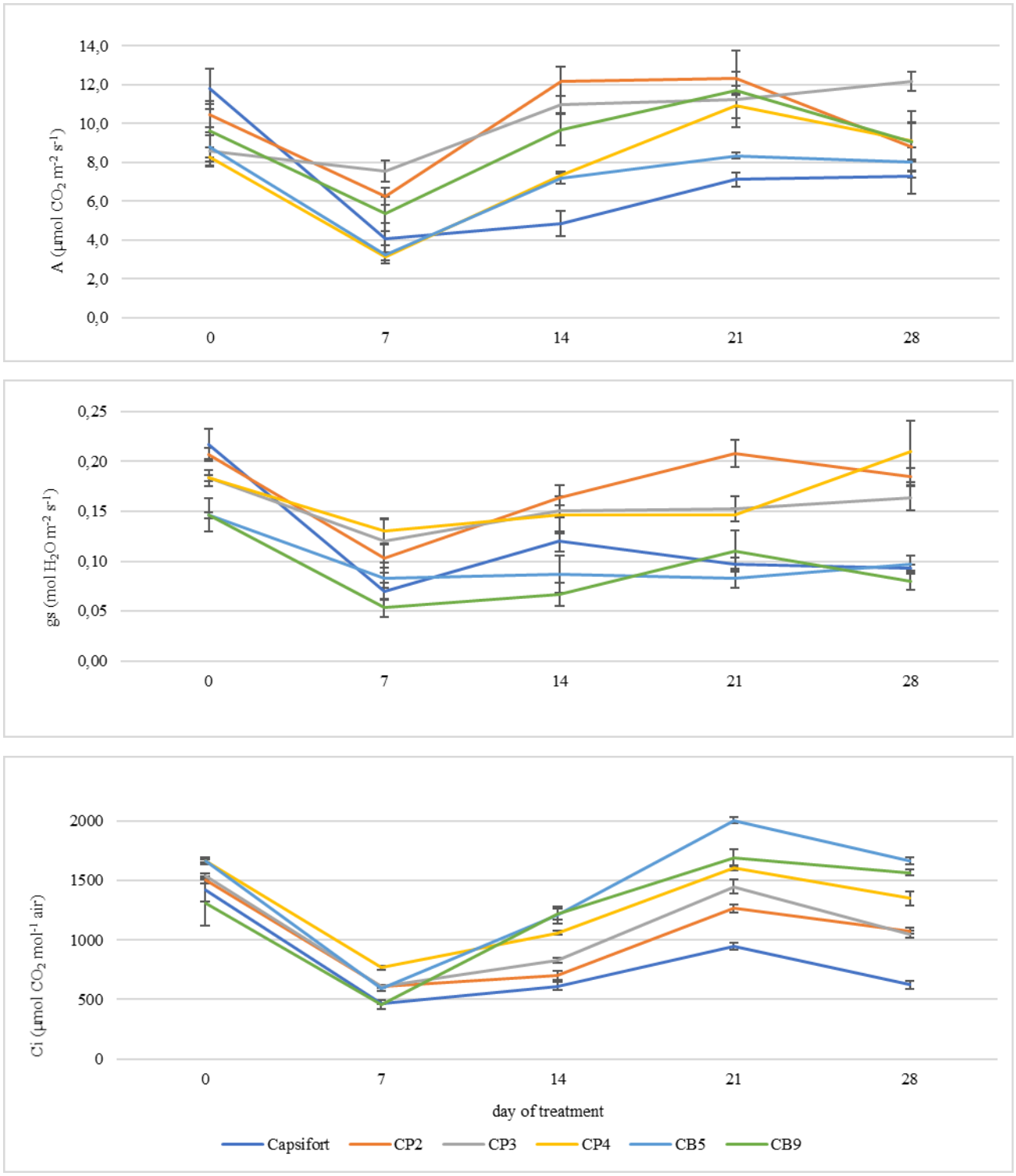



**Figure 3** - Net photosynthesis (A) (µmol $CO_2$ $m^{-2}$ $s^{-2}$), stomatal conductance (gs) (mol $H_2O$ $m^{-2}$ $s^{-1}$) and internal $CO_2$ concentration (Ci) (µmol $CO_2$ $mol^{-1}$ air) weekly recorded from day 0 to day 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

**Table 2** - Stem diameter, height, number of leaves, leaf area and specific leaf area at the end of the experiment.

| | ***Stem diameter*** | ***Height*** | ***Number of leaves*** | ***Leaf area*** | ***Stem diameter increase*** | ***Height increase*** | ***Number of leaves increase*** | ***Leaf area increase*** |
|---|---|---|---|---|---|---|---|---|
| | (mm) | (cm) | | ($cm^2$) | (%) | (%) | (%) | (%) |
| ***Genotype*** | | | | | | | | |
| *Capsifort* | 5.5 b | 30.8 d | 36.1 cd | 779 d | 61 d | 112 d | 55.1 a | 249 b |
| *CP 2* | 6.3 a | 45.9 b | 66.3 a | 1523 ab | 156 b | 247 ab | 51.9 a | 216 c |
| *CP 3* | 5.9 ab | 48.9 a | 53.8 ab | 1296 bc | 126 c | 213 b | 46.5 b | 186 d |
| *CP 4* | 5.5 b | 44.4 b | 46.3 bc | 1203 c | 127 c | 219 b | 54.2 a | 214 c |
| *CB 5* | 5.9 ab | 44.5 b | 65.7 a | 1767 a | 144 bc | 266 a | 55.3 a | 271 a |
| *CB 9* | 6.1 a | 40.9 c | 31.6 d | 1086 c | 177 a | 170 c | 54.3 a | 225 c |
| ***Temperature*** | | | | | | | | |
| *25/15 °C* | 6.8 a | 59.9 a | 82.0 a | 2138 a | 191 a | 295 a | 53.3 | 301 a |
| *15/5 °C* | 4.9 b | 25.2 b | 18.0 b | 413 b | 72 b | 114 b | 52.5 | 152 b |
| Significance | | | | | | | | |
| *Genotype (G)* | *** | *** | *** | *** | *** | *** | *** | *** |
| *Temperature (T)* | *** | *** | *** | *** | *** | *** | *** | *** |
| *G × T* | *** | *** | *** | *** | *** | *** | *** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

**Table 3** - Leaves, stem, roots and total dry weight and ratio between roots and shoot dry weight at the end of the experiment.

| | ***Leaves dry weight*** | ***Stem dry weight*** | ***Roots dry weight*** | ***Total dry weight*** | ***Root : shoot ratio*** |
|---|---|---|---|---|---|
| | (g plant$^{-1}$) | (g plant$^{-1}$) | (g plant$^{-1}$) | (g plant$^{-1}$) | (%) |
| ***Genotype*** | | | | | |
| *Capsifort* | 1,6 c | 0,7 c | 0,5 c | 2,9 c | 25,6 bc |
| *CP 2* | 3,6 a | 1,9 ab | 1,4 a | 7,0 a | 29,3 ab |
| *CP 3* | 3,2 ab | 2,1 a | 1,5 a | 6,7 a | 31,6 a |
| *CP 4* | 3,0 ab | 1,7 ab | 0,9 b | 5,7 ab | 20,6 cd |
| *CB 5* | 3,0 ab | 1,8 ab | 0,9 b | 5,6 ab | 21,0 cd |
| *CB 9* | 2,8 b | 1,5 b | 0,8 bc | 5,1 b | 19,4 d |
| ***Temperature*** | | | | | |
| *25/15 °C* | 3.8 a | 2.3 a | 1.2 a | 7.3 a | 19.0 b |
| *15/5 °C* | 1.9 b | 0.9 b | 0.9 b | 3.7 b | 30.1 a |
| Significance | | | | | |
| *Genotype (G)* | *** | *** | *** | *** | *** |
| *Temperature (T)* | *** | *** | *** | *** | *** |
| *G × T* | *** | *** | *** | *** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

**Table 4** - Specific leaf area (SLA), leaf mass ratio (LMR) and leaf area ratio (LAR) at the end of the experiment.

| | ***Specific leaf area (SLA)*** | ***Leaf mass ratio (LMR)*** | ***Leaf area ratio (LAR)*** |
|---|---|---|---|
| | ($cm^2\ g^{-1}$) | (%) | ($cm^2\ g^{-1}$) |
| ***Genotype*** | | | |
| *Capsifort* | 442 b | 55.1 a | 249 a |
| *CP 2* | 372 c | 51.9 a | 192 b |
| *CP 3* | 364 c | 46.5 b | 177 b |
| *CP 4* | 352 c | 54.2 a | 187 b |
| *CB 5* | 500 a | 55.3 a | 271 a |
| *CB 9* | 368 c | 54.3 a | 200 b |
| ***Temperature*** | | | |
| *25/15 °C* | 565 a | 53.3 | 301 a |
| *15/5 °C* | 235 b | 52.5 | 124 b |
| Significance | | | |
| *Genotype (G)* | *** | *** | *** |
| *Temperature (T)* | *** | *ns* | *** |
| *G × T* | *** | *** | ** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

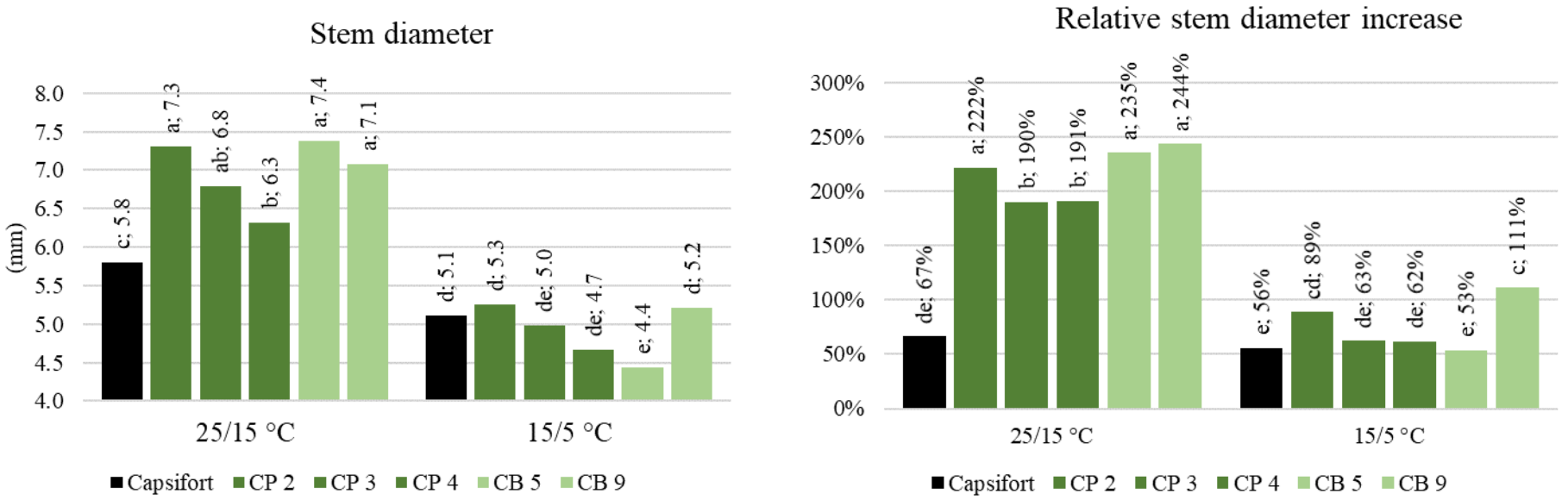



**Figure 4** - Stem diameter and its relative increase during the experiment as affected by Genotype and Temperature. For each graph column that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test ($P<0.05$).

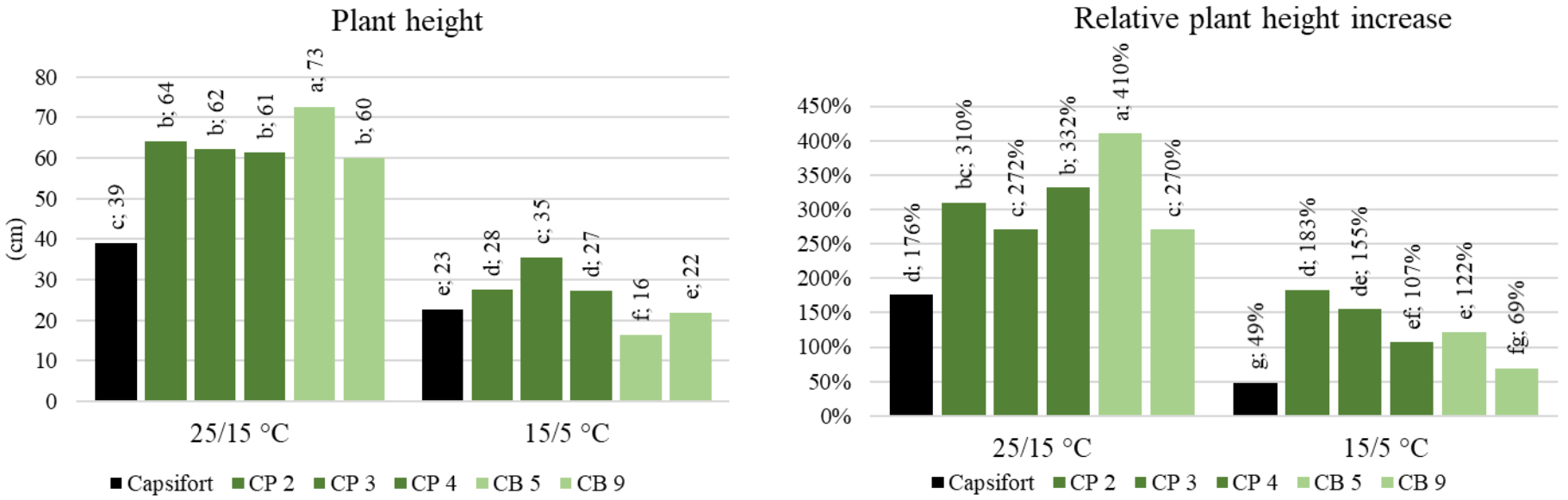



**Figure 5** - Plant height and its relative increase during the experiment as affected by Genotype and Temperature. For each graph column that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test ($P<0.05$).

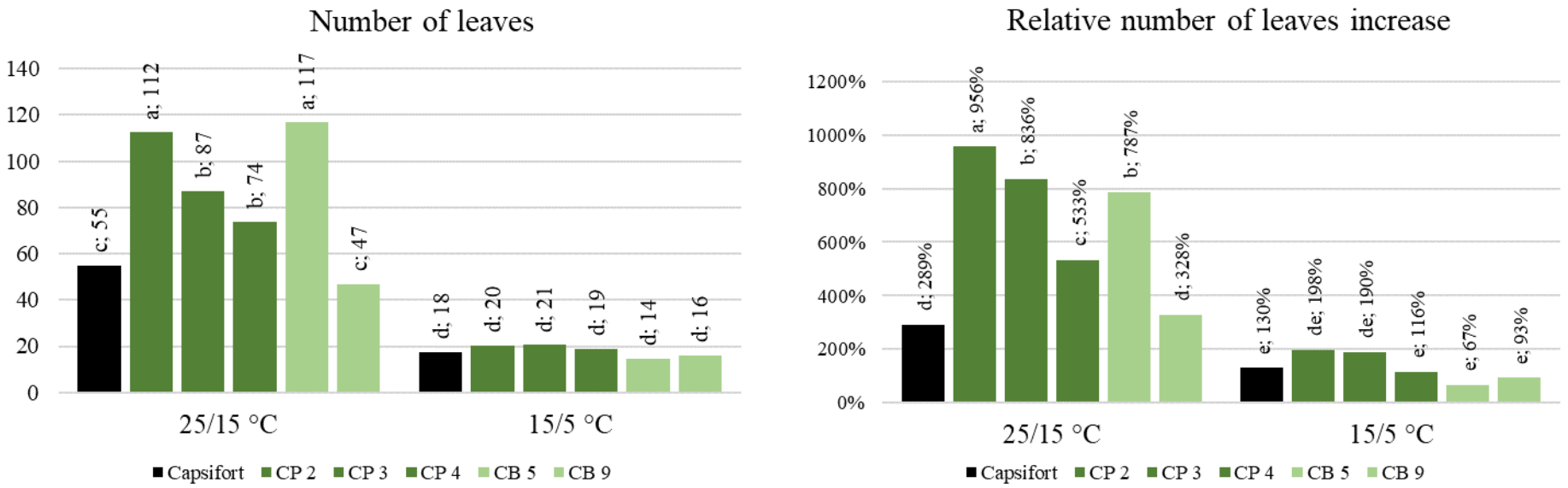



**Figure 6** - Number of leaves and its relative increase during the experiment as affected by Genotype and Temperature. For each graph columns that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test ($P<0.05$).

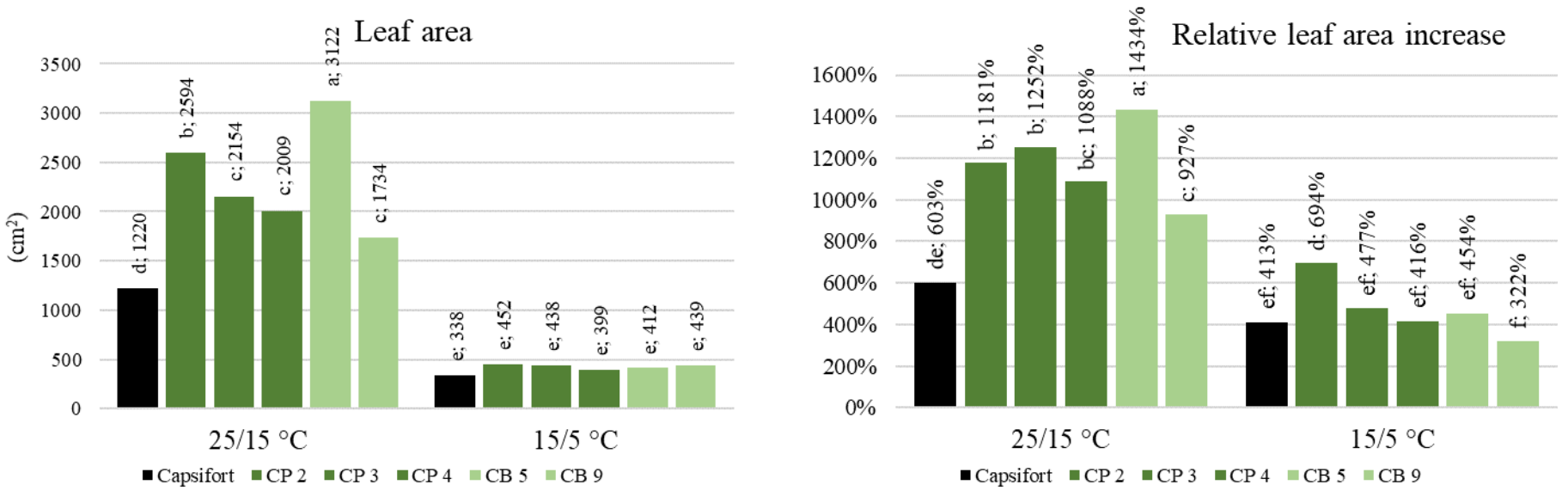



**Figure 7** - Leaf area and its relative increase during the experiment as affected by Genotype and Temperature. For each graph column that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test ($P<0.05$).

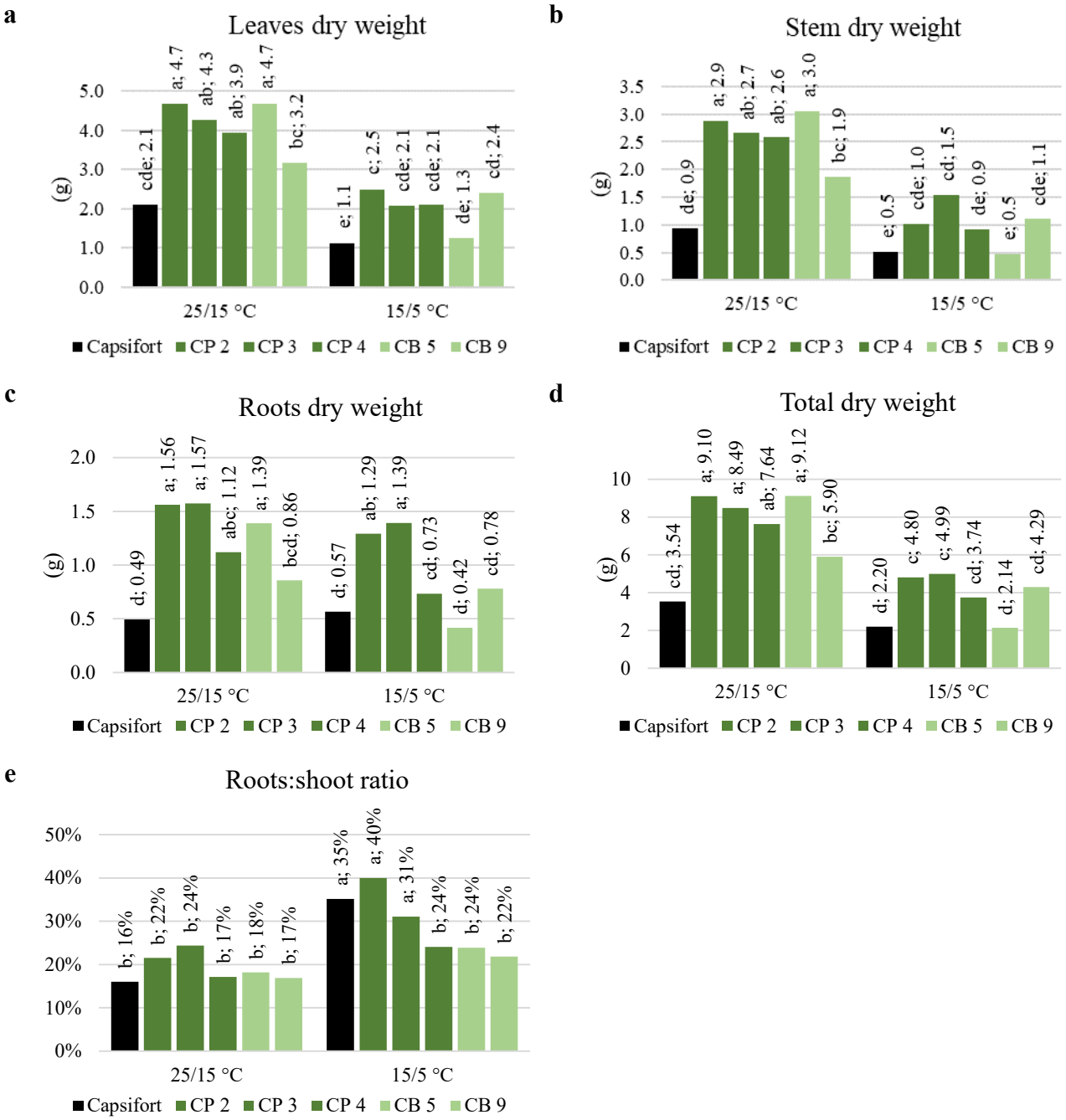
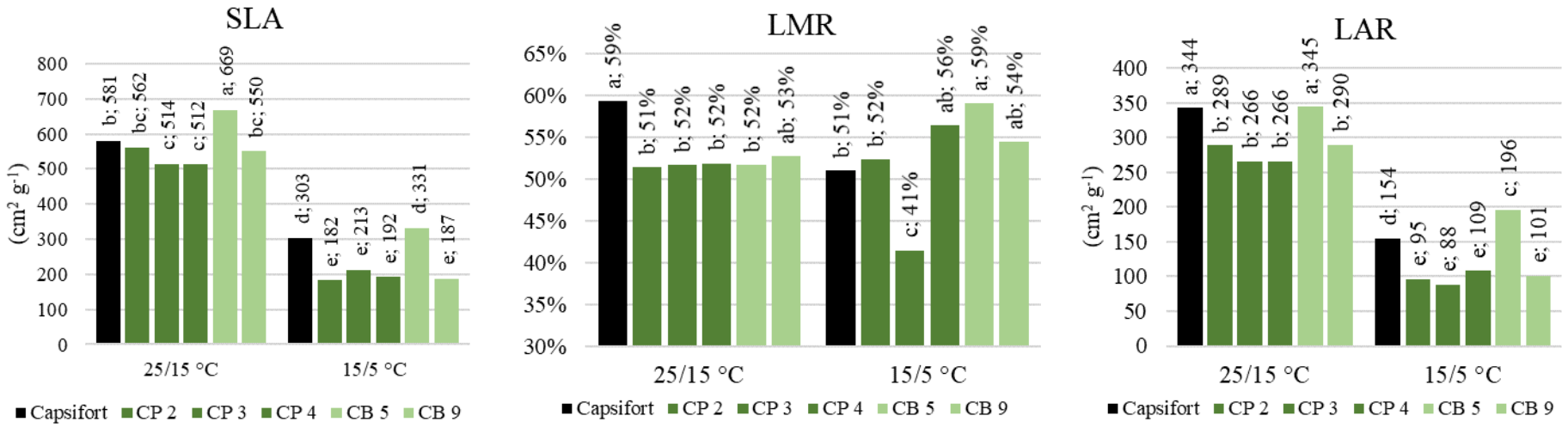



**Figure 8** – Leaves, stem, roots and total dry biomass and roots to shoot ratio, as affected by Genotype and Temperature. For each graph column that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P = 0.05).




**a** **b** **c**

**Figure 9** – Specific leaf area (SLA), leaf mass ratio (LMR) and leaf area ratio (LAR) as affected by Genotype and Temperature. For each graph column that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P = 0.05).

## ***Experiment 2 – Tables***

**Table 5** - Biometric characteristics, SPAD and xylem sap exudation of transplant of '*Tiberio'* $F_1$ grafted onto '*Capsifort'* $F_1$ and onto the tested high-altitude accessions.

| | ***ø 1 cm under grafting point*** | ***ø 1 cm above grafting point*** | ***Height*** | ***Number of leaves*** | ***Leaf area*** | ***SPAD*** | ***xylem sap exudation*** | ***Leaves FW*** | ***Leaves DW*** | ***Stem DW*** | ***Roots DW*** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **(mm)** | **(mm)** | **(cm)** | | **($cm^2$)** | | **(g)** | **(g)** | **(g)** | **(g)** | **(g)** |
| *Grafting combination* | | | | | | | | | | | |
| ***T/Capsifort*** | 3.2 a | 3.9 a | 12.3 a | 7.7 | 84.0 a | 52.2 b | 0.08 a | 2.92 a | 0.32 c | 0.151 ab | 0.25 ab |
| ***T/CP2*** | 3.3 a | 3.5 b | 8.3 c | 7.6 | 56.2 c | 52.2 b | 0.05 ab | 2.10 b | 0.36 b | 0.159 a | 0.28 a |
| ***T/CP3*** | 3.2 a | 3.4 b | 8.0 cd | 7.3 | 56.6 c | 53.5 ab | 0.02 b | 2.06 b | 0.42 a | 0.143 ab | 0.23 b |
| ***T/CP4*** | 3.2 a | 3.6 b | 8.5 c | 7.9 | 65.1 b | 51.7 b | 0.05 ab | 2.18 b | 0.36 b | 0.157 ab | 0.23 b |
| ***T/CB5*** | 2.7 b | 3.4 b | 7.3 d | 7.9 | 62.6 bc | 53.1 ab | 0.02 b | 2.03 b | 0.40 a | 0.139 b | 0.23 b |
| ***T/CB9*** | 2.9 b | 3.5 b | 9.9 b | 8.0 | 66.0 b | 55.2 a | 0.03 b | 2.16 b | 0.34 bc | 0.155 ab | 0.23 b |
| | | | | | | | | | | | |
| *Significance* | *** | *** | *** | ns | *** | *** | *** | *** | *** | ** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

**Table 6** – Significant correlations matrix reporting the Pearson's correlation coefficients among variables and their significance (ns: not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively).

| | **Ø 1 cm under grafting point** | **Ø 1 cm above grafting point** | **Height** | **Number of leaves** | **Leaf area** | **SPAD** | **Xylem sap exudation** | **Leaves FW** | **Leaves DW** | **Stem DW** |
|---|---|---|---|---|---|---|---|---|---|---|
| **ø 1 cm above grafting point** | **0.359** *** | | | | | | | | | |
| **Height** | **0.275** *** | **0.479** *** | | | | | | | | |
| **Number of leaves** | ns | ns | ns | | | | | | | |
| **Leaf area** | ns | **0.524** *** | **0.763** *** | **0.227** ** | | | | | | |
| **SPAD** | **-0.211** ** | ns | ns | ns | ns | | | | | |
| **Xylem sap exudation** | **0.328** *** | **0.273** *** | **0.363** *** | ns | **0.378** *** | ns | | | | |
| **Leaves FW** | **0.299** *** | **0.577** *** | **0.776** *** | **0.275** *** | **0.907** *** | ns | **0.470** *** | | | |
| **Leaves DW** | ns | ns | **-0.426** *** | **0.275** *** | ns | ns | **-0.256** ** | ns | | |
| **Stem DW** | **0.273** *** | ns | **0.353** *** | **0.435** *** | **0.321** *** | ns | ns | **0.411** *** | **0.240** ** | |
| **Roots DW** | **0.292** *** | **0.335** *** | ns | **0.232** ** | ns | ns | ns | **0.356** *** | ns | **0.446** *** |

## ***Experiment 3 – Tables and figures***

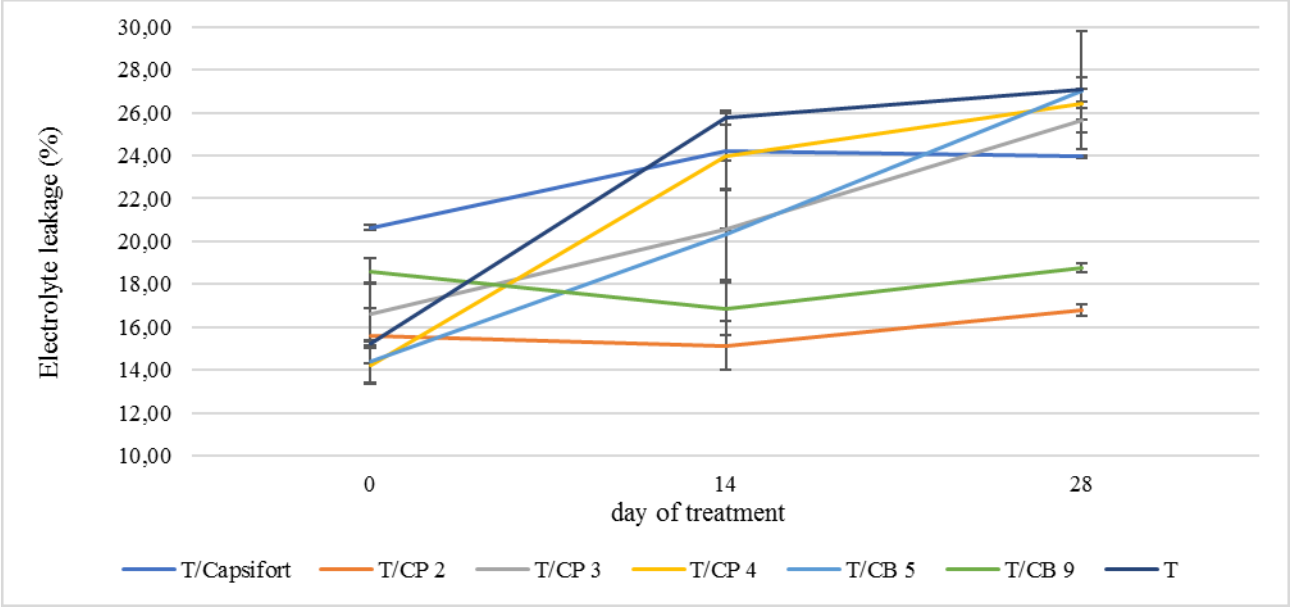



**Figure 10** - Electrolyte leakage '*Tiberio'* $F_1$ un-grafted and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study on day 0, 14 and 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

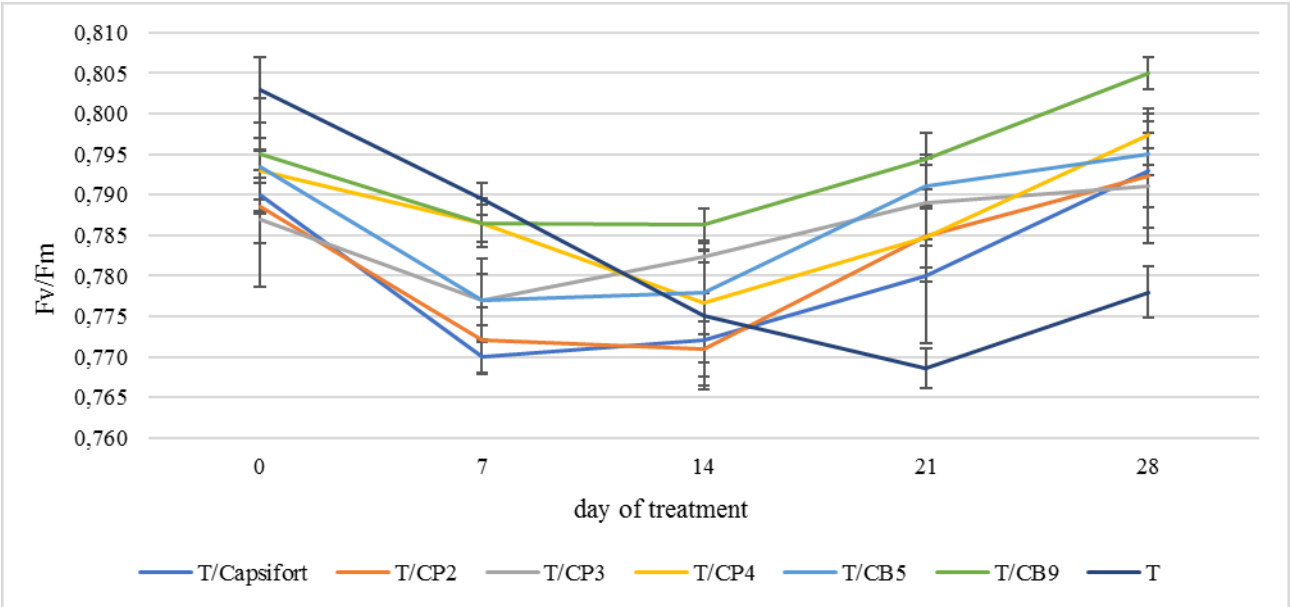



**Figure 11** - Fv/Fm of '*Tiberio'* $F_1$ un-grafted and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study weekly recorded from day 0 to day 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

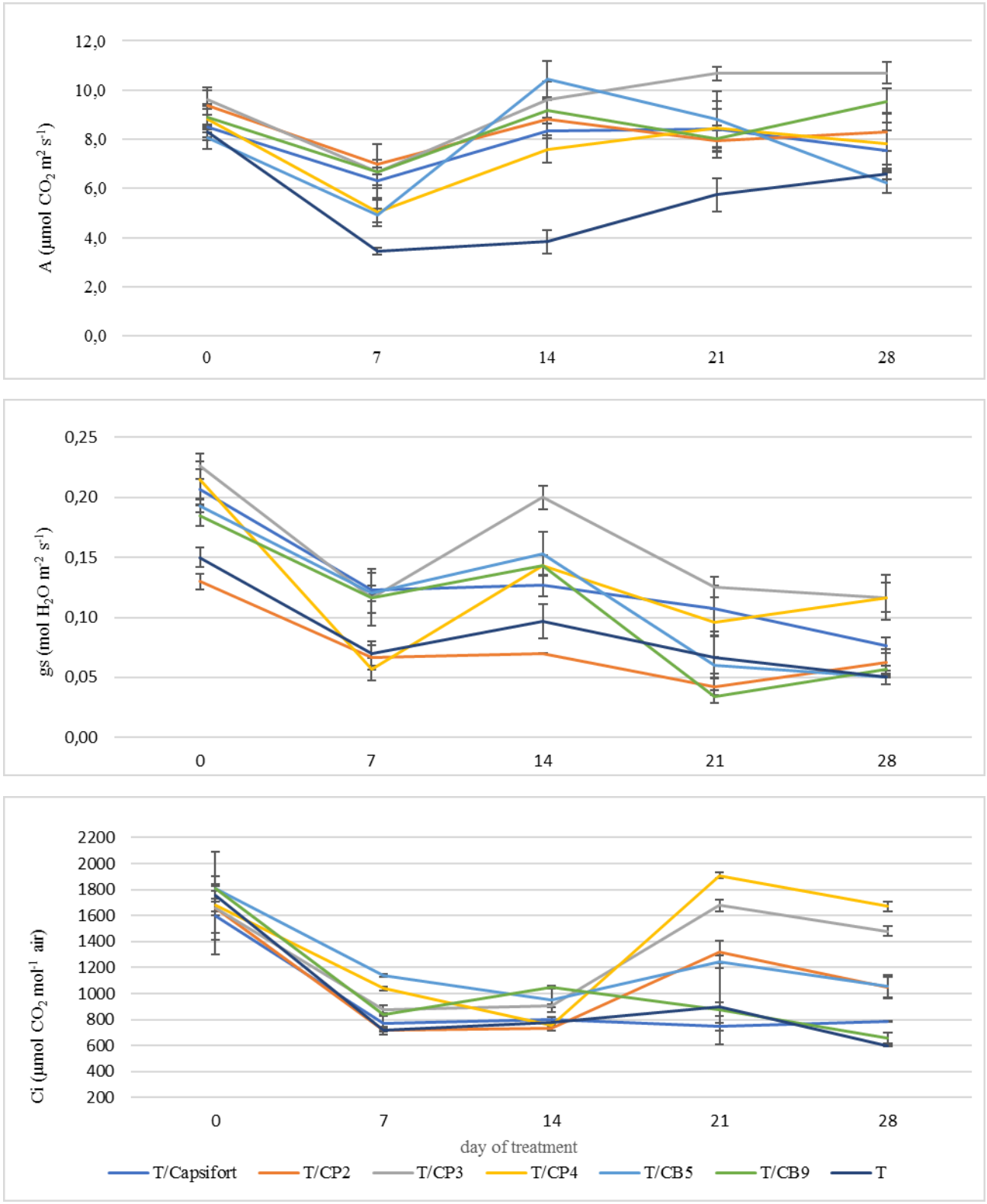



**Figure 12** - Net photosynthesis (A), stomatal conductance (gs) and internal $CO_2$ concentration (Ci) of '*Tiberio'* $F_1$ un-grafted and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study weekly recorded from day 0 to day 28 of chilling stress treatment (15/5 °C); values reported are means ± SE.

**Table 7** - Biometric measurements of '*Tiberio'* $F_1$ un-grafted and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study, after 28 days of cultivation under chilling stress conditions.

| | ***Height*** | ***Number of leaves*** | ***Leaf area*** | ***Leaves DW*** | ***Stem DW*** | ***Roots DW*** | ***Tot DW*** | ***Root : shoot ratio*** | ***SLA*** | ***LMR*** | ***LAR*** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **(cm)** | | **($cm^2$)** | **(g)** | **(g)** | **(g)** | **(g)** | **(%)** | **($cm^2\ g^{-1}$)** | **(%)** | **($cm^2\ g^{-1}$)** |
| *Grafting combination* | | | | | | | | | | | |
| ***T*** | 14.5 d | 13.7 c | 207 c | 1.0 d | 0.5 c | 0.5 bc | 2.0 d | 25.4 ab | 219 de | 49 abc | 107 d |
| ***T/Capsifort*** | 16.1 c | 16.6 ab | 411 ab | 1.6 ab | 0.6 bc | 0.7 b | 2.8 b | 24.8 ab | 258 cd | 55 a | 142 bc |
| ***T/CP2*** | 17.8 b | 15.3 bc | 262 c | 1.4 abc | 0.8 ab | 0.6 bc | 2.7 bc | 21.3 b | 195 e | 51 abc | 99 d |
| ***T/CP3*** | 18.0 b | 16.3 ab | 463 a | 1.6 a | 0.8 ab | 1.1 a | 3.5 a | 30.4 a | 297 abc | 47 bc | 138 bc |
| ***T/CP4*** | 17.3 bc | 15.7 b | 386 b | 1.3 bcd | 0.5 c | 0.7 b | 2.5 bcd | 28.4 a | 302 ab | 52 ab | 156 ab |
| ***T/CB5*** | 18.3 b | 15.7 b | 367 b | 1.1 cd | 0.6 bc | 0.4 c | 2.2 cd | 20.7 b | 325 a | 53 ab | 173 a |
| ***T/CB9*** | 19.9 a | 17.4 a | 424 ab | 1.6 ab | 0.9 a | 1.0 a | 3.5 a | 27.8 a | 273 bc | 45 c | 122 cd |
| | | | | | | | | | | | |
| *Significance* | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test ($P<0.05$).

## ***Experiment 4 – Tables***

**Table 8** - Net photosynthesis (A), stomatal conductance (gs) and internal $CO_2$ concentration (Ci) of '*Tiberio'* $F_1$ un-grafted (T), self-grafted (T/T) and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study, grown under optimal temperatures.

| | ***A*** | ***gs*** | ***Ci*** |
|---|---|---|---|
| | **(µmol $CO_2$ $m^{-2}$ $s^{-2}$)** | **(mol $H_2O$ $m^{-2}$ $s^{-1}$)** | **(µmol $CO_2$ $mol^{-1}$ air)** |
| *Grafting combination* | | | |
| ***T*** | 20.0 c | 0.53 ab | 234 bc |
| ***T/T*** | 20.2 c | 0.51 ab | 225 bc |
| ***T/Capsifort*** | 24.7 a | 0.56 ab | 254 ab |
| ***T/CP2*** | 19.9 c | 0.31 c | 220 c |
| ***T/CP3*** | 22.0 bc | 0.59 a | 279 a |
| ***T/CP4*** | 20.7 bc | 0.44 bc | 234 bc |
| ***T/CB5*** | 22.6 ab | 0.57 ab | 271 a |
| ***T/CB9*** | 23.0 ab | 0.60 a | 269 a |
| | | | |
| *Significance* | *** | *** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

**Table 9** - Yield and its components of '*Tiberio'* $F_1$ un-grafted (T), self-grafted (T/T) and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study, grown under optimal temperatures.

| | ***Marketable*** | | | ***Un-marketable*** | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Number of fruits*** | ***Fruit weight*** | ***Yield*** | ***Number of fruits*** | ***Fruit weight*** | ***Yield*** | ***Total fruits FW*** | ***Total fruits DW*** |
| | | **(g fruit$^{-1}$)** | **(g plant$^{-1}$)** | | **(g fruit$^{-1}$)** | **(g plant$^{-1}$)** | **(g plant$^{-1}$)** | **(g plant$^{-1}$)** |
| *Grafting combination* | | | | | | | | |
| ***T*** | 5.5 abc | 242.5 de | 1338.2 b | 2.7 | 75.9 abc | 204.4 | 1542.6 b | 110.0 bcd |
| ***T/T*** | 5.5 abc | 246.4 de | 1355.2 b | 1.8 | 81.8 abc | 139.2 | 1494.4 b | 103.8 de |
| ***T/Capsifort*** | 7.2 a | 288.2 a | 2064.3 a | 3.0 | 100.8 a | 305.8 | 2370.1 a | 164.7 a |
| ***T/CP2*** | 4.0 bc | 226.0 e | 905.4 b | 2.3 | 65.8 c | 148.9 | 1054.3 b | 71.0 e |
| ***T/CP3*** | 7.1 a | 285.2 ab | 2020.3 a | 2.1 | 67.8 bc | 137.4 | 2157.7 a | 145.4 abc |
| ***T/CP4*** | 5.0 bc | 251.4 cde | 1256.1 b | 2.4 | 89.9 abc | 214.1 | 1470.2 b | 107.5 cde |
| ***T/CB5*** | 7.5 a | 258.1 bcd | 1933.2 a | 3.0 | 98.1 ab | 285.1 | 2218.3 a | 151.7 a |
| ***T/CB9*** | 6.9 ab | 278.3 abc | 1925.3 a | 3.0 | 92.4 abc | 277.2 | 2202.5 a | 147.9 ab |
| | | | | | | | | |
| *Significance* | *** | *** | *** | ns | *** | ns | *** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

**Table 10** - Total soluble solids (TSS), titratable acidity (TA) and antioxidant activity (DPPH) of fruits of '*Tiberio'* $F_1$ un-grafted (T), self-grafted (T/T) and grafted onto '*Capsifort'* $F_1$ and onto the high-altitude accessions under study, grown under optimal temperatures.

| | ***TSS*** | ***TA*** | ***DPPH*** |
|---|---|---|---|
| | **(%)** | **(mg $L^{-1}$ of citric acid)** | **(µmol Trolox $kg^{-1}$ FW)** |
| *Grafting combination* | | | |
| ***T*** | 5.8 | 1.579 a | 473 bc |
| ***T/T*** | 5.4 | 1.472 ab | 408 c |
| ***T/Capsifort*** | 5.4 | 1.312 ab | 403 c |
| ***T/CP2*** | 5.1 | 1.216 b | 464 bc |
| ***T/CP3*** | 5.4 | 1.344 ab | 466 bc |
| ***T/CP4*** | 5.5 | 1.323 ab | 438 c |
| ***T/CB5*** | 5.5 | 1.280 ab | 528 ab |
| ***T/CB9*** | 5.5 | 1.451 ab | 545 a |
| | | | |
| *Significance* | ns | * | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column and experimental factor, means that share a lowercase letter did not differ statistically according to the Student-Newman-Keuls test (P<0.05).

# 2. Tomato grafting as a tool to alleviate physiological disturbances under root-zone oxygen starvation

## Introduction

Higher plants are obligate aerobic organisms needing molecular oxygen to accomplish all the oxidation reactions supporting their life. Nevertheless, they can experience excess of water in growth substrate in either natural or agricultural ecosystems, because of erratic rainfall or incorrect irrigation scheduling associated with climate change (Rouphael et al., 2017). This can often flows in flooding or submergence, which are major abiotic stresses seriously threatening growth and yield of flood sensitive crops (Armstrong et al., 1994). Flooding conditions cause oxygen starvation in roots, which arises from the slow diffusion of gases in water and from oxygen consumption by microorganisms and plant roots (Schwarz et al., 2010). Oxygen deprivation results in arrest of aerobic respiration, leading quickly to an energy deficit in plants, having in turn severe impacts on root ability to take up water and minerals from the substrate and on photosynthetic metabolism (Horchani et al., 2008). In most cultivated plant species, low oxygen availability in the rhizosphere generates symptoms often becoming visible when plants are already severely damaged and yield compromised (Kläring and Zude, 2009).

Cultivated plant species for greenhouses production were mainly selected for their high yield in optimum growing conditions. In the selection process tolerances to many stress factors is generally reduced, since such mechanisms often require extra energy and thus potentially decrease the assimilate availability for the harvest organs. This also concerns sensitivity to hypoxia in the root environment (Crawford and Braendle, 1996).

Tomato is a widely consumed vegetable crop throughout the World, with an estimated production of 159 Mt from more than 4.8 Mha cropland (Faostat, 2012). In the coastal regions of Mediterranean Basin, it is the primary field and greenhouse vegetable crop. Over recent years, the greenhouse tomato in South Italy has experienced a progressive transition to soilless culture, in order to face the exacerbating problems of soil-borne pathogens related to the phase-out of the main soil fumigants and to meet the growing demands to produce vegetables with high quality, health properties and better ecological profile (Mauro et al., 2015). Although tomato plants can grow under a wide range of conditions, it is considered one of the most susceptible vegetable species to excessive soil moisture (Bhatt et al., 2015). In soilless tomato cultivation of South Italy, this can arises from the combination of the long cropping cycle (up to 8-10 months), characterized by fast and scarcely predictable changes in water vapor pressure deficit, with the need to oversize the irrigation volumes to avoid salts accumulation in the rhizosphere, because of the wide presence of brackish groundwater (Giuffrida et al., 2013, 2014). As a result, tomato can often display suboptimal growth habit and yield performances.

An effective available method of adapting plants to counteract environmental stresses is by grafting elite, commercial cultivars onto selected vigorous rootstocks (Schwarz et al., 2010). Grafting is nowadays regarded as a rapid alternative tool to the relatively slow breeding methodology aimed at increasing environmental stress tolerance of vegetables (Leonardi and Giuffrida, 2006; Flores et al., 2010). Despite grafting has been claimed as one possible mean to improve tomato tolerance to root $O_2$ deprivation (Rouphael et al., 2012), up to now there is a scarcity of contributions dealing with grafted tomato response to such type of stress (Bhatt et al., 2015). A better understanding of the physiological modifications subtended by the increased tomato tolerance to root hypoxia may represent a useful framework in directing the choice of the best adapted rootstock genotypes and in scheduling specific breeding programs.

## Material and methods

**Site of the experiment, plant material and growing conditions**

The experiment was conducted in a greenhouse of the experimental farm of the University of Catania (Sicily; Italy) between March and May 2018. Cherry tomato cultivar Dreamer $F_1$ grafted onto the *S. lycopersicum* × *S. habrochaites* rootstocks Arnold $F_1$, Beaufort $F_1$ and Maxifort $F_1$ and onto the *S. lycopersicum* × *S. peruvianum* rootstock Top Pittam $F_1$ were used and compared with self-grafted plants. Grafting was performed at a specialized vegetable nursery through splice-grafting technique (Centro Seia s.r.l.; Italy). Two identical close hydroponic systems were installed with separate tanks, that represented the main plots of the split-plot design used in which optimal and reduced oxygen concentrations were determined. For each tank three replications of 4 plants for each grafting combination were included. The hypoxia treatment was started after 13 days of adaptation to hydroponic conditions. In the control tank the oxygen level was kept at saturation level by continuous forced aeration, whereas in the low oxygen treatment, aeration was started only when roots respiration reduced oxygen content of the nutrient solution to 2 mg $L^{-1}$ threshold and stopped again when 3 mg $L^{-1}$ concentration was reached. However, also in this case constant movement of the nutrient solution was maintained to avoid the rapid decrease in $O_2$ content in the nutrient solution layers immediately close to each root. Dissolved oxygen concentration was monitored through CS511-L sensors (Campbell Scientific, Inc.), made up of a self-polarizing galvanic cell that generated a millivolt signal proportional to the amount of oxygen present in the nutrient solution. The sensors were connected to a CR-510 data logger (Campbell Scientific, Inc.) that, in turn, activated aeration for the time that was necessary.

***Gas exchanges and chlorophyll fluorescence measurements***

Gas exchanges analysis were performed 29 days after the beginning of the hypoxia treatment on the third fully expanded leaves through an LCi Portable Photosynthesis System (ADC BioScientific Ltd.). Net photosynthesis (A), transpiration (E), stomatal conductance (gs) and internal $CO_2$ concentration (Ci) were determined and expressed as μmol $CO_2$ $m^{-2}$ $s^{-1}$, mmol $H_20$ $m^{-2}$ $s^{-1}$, mol $CO_2$ $m^{-2}$ $s^{-1}$ and μmol $CO_2$ $mol^{-1}$ air respectively.

PSII efficiency was determined in the same day of gas exchanges through Chlorophyll a fluorescence analysis using an OS1-FL fluorometer (Opti-Sciences Corporation, Tyngsboro, MA); chlorophyll fluorescence excitation was performed by a 660 nm solid-state light source coupled with filters able to block λ above 690 nm; the modulated light intensity was adjusted from 0 to 1 μE. Fluorescence detection was performed between 700 and 750 nm using a PIN silicon photodiode coupled with appropriate filtering to remove extraneous light. Saturation of the photosystem II was provided by a filtered 35W halogen lamp (350-690 nm). All the measurements were performed after a 20 minutes leaf dark-adaptation through OS cuvettes (Opti-Sciences Corporation, Tyngsboro, MA) (Liu et al. 2005).

***Electrolyte leakage***

Leaves electrolyte leakage was measured 30 days after the onset of the hypoxia treatment, before removing plants for destructive analysis. For each measurement 20 leaf discs (1 $cm^2$) were collected and placed on a 50 ml tube containing 20 ml of Millipore water, the tubes were then placed in a shaker at 100 r.p.m. for 24 hours at 25 °C. After a first EC reading (EC1) at the end of the 24 hours shaking, the test tubes were placed in an autoclave at 120 °C for 20 minutes in order to destroy cellular structures and left to cool again at 25 °C for the second EC reading (EC2). Electrolyte leakage was calculated as:

$$Electrolyte\ leakage\ (EL) = \frac{EC1}{EC2} \times 100 \qquad (1)$$

***Biometric measurements***

Plant height, number of leaves and leaf area were measured on day 30 from the onset of the stress. Roots, stem and leaves dry weights were determined using a thermo-ventilated oven at 70 °C until constant weight was reached. Specific leaf area at the end of the experiment was calculated as:

$$Specific\ leaf\ area\ (SLA) = \frac{leaf\ area\ (cm^2)}{leaves\ dry\ weight\ (g)} \quad (2)$$

Leaf area ratio was calculated as:

$$Leaf\ area\ ratio\ (LAR) = \frac{leaf\ area\ (cm^2)}{plant\ dry\ weight\ (g)} \quad (3)$$

SLA and LAR were expressed in $cm^2\ g^{-1}$

***Statistical analysis***

Bartlett's test was used to test for homoscedasticity, whereas differences among treatments were determined by applying a two-way (oxygen level × rootstock genotype) factorial analysis of variance (ANOVA), related to the experimental split-plot design adopted in the experiment. Percentage data were Bliss transformed before the ANOVA (untransformed data are reported and discussed) whereas multiple mean comparisons were performed through Fisher's protected least significant difference (LSD) test (at least for $P = 0.05$). Pearson's correlation analysis was also performed for collected and calculated data, in order to define possible relationships among variables.

## Results

***Chlorophyll fluorescence variables***

The $O_2$ availability at root level had significant effects on the chlorophyll fluorescence variables in a way that, in most cases, was rootstock-dependent (**Table 1**). Indeed, passing from optimal to low root $O_2$ level, the scion Dreamer $F_1$ showed a significant $F_0$ decrease when grafted onto Arnold $F_1$ (from 197 to 158) while showed an increase when self-grafted (from 164 to 191) (**Figure 1**). The $F_V$ values were higher under optimal (717) than under limited $O_2$ conditions (674), while regarding the rootstock genotype, such variable proved to be higher in Dreamer $F_1$ grafted onto Arnold $F_1$, Beaufort $F_1$ and Maxifort $F_1$ (714, on average), intermediate in the self-grafted test (686) and lower when grafted onto Top Pittam $F_1$ (652) (**Table 1**). The lowest $O_2$ availability caused a significant variation of the $F_M$ values only when the scion was grafted onto Arnold $F_1$, since this variable passed from 976 to 812 as the $O_2$ level decreased (**Figure 2**). As regards the ratio $F_V/F_M$, passing from optimal to low root $O_2$ availability a significant decrease was recorded for Dreamer $F_1$ self-grafted of grafted onto Beaufort $F_1$ (from 0.808 to 0.779 and from 0.810 to 0.799, respectively) (**Figure 3**).

***Photosynthetic variables***

Significant interactions between oxygen level and rootstock were observed for all the gas exchange parameters examined (**Table 2**). Under low $O_2$ conditions, the A value of Dreamer $F_1$ increased when grafted onto Arnold $F_1$ (from 18.7 to 23.5 μmol $CO_2$ $m^{-2}$ $s^{-1}$) and Top Pittam $F_1$ (from 19.9 to 22.8 μmol $CO_2$ $m^{-2}$ $s^{-1}$) rootstocks. Conversely it decreased when the scion was self-grafted (from 21.9 to 18.1 μmol $CO_2$ $m^{-2}$ $s^{-1}$) (**Figure 4**). The $O_2$ limiting conditions proved to increase the E values of the scion only when grafted onto Beaufort $F_1$ (from 6.4 to 7.5 mmol $H_2O$ $m^{-2}$ $s^{-1}$), while a significant decrease was recorded in Top Pittam (from 7.8 to 5.5 mmol $H_2O$ $m^{-2}$ $s^{-1}$), Arnold $F_1$ (from 8.8 to 6.9 mmol $H_2O$ $m^{-2}$ $s^{-1}$), as well as in the self-grafted test (from 8.0 to 6.2 mmol $H_2O$ $m^{-}$

$^{2}$ $s^{-1}$) (**Figure 5**). The root $O_2$ availability modification of gs appeared to be rootstock dependent too, since passing from optimal to low $O_2$ conditions this variable showed a significant decrease only in Dreamer $F_1$ grafted onto Beaufort $F_1$, Maxifort $F_1$ or self-grafted (-0.118, -0.112 and -0.096 mmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively) (**Figure 6**). Ci showed a general decrease passing from optimal to low $O_2$ availability, with the highest reduction recorded in Dreamer $F_1$ grafted on Beaufort $F_1$ (from 289 to 238 mmol $CO_2$ $mol^{-1}$), Maxifort $F_1$ (from 290 to 252 mmol $CO_2$ $mol^{-1}$) and Arnold $F_1$ (from 293 to 268 mmol $CO_2$ $mol^{-1}$) (**Figure 7**).

***Plant growth variables***

*Leaves growth*

Excepting Arnold $F_1$, all the rootstock significantly increased the number of leaves per plant in Dreamer $F_1$ scion as compared to self-grafted test, irrespective of the root $O_2$ availability. This was more evident for Beaufort $F_1$, Maxifort $F_1$ and Top Pittam $F_1$, for which an average increase of 2.1 leaves $plant^{-1}$ was recorded when compared to the self-grafted test (**Table 3**). Differently, leaf area and leaf area ratio were both affected by the "oxygen level × rootstock" interaction (**Table 3**). Passing from optimal to low $O_2$ root availability, the former variable dramatically decreased in Dreamer $F_1$ self-grafted (from 5,607 to 2,628 $cm^2$ $plant^{-1}$) (**Figure 8**). Similarly, leaf area ratio showed significant variations across $O_2$ root availability levels only when the scion was self-grafted (where decreased from 104 to 73 $cm^2$ $g^{-1}$) (**Figure 9**). The root $O_2$ availability affected the specific leaf area which, passing from optimal to low $O_2$ level, decreased from 222 to 198 $cm^2$ $g^{-1}$, and was higher in Dreamer $F_1$ grafted onto Maxifort $F_1$ and Top Pittam $F_1$ (225 $cm^2$ $g^{-1}$, on average) as compared to the other rootstocks (200 $cm^2$ $g^{-1}$, on average) (**Table 3**).

*Plant growth*

Plant, shoot and root biomass were affected by the root $O_2$ availability since, passing from optimal to low $O_2$ level, these variables decreased by 13 (from 63 to 55 g DW $plant^{-1}$), 10 (from 52 to 47 g DW $plant^{-1}$) and 23% (from 11.0 to 8.5 g DW $plant^{-1}$), respectively (**Table 4**). Concerning the rootstock, Maxifort $F_1$, Beaufort $F_1$ and Top Pittam $F_1$ showed higher values of both plant biomass (71, 62 and 58 g DW $plant^{-1}$, respectively) and root biomass (11.0, 10.1 and 9.8 g DW $plant^{-1}$, respectively) as compared to self-grafted test (Table 4). Differently, the effect of $O_2$ availability on root:shoot ratio was rootstock-dependent (Table 4). Indeed, passing from optimal to low $O_2$ availability, the root:shoot ratio significantly increased in the self-grafted Dreamer $F_1$ (from 0.21 to 0.26), while decreased in the grafted ones onto Maxifort $F_1$ (from 0.22 to 0.15) and Arnold $F_1$ (from 0.23 to 0.19) (**Figure 10**).

*Electrolyte leakage*

All the main factors studied, namely $O_2$ level and rootstock genotype, significantly affected the electrolyte leakage, without interactive effect (**Table 4**). Indeed, passing from optimal to low $O_2$ root availability, electrolyte leakage increased from 24.9 to 26.9%, while comparing the rootstock genotypes, this variable was higher in Dreamer $F_1$ self-grafted or grafted onto Beaufort $F_1$ and Top Pittam $F_1$ (26.9%, on average) followed by that grafted onto Arnold $F_1$ (25.4%) and Maxifort $F_1$ (23.4%) (**Table 4**).

***Correlation among variables***

Table 6 reports the significant correlations found among variables. $F_0$ was positively correlated to $F_V$ (0.670 *), $F_M$ (0.800 **) and root:shoot ratio (0.694 *), but negatively correlated to $F_V/F_M$ (-0.656 *) and A (-0.642 *). $F_V$ was strongly and positively correlated to $F_M$ (0.981 ***), while $F_V/F_M$ was positively correlated to leaf area (0.815 **), number of leaves $plant^{-1}$ (0.727 *) and plant biomass (0.858 **), and negatively correlated to root:shoot ratio (-0.658 *) and HI (-0.646 *). The A was positively correlated to leaf area (0.657 *) and leaf area ratio (0.688 *) and negatively correlated to electrolyte leakage (-0.827 **). Ci resulted significantly correlated to specific leaf area

(-0.639 *) and root:shoot ratio (0.696 *), while the number of leaves plant$^{-1}$ was correlated to leaf area (0.745 *), plant biomass (0.4790 **) and root:shoot ratio (-0.664 *). The leaf area was positively correlated to plant biomass (0.924 ***) and leaf area ratio (0.875 ***) and negatively correlated to root:shoot ratio (-0.642 *) and electrolyte leakage (-0.665 *). Root dry weight was positively correlated to plant biomass (0.754 *) and negatively correlated to HI (-0.659 *) while plant biomass was positively correlated to specific leaf area (0.643 *).

## Discussion

One of the earliest responses of tomato plants exposed to root hypoxia is the reduced ability of roots to take up water from the growth substrate. This is followed by a variety of physiological dysfunctions concerning plant growth, photosynthesis, hormonal balances, distribution of carbohydrates, nutrient uptake, early senescence or injury in organs, which sometimes precede plant death (Kramer, 1969; Schildwacht, 1989; Else et al., 1995; Else et al., 2001; Else et al., 2009). To cope with this stress typology, tomato plants display a strategy involving drastic metabolic modifications leading to biochemical, anatomical and morphological changes (Rodríguez-Gamir et al., 2011).

In the present experiment, the low root $O_2$ availability affected all the recorded physiological and developmental characteristics of tomato plants, showing systemic effects involving both rootstock and scion. As regards the chlorophyll fluorescence variables, the main effects of root hypoxia were recorded on variable fluorescence ($F_V$), maximum fluorescence ($F_M$) and the ratio among them ($F_V/F_M$). $F_V$ is a fluorescence variable reflecting the reduction at a given time of the primary electron acceptor, which, in the oxidized state, quenches fluorescence (Jefferies, 1992). $F_M$ is obtained at the fully saturating irradiance for the plant when the electron acceptor QA is fully reduced, while $F_V/F_M$ is a useful ratio which has been shown to be proportional to the quantum yield of photosystem II (PSII) photochemistry and exhibits a high degree of correlation with the quantum yield of net photosynthesis (Kitajima and Butler, 1975). Beyond the differences among rootstock genotypes, root hypoxia acted to reduce both $F_V$ and $F_M$, a condition which, in turns, lowered the $F_V/F_M$ ratio. The lowering of this fluorescence variable is often associated with unrepaired damage to PSII (Else et al., 2009; Bhatt et al., 2015), the results of this experiment suggest that under hypoxically treated roots there was a progressive impairment of photosynthetic machinery, starting with the reduction in efficiency of the light-harvesting complexes. The reason why of such impairment is perhaps to be found in the disruption of the fine tuning among the light-depending processes and the dark reactions of photosynthesis. Indeed, under hypoxically conditions, roots experience a reduction of their hydraulic conductance, with subsequent reduction of stomatal conductance to prevent water loss and cavitation vulnerability of the xylem. However, such modification also induces a decrease in $CO_2$ availability for the leaves (Rodríguez-Gamir et al., 2011). In this experiment there was a general reduction of E, gs and Ci after root hypoxia, suggesting the occurrence of stomatal limitations to photosynthetic processes. Hence the working hypothesis is that with less $CO_2$ available for photosynthesis, surplus reducing power is diverted to $O_2$ and the generation of potentially damaging superoxide anions ($O_2$) and $H_2O_2$, with subsequent alteration of the operational status of light-harvesting complexes (Yan et al., 1996; Yordanova and Popova, 2007). Accordingly under conditions of root hypoxia we found a significant increase in electrolyte leakage, a conditions which is strongly associated to a loss of integrity of the cell membranes in tomato (Beckles, 2012). Interestingly, the self-grafted tomato plants, i.e. those showing the highest $F_V/F_M$ reduction, displayed the highest reduction in gs, E and A, but the least reduction in terms of Ci, so suggesting an involvement of reduced carboxylation potential too (non-stomatal factors) in generating damages to the antenna complexes. This hypothesis is consistent with the observed promoting effect of lipid peroxidation in leaf cells under root hypoxia (Rasheed et al., 2018). On the contrary, all the rootstocks used in this experiment were able to minimize the variation of $F_V/F_M$ ratio and A under condition of root hypoxia, indicating their ability to maintain

an overall better functionality of the photosynthetic apparatus, likely as a consequence of superior biological perfomances of the root under condition of $O_2$ deprivation. Although the layout of this experiment does not allow to ascertain which mechanism is linked to this better root functionality, according to Schwarz et al., (2010) and Aloni et al., (2010) we can argue that mechanisms such better nutrient absorption, well developed root aerenchyma or improved hormonal balance could be responsible for a better root hypoxia tolerance of the grafted tomato plants.
Under conditions of root $O_2$ stress, it was recorded an average 13%, 10% and 23% decrease of plant, shoot and root biomass, respectively, together with a modification of the root:shoot ratio, indicating a decrease of the synthesis of carbohydrate and an alteration in their allocation, respectively. In particular the root:shoot ratio appeared a discriminant variable among graft combinations, as the self-grafted test was the only in which this ratio increased under root hypoxia. This could be due to its highest need of energy and carbohydrate for root cell turnover and regeneration, leading roots acting as high priority organs competing with the leaves for carbohydrate allocation. Indeed the primary effect of soil flooding is to slow down oxygen transfer to the roots (Mommer et al., 2004), which results in a degradation process and in the death of a part of root tissues. This leads to limited aerobic respiration and dramatically depresses root growth which, in the self-grafted test, was counteracted by diverting more photosynthates toward roots (Henshaw et al., 2007a, 2007b). On the contrary, the other graft combinations showed a higher root weight, a decreased root:shoot ratio and higher values of leaf area and leaf area ratio under $O_2$ stress, overally indicating a better functionality of the root system and the mantainance of a higher photosynthetic potential than self-grafted test under $O_2$ stress. Such a shift in carbohydrate allocation strongly suggests the involvement of hormonal messages mediating a root-shoot communication at whole plant level. A partial confirmation of a hormonal involvement can be found also in the $O_2$ stress-induced increase in specific leaf area, which indicates an increase in leaf thickness under hypoxia conditions likely driven by an accumulation of ethylene in plant tissues leading to an alteration of cell expansion (Horchani et al., 2010).

## Conclusions

The outcome of this experiment showed that grafting is a useful technique to improve tolerance to root hypoxia in tomato plants. Grafted tomato plants exhibited better photosynthetic performances under conditions of $O_2$ stress as compared to self-grafted test, as a consequence of reduced impairment of the photosynthetic machinery deriving from both stomatal and non-stomatal limitations. These better performances are likely linked to a better functionality of the roots under conditions of $O_2$ deprivation, a condition that was mirrored also in the greater extension and functionality of the assimilating leaf apparatus which, in turn, derived from a better carbohydrate partitioning inside the plant. To ascertain the origin of this better functionality of root system and sink-source arrangement inside the plant, further investigations are needed, considering histological (development of aerenchymas) and hormonal cues.

**Table 1**. Chlorophyll fluorescence variables of tomato plants as affected by rootstock and oxygen level.

| Variable | $F_0$ | $F_V$ | $F_M$ | $F_V/F_M$ |
|---|---|---|---|---|
| | Adimensional | | | |
| Oxygen level | | | | |
| *Low* | 169 a | 674 b | 843 b | 0.799 b |
| *Optimal* | 173 a | 717 a | 891 a | 0.805 a |
| Rootstock | | | | |
| *Arnold* $F_1$ | 177 a | 717 a | 894 a | 0.801 bc |
| *Beaufort* $F_1$ | 174 a | 724 a | 898 a | 0.804 ab |
| *Maxifort* $F_1$ | 165 b | 701 a | 866 a | 0.809 a |
| *Top Pittam* $F_1$ | 162 b | 652 b | 814 b | 0.801 bc |
| *Self-grafted* | 177 a | 686 ab | 863 ab | 0.794 c |
| Significance | | | | |
| *Oxygen level (O)* | ns | ** | ** | * |
| *Rootstock (R)* | ** | * | * | ** |
| *O* × *R* | *** | ns | ** | *** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column, means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

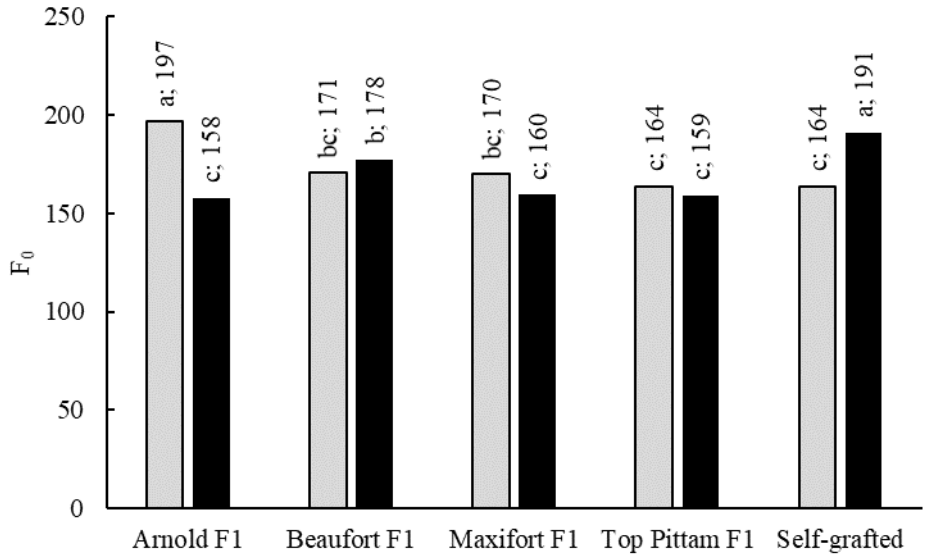



**Figure 1**. $F_0$ values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

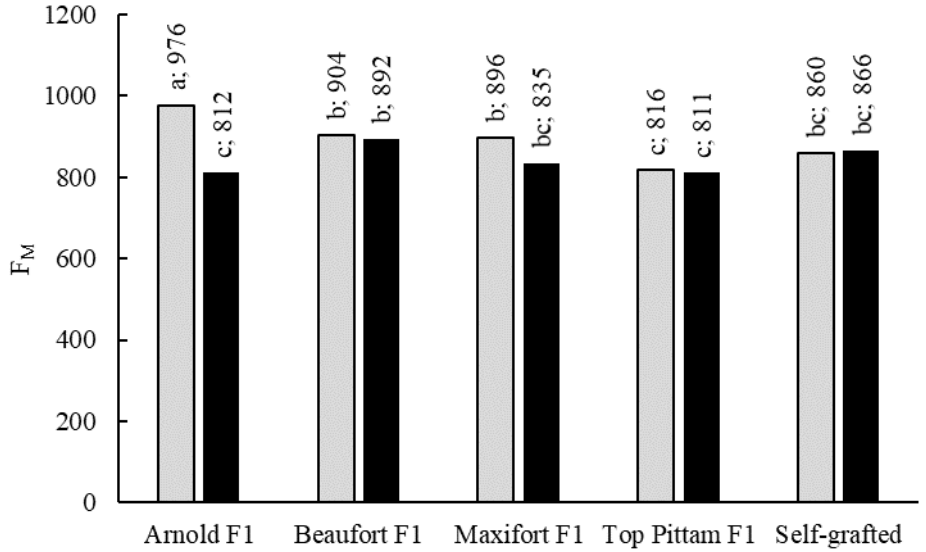



**Figure 2**. $F_M$ values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

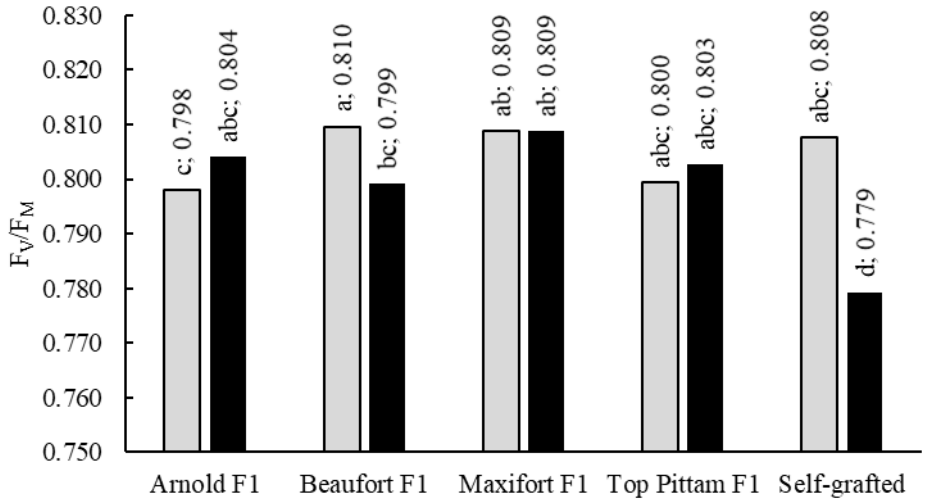



**Figure 3**. $F_V/F_M$ values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

**Table 2**. Instantaneous leaf photosynthetic rate (*A*), leaf transpiration (*E*), stomatal conductance (*gs*) and leaf intercellular $CO_2$ concentration (Ci) of tomato plants as affected by rootstock and oxygen level.

| Variable | ***A*** ***(μmol $CO_2$ $m^{-2}$ $s^{-1}$)*** | ***E*** ***(mmol $H_2O$ $m^{-2}$ $s^{-1}$)*** | ***gs*** ***(mol $CO_2$ $m^{-2}$ $s^{-1}$)*** | ***Ci*** ***(μmol $CO_2$ $mol^{-1}$ air)*** |
|---|---|---|---|---|
| Oxygen level | | | | |
| *Low* | 21.3 a | 6.9 b | 0.445 b | 265 b |
| *Optimal* | 20.6 a | 7.9 a | 0.505 a | 290 a |
| Rootstock | | | | |
| *Arnold $F_1$* | 21.1 ab | 7.8 ab | 0.505 a | 281 ab |
| *Beaufort $F_1$* | 22.1 a | 6.9 c | 0.477 ab | 263 c |
| *Maxifort $F_1$* | 20.2 b | 8.5 a | 0.439 b | 271 bc |
| *Top Pittam $F_1$* | 21.4 ab | 6.7 c | 0.445 b | 279 b |
| *Self-grafted* | 20.0 b | 7.1 bc | 0.510 a | 294 a |
| Significance | | | | |
| *Oxygen level (O)* | ns | *** | ** | *** |
| *Rootstock (R)* | * | *** | * | ** |
| *O × R* | *** | *** | * | ** |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column, means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

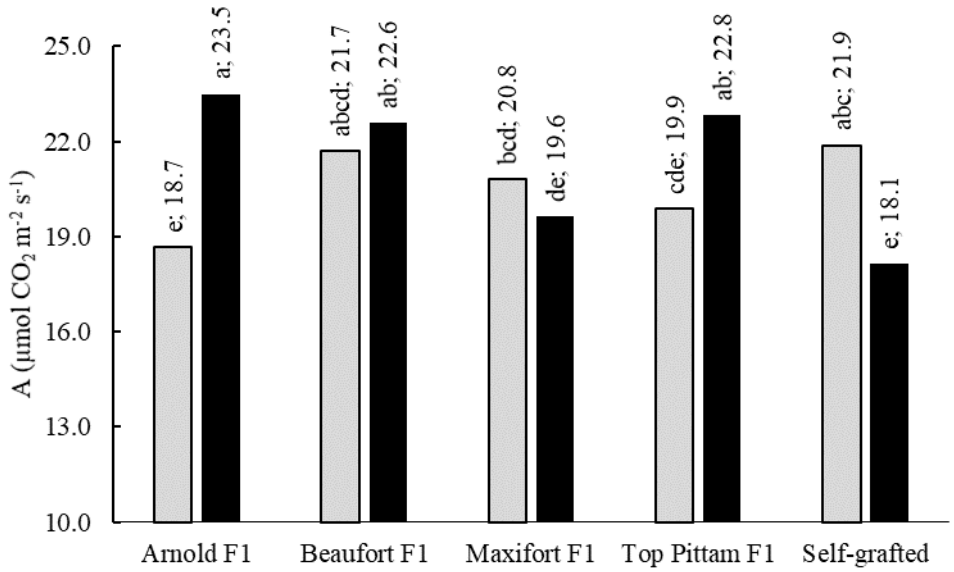



**Figure 4**. Net photosynthesis (A) values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

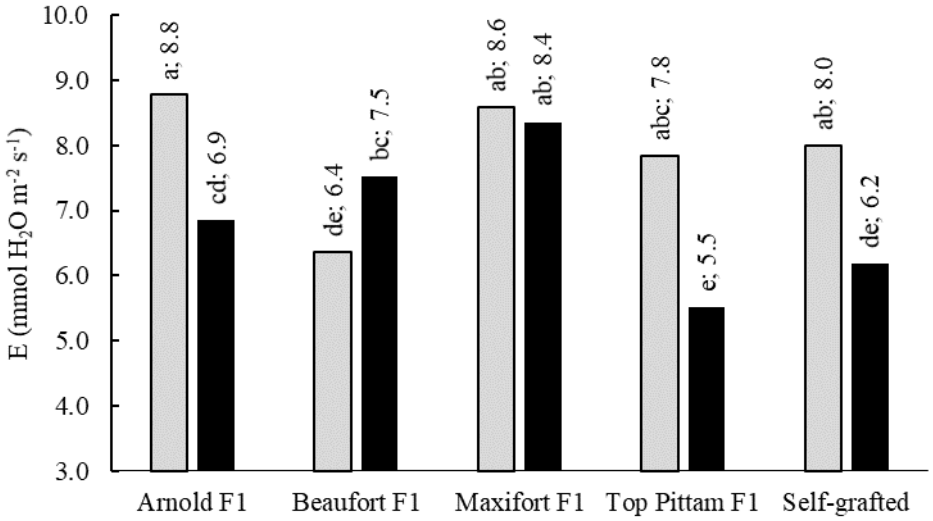



**Figure 5**. E values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

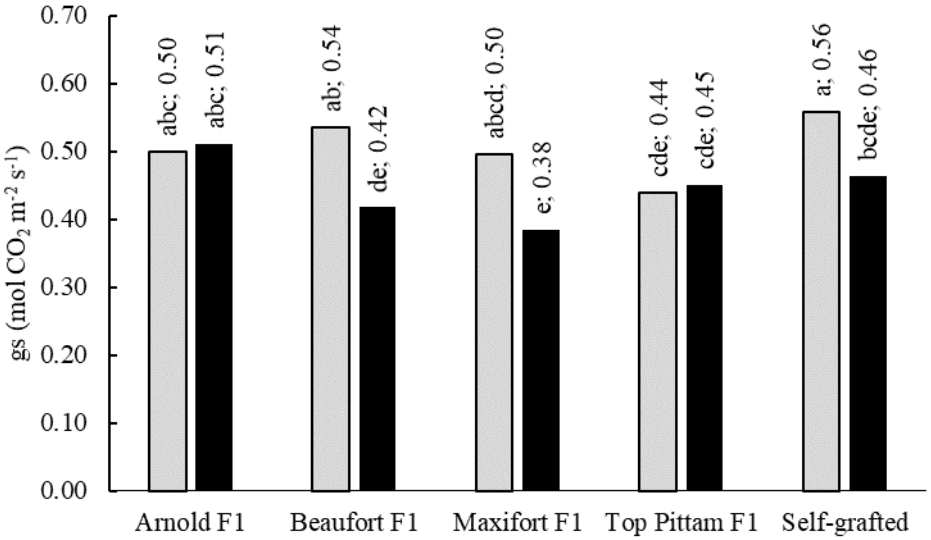



**Figure 6**. gs values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

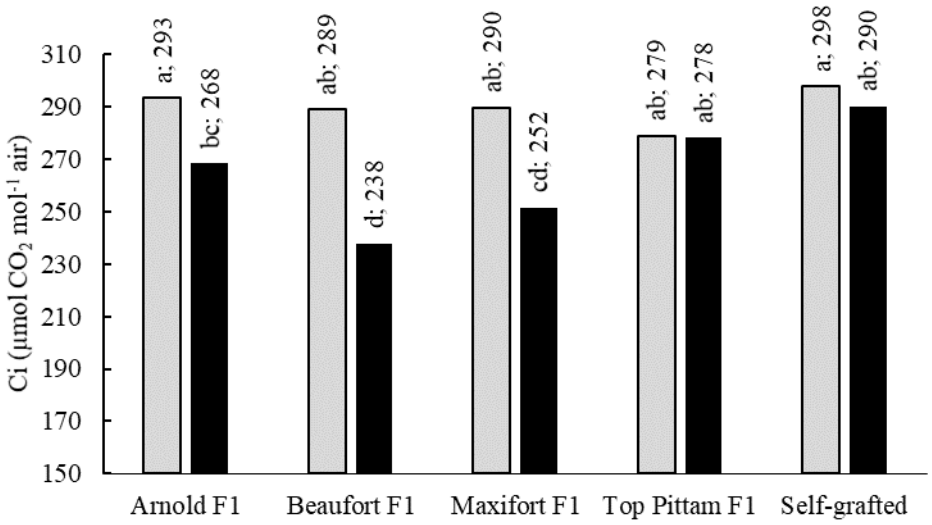



**Figure 7**. Ci values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

**Table 3**. Leaves development variables of tomato plants as affected by rootstock and oxygen level.

| Variable | *Number of leaves* (*n. plant$^{-1}$*) | *Leaf area* (*cm$^2$ plant$^{-1}$*) | *Leaf Area Ratio* (*cm$^2$ g$^{-1}$ DW plant*) | *Specific leaf area* (*cm$^2$ g$^{-1}$ DW leaves*) |
|---|---|---|---|---|
| Oxygen level | | | | |
| *Low* | 19.4 a | 4911 b | 90 a | 222 a |
| *Optimal* | 19.9 a | 5645 a | 93 a | 198 b |
| Rootstock | | | | |
| *Arnold $F_1$* | 19.2 bc | 4859 bc | 89 b | 200 b |
| *Beaufort $F_1$* | 20.4 a | 5171 bc | 86 b | 200 b |
| *Maxifort $F_1$* | 20.3 ab | 7126 a | 104 a | 223 a |
| *Top Pittam $F_1$* | 20.2 ab | 5117 b | 91 b | 226 a |
| *Self-grafted* | 18.2 c | 4117 c | 88 b | 201 b |
| Significance | | | | |
| *Oxygen level (O)* | ns | * | ns | *** |
| *Rootstock (R)* | *** | *** | ** | * |
| *O × R* | ns | ** | *** | ns |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column, means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

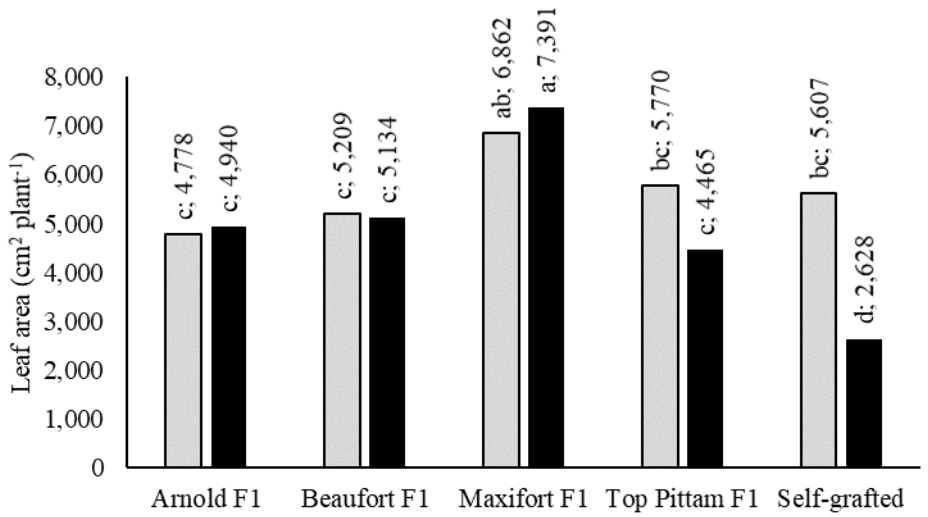



**Figure 8**. Leaf area values of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

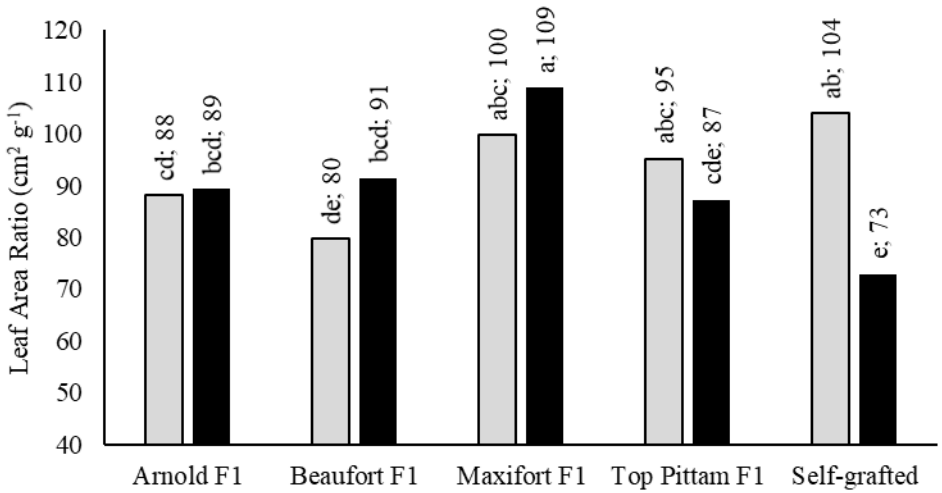



**Figure 9**. Leaf area ratio of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

**Table 4**. Plant development variables and leaf electrolyte leakage in tomato plants as affected by rootstock and oxygen level. Different letters within columns and within factors indicate significantly different means according to Fisher's protected LSD test ($P \leq 0.05$).

| Variable | *Plant biomass (g DW plant$^{-1}$)* | *Root weight (g DW plant$^{-1}$)* | *Root : shoot ratio (adimensional)* | *Electrolyte leakage (%)* |
|---|---|---|---|---|
| Oxygen level | | | | |
| *Low* | 55 b | 8.5 b | 0.20 b | 26.9 a |
| *Optimal* | 63 a | 11.0 a | 0.22 a | 24.9 b |
| Rootstock | | | | |
| *Arnold $F_1$* | 56 bc | 9.5 bc | 0.21 b | 25.4 b |
| *Beaufort $F_1$* | 62 ab | 10.1 ab | 0.20 b | 27.5 a |
| *Maxifort $F_1$* | 71 a | 11.0 a | 0.19 b | 23.4 c |
| *Top Pittam $F_1$* | 58 b | 9.8 ab | 0.21 b | 27.1 ab |
| *Self-grafted* | 47 c | 8.4 c | 0.24 a | 26.2 ab |
| Significance | | | | |
| *Oxygen level (O)* | * | *** | ** | ** |
| *Rootstock (R)* | *** | * | ** | *** |
| *O × R* | ns | ns | *** | ns |

Significance: ns - not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For each column, means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

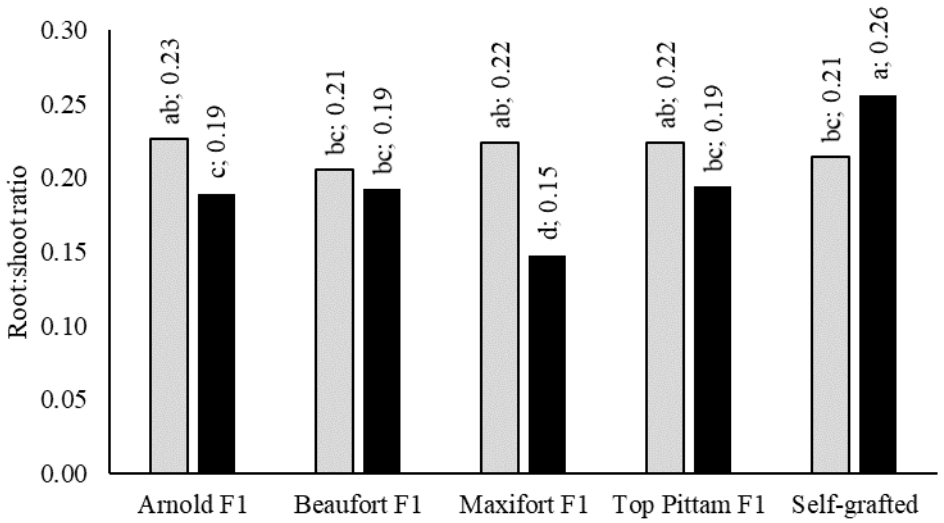



**Figure 10**. Root:shoot ratio of tomato as affected by "oxygen level × rootstock" interaction. Grey bars: optimal root $O_2$ availability; black bars: suboptimal root $O_2$ availability. The means that share a lowercase letter did not differ statistically according to Fisher's protected LSD test ($P \leq 0.05$).

**Table 5**. Correlation matrix reporting the Pearson's correlation coefficients among variables. NS: not significant; *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

| | $F_o$ | $F_V$ | $F_M$ | $F_V/F_M$ | A | E | gs | Ci | A/E | N. leaves | Leaf Area | LAR | SLA | Plant biomass | Root weight | Root:Shoot ratio | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$F_V$** | 0,670 * | | | | | | | | | | | | | | | | |
| **$F_M$** | 0,800 ** | 0,981 *** | | | | | | | | | | | | | | | |
| **$F_V/F_M$** | -0,652 * | ns | ns | | | | | | | | | | | | | | |
| **A** | -0,642 * | ns | ns | ns | | | | | | | | | | | | | |
| **E** | ns | ns | ns | ns | ns | | | | | | | | | | | | |
| **gs** | ns | ns | ns | ns | ns | ns | | | | | | | | | | | |
| **Ci** | ns | ns | ns | ns | ns | ns | 0,741 * | | | | | | | | | | |
| **A/E** | ns | ns | ns | ns | 0,705 * | -0,901 * | ns | ns | | | | | | | | | |
| **N. leaves** | ns | ns | ns | 0,721 * | ns | ns | ns | ns | ns | | | | | | | | |
| **Leaf Area** | ns | ns | ns | 0,816 ** | ns | 0,656 * | ns | ns | ns | 0,745 * | | | | | | | |
| **LAR** | ns | ns | ns | 0,656 * | ns | 0,688 * | ns | ns | ns | ns | 0,875 *** | | | | | | |
| **SLA** | ns | ns | ns | ns | ns | ns | -0,863 * | -0,639 * | ns | ns | ns | ns | | | | | |
| **Plant biomass** | ns | ns | ns | 0,857 ** | ns | ns | ns | ns | ns | 0,789 ** | 0,924 *** | 0,643 * | ns | | | | |
| **Root weight** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | 0,755 * | | | |
| **Root:Shoot ratio** | 0,639 * | ns | ns | -0,633 * | ns | ns | ns | 0,695 * | ns | -0,644 * | ns | ns | ns | ns | ns | | |
| **HI** | ns | ns | ns | -0,644 * | ns | ns | -0,647 * | ns | ns | ns | ns | ns | ns | ns | -0,662 * | ns | |
| **Electrolyte leakage** | ns | ns | ns | ns | ns | -0,827 * | ns | ns | 0,808 ** | ns | -0,665 * | ns | ns | ns | ns | ns | ns |

# 3. The effects of grafting combination on cherry tomatoes yield and quality

## Introduction

The large-scale spread of vegetable grafting was initially promoted to meet restrictions on the use of fumigants for soil disinfection, thanks to the higher tolerance of wild or hybrid rootstocks to many soil pathogens and pests (Bie et al., 2017; Keatinge et al., 2014). Beyond soil phytopathology, grafting onto appropriate rootstocks have been performed to confer tolerance to many abiotic stress conditions including salinity, drought, flooding, alkalinity, nutrient deficiency and heavy metals pollution (Savvas et al., 2010; Schwarz et al., 2010). Throughout the years, breeding companies put many efforts in the selection of rootstocks aiming to an improvement of crops productivity; anyway, productivity and fruit quality usually show contradictory trends. For that reason, grafting-induced improvements of yield are often accompanied by a loss in terms of product quality (Kyriacou et al., 2017).

"Fruit quality" assumes different meanings basing on the perspective of the specific stakeholder involved; for example, the quality traits hierarchy of growers, traders, retailers and consumers can be completely different and there are cases in which an improvement in tomato "quality" for one stakeholder implies quite automatically a loss of "quality" for another one (Dos-Santos et al., 2013; Pech et al., 2008).

To express quality traits from a product-oriented perspective, unlike basing on some stakeholder preference, however, allows giving measurable, objective and universal information. Using this approach, "fruit quality" is used in scientific literature to indicate a number of external traits including shape, size, texture, colour, the absence of defects and decay, as well as many internal characteristics. Among them, besides the safety requirements related to the absence of pesticides concentration exceeding the maximum residue limits, the contents of soluble sugars, acids, minerals, aroma volatiles and bioactive compounds are included (Leonardi et al., 2017).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide and has been largely used as a model plant for research purposes; moreover, grafting is now considered as a standard practice in specialized tomato cultivation (Ezura, 2009; Keatinge et al., 2014). For that reasons the effects of grafting on tomato yield and quality has been studied during recent years and is still under investigation (Flores et al., 2010; Savvas et al., 2011).

An increased yield is usually reported in tomato grafted onto appropriate rootstocks as a combined result of their higher performance under biotic and abiotic stress conditions. The increased productivity observed is more often related to an increase in fruit size than to a higher number of fruits (Kyriacou et al., 2017; Leonardi et al., 2017; Savvas et al., 2011). Even if the effects of grafting on fruit weight and size have been reported to be dependent on the specific rootstock-scion combination, it seems that, besides grafting-incompatibility events, vigorous rootstocks tend to increase scion fruits size, probably through enhanced water and nutrient uptakes (Colla et al., 2006). This increase in fruit size has been specially reported in small fruit types (fruit weight < 40 g), like cherry tomato (Kyriacou et al., 2017). A corresponding increase in the ratio between equatorial and polar diameter of the fruit highlighted the effect of grafting on shape beyond fruit size in some tomato types (Schwarz et al., 2013).

Grafting-induced worsening in tomato colour has been sometimes reported as a result of a reduced carotenoids content by grafting onto vigorous rootstocks (Di Gioia et al., 2010; Nicoletto et al., 2013), however, this has not always been confirmed (Schwarz et al., 2013).

Although the texture of the fruits, usually described through the analysis of fruit firmness, seems to be also reduced by grafting in tomato, the results in this regard are not always unambiguous (Khah et al., 2006; Kyriacou et al., 2017; Riga, 2015). This is not surprising considering the multiple mechanisms involved, including $Ca^{++}$ concentration, $K^{+}$/ $Ca^{++}$ interaction, water relations, cell-wall proteins and cell-wall structure and solubilisation (Leonardi et al., 2017; Saladie et al., 2007).

Conflicting findings have also been reported about grafting effects on total soluble solids (TSS), and titratable acidity (TA); anyway, a decrease in TSS and an increase in TA in tomato grafted onto vigorous rootstocks are often observed (Barrett et al., 2012; Kumar et al., 2015; Kyriacou et al., 2017; Schwarz et al., 2013). The TSS decrease induced by vigorous rootstocks can be due to increased crop load, sink strength of the larger root system or dilution effects in the fruits due to higher water uptake efficiency of the rootstock. Moreover, rootstock-induced changes in the time of flowering, fruit load and ripening timeline can occur especially for interspecific grafting, since intricated long-distance communications mediated by hormones and nucleic acids exchanges between scion and rootstock do exists. In particular, when similar changes occur, the sampling procedures should be carefully standardized to avoid misleading results (Kyriacou et al., 2017). The cause of the increase in TA often reported has not been fully elucidated, however, an increase in organic acids in developing fruits could be a compensatory mechanism to provide respiratory substrates under low sugar content (Kyriacou et al., 2017; Leonardi and Giuffrida, 2006).
Among tomato functional compounds, the majority of researches report a reduction in ascorbic acid compared to un-grafted or self-grafted plants (Djidonou et al., 2016; Di Gioia et al., 2010; Riga et al., 2016; Vinkovic-Vrcek et al., 2011). The reasons are not clear but a redistribution or an accumulation in other parts of the plant has been suggested (Kyriacou et al., 2017).
The fact that many of the results reported about the effect of tomato grafting on yield and fruit quality are conflicting is related to the high number of factors involved, including environmental conditions, rootstocks genetic origin. Moreover, many of the researches in this field have been conducted using different scion cultivars or even types of tomato, that makes not always possible to compare the relative findings indiscriminately. In the past, the most popular approach was to examine the effects of different rootstocks on one or few scions; although giving useful information, this may not consider the typical characteristics of the scion, which actively supports the rootstock with photosynthesis products and is part of an intricate network of hormonal communications with the root system. To observe the rootstocks-induced changes in tomato yield and fruit quality without missing the scion active role, large experimental designs including many rootstocks-scion combinations could be useful.
The present experiment was designed to achieve a deep examination of the effects on yield and quality of seven cherry tomato cultivars, grafted onto eight rootstocks and compared with un-grafted plants. Among the large number of rootstocks today available for tomato, hybrids of *S. lycopersicum* × *S. habrochaites* are the most popular, however hybrids between tomato and other related species together with intraspecific tomato rootstocks have been released by breeding companies (Fullana-Pericàs et al., 2018; King et al., 2010; Kyriacou et al., 2017). For that reason, interspecific hybrids between tomato and *S. habrochaites*, *S. peruvianum* and *S. pimpinellifolium*, as well as an intraspecific hybrid have been used.
Above ground biomass partitioning, yield and its components have been recorded. Quality traits including colour, firmness, TSS, TA and ascorbic acid have also been analysed.
Besides the classic results discussion in relation to the specific grafting combinations, a new approach was adopted to analyse the large amount of data obtained in order (1) to highlight to what extent each examined variable was actually affected by rootstock and scion respectively, both in an overall analysis of the scions and focusing on the most common group of productive vigour (intermediate) and (2) to understand the active role of the scion in controlling yield and quality traits changes due to the rootstock, by quantifying the ability of each scion to impose its typical characteristics for the different examined variables.
This will also answer if potential cultivar able to impose their characteristics, would do that in a variable-dependent or -independent way.

## Materials and methods

### Site and growing cycle

The experiment was conducted in Sicily (Italy) (36°59'11.9" N; 14°21'35.8" E; 40 m a.s.l.) in an unheated greenhouse of around 1000 $m^2$ with sandy soil and an electrical conductivity of the water of 2.5 mS $cm^{-1}$. Transplant date was 05/10/2016 and 2.5 plants $m^{-2}$ were used, growing two stems per plant; the cultivation cycle lasted for 224 days, with a production of 11 trusses per stem.

***Plant material and experimental design***

Scions and rootstocks used in the experiment are reported in **Table 1**. From previous information, productive vigour data of un-grafted plants under comparable growing conditions for a number of cherry tomato cultivars were known. Among the seven scions included in the experiment, two had an average fruit diameter around 26-29 mm, four of 31-34 mm and one of 34-37 mm, and a low, intermediate and high productive vigour respectively.

Since they represent the majority of the rootstocks today available for tomato, four *S. lycopersicum* × *S. habrochaites* were used, together with two *S. lycopersicum* × *S. peruvianum* one *S. lycopersicum* × *S. pimpinellifolium* and one intraspecific tomato hybrid, whereas un-grafted plants were used as a control. A randomized blocks design was used with 63 rootstock/scion combinations and 3 replicates for each combination.

***Biomass, yield and fruit quality***

Dry matter of the different parts of the plant was obtained with an oven at 70 °C until constant weight. The dry weight of leaves was determined at each leaf removal and stem dry weight was determined 224 days after transplant. The fresh weight of the fruits was recorded at each harvest time; moreover, in the second and third trusses, all the fruits were counted to calculate the average weight. No changes occurred in fruit weight throughout the growing cycle, as it was also determined from the fourth truss to the end of the experiment considering representative samples (5 kg per replication at least). To assess potential differences in fruit ripening timeline the maturation of the first two trusses was monitored to harvest the third truss at a homogeneous stage of ripening. However, the differences observed during the first truss maturation really lowered for the second truss and almost disappeared for the third one. Fruit colour was measured by a Minolta colorimeter mod. CR-200; the firmness of the fruits was measured by a texture analyser (Stable Micro Systems model TA-XT2). Total soluble solids (TSS) content was determined by a digital refractometer DBX-55° (Atago CO, Ltd, Japan) provided with an automatic temperature compensation system. Titratable acidity was determined by neutralization of the free acids with a titration solution of 0.1 N NaOH up to the changing colour of phenolphthalein; the results were then expressed as mg/L of citric acid equivalents.

For the ascorbic acid determinations, 0.1 g of freeze-dried material was extracted in 2 ml of $H_3PO_4$ 0.05 N through sonication for 6 minutes. After centrifugation at 4 °C for 15 minutes at 13,000 ×g, the supernatant was collected, filtered through 0.45 μm nylon filters and analysed by HPLC-UV (λ = 245 nm) equipped with an autosampler. The mobile phase was $KH_2PO_4$ buffer at 2.3 pH, and a reverse phase C18 column has been used, with a 0.5 ml $min^{-1}$ flow rate. Peak areas were converted to ascorbic acid through a standard curve prepared with L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA).

***Statistical analysis***

The homoscedasticity of the variances was assessed through Bartlett's test and, when it was necessary, the data were log-transformed to allow the application of further parametric analysis. The differences among treatments were determined by a two-way ANOVA, and the multiple mean

comparisons were performed using Student–Newman–Keuls test (at least for P = 0.05); these analyses were performed through CoStat 6.451 (CoHort).
In the analysis of the scion and rootstock relative contributions, the sum of squares (SS) data obtained from the two-way ANOVA were used. The total SS includes the explained sum of square as well as SS of the residual error. The first tells how much of the variation in a certain dependent variable is explained by the proposed model and includes the SS explained by each factor, their interaction and repetitions. The SS explained by scion (*S*), rootstock (*R*) and their interaction (*S*×*R*) were one by one divided to total SS and expressed as a percentage, both considering all the grafting combinations or only the ones relative to the intermediate vigour rootstocks.
The coefficients of variation defined as the ratio between the standard deviation and the mean were calculated through Minitab 16 statistical software (Minitab Inc.) and reported as a percentage.

## Results

***Biomass, harvest index and leaf area***

A strong interaction between factors was observed in the biomass accumulation and partitioning, harvest index and total leaf area (**Table 2**). Regarding total epigeous biomass accumulation, the main reason for the significant interaction was that differently to the other scions Caprice epigeous biomass production was significantly lower in grafted plants. This reduction ranged from -7.0 % when grafted onto Optifort, Kaiser, Interpro and Pittam, to -13.5 % when grafted onto Mozart, Bental, Dynafort and 8614.
Total aerial biomass production did not reflect the productive vigour of the scion neither in grafted nor in un-grafted plants, as the lowest values were recorded in 5525 un-grafted or grafted onto Mozart and Dynafort (**Figure 1**). On the contrary, the epigeous biomass production in un-grafted Caprice (low productive vigour) was the same as Porpora, and higher than all the other un-grafted scions.
Among the other scions, several grafting-combination increased dry biomass, especially in Eletta, where it ranged from +7.7 % when grafted onto the intra-specific rootstock 8614 to +27.5 % in the average of *S. lycopersicum* × *S. habrochaites* rootstocks Kaiser and Interpro. This cultivar showed no increase in biomass production only when grafted onto Dynafort (*S. lycopersicum* × *S. pimpinellifolium*). Except for Caprice, each tested scion showed higher biomass when grafted onto Optifort, compared with un-grafted plants. This increase ranged from +10.2 % in Dreamer to +23.6 % in Creativo. However, although with few exceptions in 5525, Dreamer and Beka, also grafting onto the other *S. lycopersicum* × *S. habrochaites* increased epigeous biomass production.
The biomass accumulated in leaves and stems (vegetative fraction) represented around 65-70 % of the total epigeous biomass, so it largely followed a similar trend (**Figure 2**). However, Caprice showed, in this case, a higher biomass accumulation in the vegetative portions when grafted onto Optifort and Interpro (+10 % in the average) compared to control. For this variable, in fact, plants grafted onto Optifort showed increased values in every scion when compared to un-grafted plants (from +9.5 % in Caprice to +47.0 % in Creativo). Grafting onto *S. lycopersicum* × *S. habrochaites* rootstocks increased biomass allocation in vegetative structures in the majority of grafting combination, for example in Porpora it determined a +26.8 % increase in the average of the rootstocks. Also grafting onto *S. lycopersicum* × *S. peruvianum* hybrids was often associated with increases in dry matter accumulation in vegetative parts, with a maximum in Porpora grafted onto Pittam (+35.0 %), and only in Caprice grafted onto Bental significant reduction was observed (-13.0 %). Variations compared to un-grafted ranged from +16.4 % (Creativo) to -10.9 % (Caprice) when the intraspecific hybrid 8614 was used.
The biomass accumulation in the trusses (reproductive fraction) did not reflect the productive vigour of the scion (**Figure 3**). For example, in un-grafted plants, Beka showed no differences with 5525, Dreamer and Eletta; moreover, even un-grafted Caprice total dry matter accumulation in fruits was not different from the one of Porpora. However, it was strongly reduced in grafted

Caprice compared to un-grafted plants (from 16.3 % to 35.4 % depending on the rootstock). Biomass accumulation in fruits was lowered by grafting onto Interpro, Mozart and Dynafort in Porpora (-19.1 % in the average).
Among the intermediate productive vigour group of scions and in Beka, the few cases of reduction in dry matter accumulation in fruits occurred in plants grafted onto *S. lycopersicum* × *S. habrochaites* hybrids (-13.3 % in Creativo grafted onto Optifort; -14.4 % in Dreamer in the average of grafting onto Optifort, Kaiser and Mozart, and -14.2 % in Beka grafted onto Optifort). The only increase in biomass allocation in the reproductive fraction of the plant were observed in the intermediate vigour group, and more specifically, in 5525 grafted onto the intra-specific rootstock 8614 (+12.0 %), Dreamer grafted onto Interpro (+11.0 %) and Eletta grafted onto Pittam and 8614 (15.6 %).
A strong interaction was reported between main factors for harvest index (HI). The majority of grafting combinations showed lower HI values as compared with un-grafted plants (**Figure 4**). The highest HI reductions for each scion ranged from -8.2 % in 5525 grafted onto Interpro and Bental, to -29.9 % in Creativo grafted onto Optifort. However, Porpora showed no decrease in HI from the control when grafted onto Bental. In a similar way, no reduction was reported in Creativo grafted onto Bental and Dynafort, 5525 grafted onto Kaiser, Mozart, Dynafort and 8614, Dreamer grafted onto Interpro, Pittam, Dynafort and 8614, and Eletta grafted onto Pittam, Dynafort and 8614. Among the low productive-vigour scions, HI levels of un-grafted plants were maintained in Beka grafted onto Interpro, Pittam, Dynafort and 8614 and in Caprice grafted onto Bental and onto the intra-specific rootstock 8614.
The total leaf area of the different scions sometimes showed different responses when grafted on the same rootstock and it made the scion-rootstock interaction (*S*×*R*) significant (**Figure 5**). For example, Caprice grafted onto Optifort showed no difference with the un-grafted but, in every other scion grafted onto the same rootstock a strong increase was observed (ranging from +21.7 % in 5525 to +108 % in Creativo). In Porpora, Creativo and Eletta leaf area was always higher in grafted plants compared to the un-grafted, although to a different extent depending on the rootstock used. The highest leaf area values for the different scions always included grafting combinations with some *S. lycopersicum* × *S. habrochaites* hybrid.

***Yield and its components***

Significant interactions between the main factors were observed for yield and its components as reported in **Table 3**. The yield of un-grafted plants was consistent with the productive vigour expected basing on previous studies, except for Eletta and Caprice that showed similar values (112 t/ha in the average) (**Figure 6**). However, when grafted onto appropriate rootstocks, the productivity of the scions belonging to the intermediate vigour group reached yield values not statistically different from un-grafted Porpora (137 Mg $ha^{-1}$). In Creativo it was observed regardless the rootstock used; however, it was also the case of 5525 grafted onto Optifort, Dreamer grafted onto every rootstock used except for Mozart and Dynafort as well as Eletta grafted onto Optifort and Pittam. Porpora showed an average yield of 156 t/ha when grafted onto Optifort, Bental and 8614 (+14.1 % in the average) compared to un-grafted plants. Among the intermediate vigour group, Creativo showed no significantly higher yield when grafted onto Dynafort, whereas in the other combinations were reported an average increase of +13.2 % compared to control. 5525 grafted onto Optifort showed an increase of productivity of +16.0 %, and Dreamer yield was 13.5 % higher than un-grafted plants when grafted onto Optifort, Interpro, Bental, Pittam and 8614. Eletta showed an average +18.0 % of yield increase compared with un-grafted plants when Optifort or Pittam were used as rootstocks. Among the most important reasons of the high significance of the *S*×*R* interaction on yield there was a different effect of grafting on scions characterized by a low productive vigour and by a small size of the fruits compared to the other ones. In Beka and Caprice, in fact, no grafting combination exceeded the yield of un-grafted plants. On the contrary, when grafted onto the *S. lycopersicum* × *S. pimpinellifolium* rootstock Dynafort, they showed a significant

yield decrease compared to the control (-5.8 % and -17.3 % in Beka and Caprice respectively). In Caprice, yield was also reduced to a different extent in plants grafted onto Optifort and Interpro (-14.5 % in the average) and onto Mozart and Pittam (-8.6 % in the average).
The fruit weight was almost unaffected by grafting in the scions belonging to the high and low productive vigour groups (**Figure 7**). In fact, among them, it only slightly increased in Porpora grafted onto Kaiser, Bental and Dynafort (+5.3 % in the average) compared to un-grafted plants.
On the other hand, in the intermediate productive vigour group, which represents the majority of the available cultivars, fruit weight was generally increased by grafting. To a different extent, fruit weight increases were observed in all the grafted combinations of 5525 (+22.9 % when grafted onto Optifort or Kaiser, and +11.1 % in the other grafting combinations) and Eletta (+8.3 % when grafted onto 8614 and +20.1 % in the other grafting combination) compared to un-grafted plants. In Creativo a higher fruit weight compared to un-grafted plants (from +10.7 % to +21.4 basing on the rootstock used) was observed in every grafting combination except when grafted onto Dynafort. However, Dreamer fruit weight was increased only by grafting onto 8614 (+15 %).
In general, fruit number per plant was generally lower in the scions characterized by a higher fruit size and vice versa (**Figure 8**). In Porpora the number of fruits was higher in plants grafted onto Optifort and 8614 (+14.1 % in the average) compared to un-grafted. In Creativo no grafting combination exceeded the number of fruits of un-grafted; on the contrary, plants grafted onto Optifort showed a lower number of fruits (-9.1 %). When grafted onto each *S. lycopersicum* × *S. habrochaites* rootstocks as well as onto Bental and Dynafort, the number of fruits of 5525 was (in the average) -14.2 % compared to un-grafted. In Eletta, grafting onto Interpro, Mozart, Bental and Dynafort also caused a reduction in fruit number compared to un-grafted plants (-10.8 % in the average). In Beka, fruit number was lowered by grafting onto Optifort Dynafort and 8614 (-7.0 %) and increased by grafting onto Interpro (+8.3 %), whereas in Caprice it was reduced by grafting onto Interpro, Dynafort and 8614 (-11.4 % in the average) and increased by grafting onto Kaiser (+9.2 %).

***Quality traits***

Among the quality traits, the two-way ANOVA showed that only the scion was significant for shape index and CIELab coordinates b* and L* (**Table 4**). Total soluble solids (TSS) and sugar/acid ratio (TSS/TA) showed the high significance of both main factors whereas, for titratable acidity, scion was significant and a little *S*×*R* interaction was observed. Both the main factors and their interaction were significant in firmness, a* CIELab coordinate and ascorbic acid content. Shape index was only influenced by the scion, with higher values in 2252, Beka and Caprice (1.20 in the average). Firmness was measured as the strength necessary to determine a 2-mm deformation in the height of the fruits, and it was reported in grams (g). The main influence on firmness has been determined by scion since the only significant reduction in fruits firmness in grafted plants regardless the rootstock used was observed in Creativo (-36.18 % in the average) (**Figure 9**). Among the other grafting combinations, only Porpora grafted onto Interpro (-17.1 %) and Beka grafted onto Kaiser and Dynafort (-28.0 % in the average) showed some significant reduction in firmness compared to un-grafted.
The a* coordinate refers to higher amounts of red as long as it assumes higher positive values. The significant interaction was caused by the fact that a* was occasionally reduced, even strongly, in some specific grafting combination compared to un-grafted plants (**Figure 10**). For example, lower a* values compared to un-grafted were observed in Porpora grafted onto the *S. lycopersicum* × *S. habrochaites* Optifort and Mozart (-11 % in the average), Creativo grafted onto the *S. lycopersicum* × *S. peruvianum* Pittam (-33.6 %), 5525 grafted onto Kaiser, Interpro and Dynafort (-17.3 % in the average) and Eletta grafted onto Mozart (-14.7 %). In a similar way Dreamer and Beka showed significantly lower values of a* when grafted onto the *S. lycopersicum* × *S. peruvianum* Bental (-47.8 and -12.4 % in the average) as well as Caprice grafted onto Optifort and Pittam (-11.9 % in the average). However, the intraspecific rootstock 8614 was the only one that was never associated to

decreases in the a* coordinate of the fruits; on the contrary, the only two grafting combination in which a* was increased compared to un-grafted were 5525 and Eletta grafted onto 8614 (+11.9 and + 14.0 % respectively).
The b* coordinate of CIELab colour space showed higher values in 5525 and Dreamer (19.15 in the average) than Eletta (18.0) in the average of the grafting combinations. The highest value of L* in the average of the grafting combinations was recorded in 5525 (40.7), followed by Dreamer (39.7), whereas Porpora, Eletta, Beka and Caprice showed an average value of 38.4.
TSS differences between scions have been strongly related to the cv vigour, with values almost inversely proportional to the productive vigour and to the mean fruit size. Higher values were observed, in fact, in the scion cultivars with a typical low productive vigour and small fruit size, with the maximum on Caprice (6.25 %) followed by Beka (5.98 %). On the contrary, Porpora, which has got a high fruit size and vigour, showed the lowest value together with 5525 and Dreamer (4.93 % in the average). Un-grafted plants, as well as plants grafted onto the intraspecific rootstock 8614, showed higher TSS (5.78 % in the average) compared to Optifort, Kaiser, Mozart, Pittam and Bental (5.16 % in the average).
TSS/TA ratio was the highest in Eletta (1.73), whereas the lowest value was recorded in 5525 (1.08). Between the rootstocks, un-grafted plants and plants grafted onto 8614 showed a higher TSS/TA ratio (1.42) compared to Kaiser, Mozart, Interpro and Bental (1.28 in the average).
Regarding the ascorbic acid content of the fruits beside the main factors' significance, a strong *S*×*R* interaction was observed. Except for 5525, in which grafting onto the intraspecific 8614 significantly increased it (+29.9 %) compared to un-grafted plants, grafting often caused a reduction in ascorbic acid (AA). Un-grafted Porpora showed the highest content (403 mg $kg^{-1}$) followed by Caprice (345 mg $kg^{-1}$), and they showed the most severe reductions when grafted. AA content in Porpora, for example, showed reductions between -47.1 % (when grafted onto Pittam and 8614) and -65.6 % (when grafted onto the *S. lycopersicum* × *S. habrochaites* Optifort, Kaiser and Interpro). In Creativo and 5525, only grafting onto Optifort reduced AA content compared to un-grafted. In Dreamer this reduction ranged from -23.0 % (in the average of Optifort, Interpro, Mozart, Pittam, Dynafort and 8614) to -46.0 % (when grafted onto Kaiser), whereas in Eletta only Optifort (-38.9 %) and the *S. lycopersicum* × *S. peruvianum* Bental and Pittam (-22.7 %). AA content was lower in Beka grafted onto Optifort, Kaiser, Mozart and Bental (-28.5 % in the average) than in un-grafted plants, whereas all the grafting combinations affected AA accumulation in fruits compared to control in Caprice (-45.5 %).

***Contribution of scion, rootstock and their interaction***

For each dependent variable, the percentage of SS explained by scion, rootstock and their interaction considering all the scions (overall analysis) and the intermediate productive vigour group are reported in **Tables 5** and **6** respectively. Total epigeous biomass, its vegetative component and total leaf area were mainly determined by scion both in the overall analysis (55.9, 45.8 and 42.6 % respectively), and in the intermediate productivity group (44.8, 37.3 and 41.6 % respectively).
Although reproductive fraction, representing the total dry matter accumulated in the different trusses, and the harvest index were strongly dependent on *S*×*R* interaction (36.4 and 34.7 %) in the overall analysis, the relative importance of interaction was reduced when only the intermediate productivity group of scions was considered. In this case relative contributions of *S*, *R* and *S*×*R* on reproductive fraction SS were much more equilibrated (26.8, 20.1 and 25.1 % respectively), and the percentage of Harvest index explained by the rootstock exceeded the other ones (42.3 % vs 23.9 and 32.5 % for *S* and *S*×*R* respectively).
The scion determined almost the entire explained SS of yield and its components (77.6, 83.1 and 94.0 % for yield, number of fruits and fruit fresh weight) in the overall analysis but lost a lot of importance when the only intermediate vigour scions were examined. In this group S and R had almost the same importance for yield (27.1 and 29.6 % respectively), and fruit number (13.7 and

15.1 % respectively). However, even though also in the intermediate group fruit weight depended mainly form the scion (31.2 %), the relative contribution of *R* and *S*×*R* increased dramatically compared to the analysis conducted on all the scion-vigour groups (from 0.6 to 19.5 % and from 2.2 to 19.2 % respectively). The only significant influence in shape index SS was the one of the scions in both the analysis, with 51.9 and 43.4 % in the overall analysis and in intermediate vigour group respectively. The percentage of firmness SS explained by the factors and their interaction, almost didn't change between the two groups, with a clearly higher contribution of scion (45.4 % in the average).
Scion contribution was also significant for all the colour CIELab coordinates, whereas rootstock and *S*×*R* interaction was only significant for a*. The a* SS explained by scion was 47 % when all the scions were considered, and 25.9 % for the intermediate vigour group. The *R* and *S*×*R* contribution to a* SS showed the opposite trend, increasing from 8.3 to 12.4 % and from 16.0 to 23.4 % respectively, passing from the overall analysis to the intermediate vigour group.
The scion showed higher contribution to the explanation of TSS sum of squares (45.1 %) in the overall analysis, but it lost importance in the intermediate vigour scion group (13.3 %) in favour of the rootstock (29.3 %). Regarding TA and TSS/TA ratio, the scion determined the higher amount of explained SS regardless the group of scions considered.
On the contrary, the ascorbic acid content in the fruits was mainly determined by the rootstocks both in the overall scion analysis (34.5 %) and in the intermediate vigour group (30.2 %).

***Coefficients of variation***

Aiming to quantify the ability of each scion to impose its typical characteristics, the coefficients of variation for the different examined variables were analysed. However, the potential ability to left unchanged its typical traits despite the rootstocks induced alterations in many physiological processes, can both represent a strength or a weak point depending on the variable examined. In fact, for the different variables the main goal could be to obtain some grafting-induced change or not; for example, yield increase is desired, but the maintenance of high-quality traits showed by some cultivar when un-grafted is desired in grafted plants as well. Moreover, in the latter case, a really low CV assumes importance only when un-grafted plants show excellent traits to maintain; for these reasons the CVs results for the different tested variables will be successively analysed considering also the un-grafted absolute values and the context.
The coefficients of variation of biomass production and partitioning, harvest index and total leaf area are reported in **Figure 13**. Among the scions, 5525 showed the lowest CV in total epigeous biomass (5.02 %) (**Figure 13 a**), biomass accumulation in leaves and stems (7.85 %) (**Figure 13 b**) and in fruits (6.65 %) (**Figure 13 c**) as well as in the harvest index (7.49 %) (**Figure 13 d**). However, in biomass production, the CV never exceeded 10 %, with the highest values in Creativo and Eletta (9.12 and 9.32 %). Representing the 65-70 % part of aerial biomass, vegetative fraction CV followed the same trend of total epigeous biomass, with the highest value reported in Creativo (14.37 %). Regarding biomass accumulation in fruits, the higher variability was observed in Caprice (15.78 %), and the lowest values were the ones of the above mentioned 5525 and Beka (6.98 %).
The CVs of total leaf area indicated almost the double of variability in Creativo (highest value; 21.21 %) compared to Caprice (lowest value; 10.43) (**Figure 13 e**).
The CV of yield was 7.35 % in Porpora and ranged from 5.87 % to 6.48 % between the intermediate vigour group of the scions (**Figure 14 a**). Caprice and Beka showed the highest (8.34 %) and the lowest (4.65 %) values respectively. Porpora was the only scion cultivar showing opposite trend between the CVs of the fruits number and of the fruit weight (7.59 % vs 4.67 %). 5525 and Eletta showed higher variability than Creativo and Dreamer among the intermediate productive vigour group (**Figures 14 b; 14 c**). In a similar way, CVs of the number of fruits and fruit weight was lower in Beka (6.96 and 5.37 % respectively) than in Caprice (11.32 and 9.25 % respectively).

Variability in shape index was very low and its CVs ranged from 1.9 to 3.4 % in the different scion cultivars (data not shown). The CV of firmness was maximum in Creativo (23.22 %) and minimum in 5525 (13.09 %), however, also Beka, Dreamer and Eletta showed a CV higher than 20 % (22.14, 21.51 and 20.11 % respectively) (**Figure 15**).
Among fruits CIELab coordinates, the a* coefficients of variability (CV) showed higher values compared to L* and b* (**Figure 16**). Lightness showed only little variability with CVs that never reached 5%; ranging from the minimum of 2.78 % in Caprice to the maximum of 4.76 % in Dreamer. CV of b* was also relatively low ranging between the minimum value of Eletta (5.54 %) to 8.76 % in Porpora. However, the a* CVs highlighted different variability among scion genotypes, with a minimum (8.54 %) in Caprice and the highest value in Dreamer (22.86 %). Creativo and 5525, also showed a relatively high a* CV (18.6 and 18.8 % respectively).
The coefficients of variation for TSS, TA and their ratio are reported in **Figure 17**. TSS CVs ranged from 7.70 % in 5525 to 12.33 % in Dreamer; minimum CV for TA was 10.48 % reported in Eletta whereas 5525 and Creativo had the highest values (14.09 and 14.39 % respectively). Regarding CVs of TSS/TA ratio, really low differences were found among scions, with a minimum in Dreamer (10.05 %) and the highest values in Caprice and 5525 (12.44 and 12.65 % respectively).
The CV of ascorbic acid content highlighted a strong variability in Porpora (42.19 %), followed by Caprice (30.70 %). The remaining scions had a CV ranging from 19.27 to 22.95 % for this variable (**Figure 18**).

## Discussion

Except for Caprice, that probably underwent grafting incompatibility events, grafted plants often showed higher biomass accumulation. When similar increases in total epigeous biomass occurred, they were due to a higher biomass in the vegetative parts of the plants but biomass allocation in the fruits was usually reduced or at least maintained in grafted plants. Among the few exceptions, in 5525 and Eletta biomass accumulation in the reproductive structure was higher in plants grafted onto the intraspecific rootstock 8614.
The higher biomass accumulation in leaves and stems in every scion when grafted onto Optifort and the higher epigeous biomass production that we in most cases observed in plants grafted onto *S. lycopersicum* × *S. habrochaites* hybrids, is consistent with what previously reported for other *S. lycopersicum* × *S. habrochaites* rootstocks (Leonardi and Giuffrida, 2006). The rootstocks obtained from its breeding with *S. habrochaites* represent the larger part of the available rootstocks for tomato since their high tolerance to many biotic stresses promoted their spread through the years although higher vegetative tendency of plants grafted onto these vigorous rootstocks is often observed.
However, as expected, the responses were often dependent on the specific grafting combination and not simply rootstock-specific, so it was not possible to generalize the expected biomass production and partitioning, as well as vegetative vigour basing on the rootstock botanical origin only.
The high significance of the *S*×*R* interaction in harvest index (HI) was related with the reduced biomass allocation in fruits in Caprice and, at the same time, with the higher biomass accumulation in vegetative parts in many grafting combinations in other scions. However, reduced yield was reported in both the low productive vigour cultivars when grafted onto *S. lycopersicum* × *S. pimpinellifolium*.
Scion explained the higher amount of variability in total epigeous biomass and in the vegetative fraction, however, rootstock and S×R interaction explained more variability in harvest index.
Interesting fact, among the scions, one cultivar (5525) emerged as the cultivar simultaneously showing the lowest CV for epigeous biomass production, its partitioning and HI, although having shown yield increase when grafted onto Optifort, Kaiser and 8614.
Since intrinsic characteristics of cultivar belonging to different productive vigour groups made predictable the strongest scion effectiveness in explaining yield variability on an overall scion

analysis, the attention should be focused in the completely different results observed considering the most common group of productive vigour only (intermediate). In this case, rootstock and scion explained almost the same amount of variability, and the *S*×*R* interaction showed a lower significance. Among the most important reasons of the high significance of the *S*×*R* interaction on yield in the overall scion analysis, there was the different effect of grafting on scions that are characterized by a low productive vigour and by a small size of the fruits compared to the other ones. In Beka and Caprice, in fact, no grafting combination exceeded the yield of un-grafted plants.
Anyway, the majority of the grafting combinations tested for high and intermediate productive vigour cultivars significantly increased yield compared to un-grafted plants. Moreover, yield increase is usually reported to be more pronounced under environmental limiting conditions since the higher tolerance of appropriate rootstocks can help grafted plants to cope with abiotic and biotic stress conditions (Colla et al., 2010; Schwarz et al., 2010). However, according to the main objectives of the study, we tried to avoid the introduction of additional variability due to salinity or high presence of soil pathogens and pests, through an accurate choice of the experimental site. The absence of nematodes infection at the end of the experiment was assessed by plants uprooting. The yield gap between grafted and un-grafted plants could have been even higher under stress condition.
In our case, grafting onto the *S. lycopersicum* × *S. lycopersicum* hybrid 8614 increased yield in all the high and intermediate vigour scion cultivars, and, unlike grafting onto *S. lycopersicum* × *S. pimpinellifolium* Dynafort and some grafting combination with *S. lycopersicum* × *S. habrochaites*, it didn't affect yield in low productive vigour rootstocks. Only in Porpora (high vigour), this increased production was caused by a higher number of fruits set per plant, whereas in all the intermediate vigour scions it was related to an increase in fruits weight.
The high yield CV observed in Caprice could be misleading if not considered together with the absolute yield values of its different grafting combinations. Excluding the yield decrease in the other low productive vigour scion Beka grafted onto the *S. lycopersicum* × *S. pimpinellifolium* Dynafort, in fact, Caprice was the only cv in which grafting-induced reduction of yield was observed. Only when grafted onto Kaiser, Bental and 8614, Caprice had the same productivity of un-grafted plants.
The analysis of the yield CV among the intermediate vigour group of scion cultivars showed similar values instead. In 5525 and Eletta higher CVs were observed in the number of fruits and in fruit weight, but they were due to an increase in fruit weight and to a reduction in fruit number respectively. In this way, through a plant autoregulation mechanism, the final yield gap between grafted and un-grafted was similar to the Creativo and Dreamer one, in turn leading to the similar yield CV above mentioned.
Fruits shape modifications have been reported in grafted tomato together with an increase in fruits size and some authors stated that increases in the ratio between diameter and height of the fruits are usually more evident on scion cultivars characterized by an average fruit weight under 40 g (Kyriacou et al., 2017). However, in our experiment, we didn't observe any change in fruits shape between grafted and un-grafted plants, although fruit weight increases were often observed.
Although the fruit weight was often increased by grafting in the scions belonging to the intermediate productive vigour group, no changes in fruit shape was observed, as demonstrated by the non-significant effect of *R* and *S*×*R* on shape index, and even more by the extremely low CV for this variable (1.9 to 3.4 %) indicating a strong maintenance of the typical shape in all the scions used regardless grafting combination.
Although the texture of the fruits, usually described through the analysis of fruit firmness, is reported to be often reduced by grafting in tomato, especially when vigorous rootstocks are used, the results in this regard are not always unambiguous (Khah et al., 2006; Kyriacou et al., 2017; Riga, 2015). In our experiment the percentage of firmness variability SS explained by the factors and their interaction, almost didn't change between the overall analysis and the intermediate vigour group, with a higher contribution of the scion, followed by *S*×*R* interaction. In our study, the only scion cultivar in which firmness was affected in every grafting combination compared to un-grafted

was Creativo. It showed both the highest value of firmness in un-grafted plants and the strongest grafting-induced loss of firmness, and it caused the significance and the contribution of *S*×*R* interaction. Basing on our findings it seems that the reduction of firmness in grafted cherry tomato plants becomes evident only in cultivar characterized by particularly high firmness values. Moreover, in these cases, it seemed to be almost unaffected by the choice of the rootstock.
When positive, the CIELab coordinate a* refers to higher amounts of red as long as it assumes higher values, finally representing, in our case, the top contributor to the perceived "redness" of the fruits. In red tomatoes both a* and b* assume positive values, however, the higher b* value is, the more perceived colour turns to orange through progressive yellow addition. In the range of data obtained in our experiment higher, also higher L* values represent a deterioration in the colour of the fruits.
The a* coordinate of tomato fruits depends on ripening processes and in carotenoids accumulations. Grafting-induced worsening in tomato colour has been sometimes reported as a result of a reduced carotenoids content by grafting onto vigorous rootstocks (Di Gioia et al., 2010; Nicoletto et al., 2013), however, it was not always confirmed (Schwarz et al., 2013).
Among the rootstocks included in our experiment, the intraspecific rootstock 8614 was the only one that was never associated to decreases in the a* coordinate of the fruits, on the contrary in two scions grafted onto 8614 even an increase in a* coordinate was observed. Among scion cultivars, Eletta, Beka and Caprice, showed the lowest a* CVs and, at the same time, high absolute values of a* in un-grafted plants, indicating the higher ability of these scions to maintain the typical carotenoids accumulation and so reporting an optimal colour almost regardless the rootstock used.
Total soluble solids (TSS) and sugar/acid ratio (TSS/TA) showed high significance of both main factors without interaction, whereas, for titratable acidity, scion was significant and a little *S*×*R* interaction was observed. TSS differences between scions have been strongly related to the cv vigour, with values almost inversely proportional to the productive vigour and to the mean fruit size. Although both scion and rootstock were highly significant, the analysis of the relative contribution to final TSS sum of squares showed a predominant effect of the scion in the overall scion analysis. However, it lost importance focusing on the widespread intermediate vigour only, in which case rootstocks explained much than the double of TSS variability. This shows the higher importance of the right choice of the scion cv, considering that higher productive vigour could be associated with lower TSS but, at the same time, the inaccurate choice of the rootstock can affect TSS content especially when intermediate vigour scion cv is used.
Among the rootstocks lower TSS values were often observed in plants grafted onto the majority of *S. lycopersicum* × *S. habrochaites*, and considering the higher vegetative trend that they also induced, it could be related to an unbalanced requirement of photosynthesis products due to the modification of sink-source ratio. On the other hand, the intraspecific hybrid 8614 often induced yield increases, but showed no induced decrease in TSS; basing on these results, to introduce useful traits in introgression lines that can lead to the selection of *S. lycopersicum*-like parental lines could be a useful way to obtain candidate rootstocks to increase yields without affecting fruit quality traits.
Fruits TA and TSS/TA ratio variability, in turn, were mainly explained by scion both in the overall analysis and in the intermediate vigour group analysis. The significance of *S*×*R* interaction in TA was related to a scion vigour-dependent trend of grafted plants TA compared with un-grafted ones. More specifically cv with a low productive vigour usually showed a strong TA increase when grafted, but it is not consistent with the proposed role of organic acids accumulation as a mechanism to cope with the grafting-induced decrease in fruits TSS, since no significant grafting-induced TSS reduction was observed in this two cultivars (data not shown).
The percentage of variability in the ascorbic acid content of the fruits explained by rootstock was higher than the one explained by scion or by *S*×*R* interaction, and this is consistent with the strong grafting-induced decrease reported in literature (Kyriacou et al., 2017). AA synthesis and accumulation is reported to be strongly dependent on the intensity, duration and quality of light;

specifically, it is reported to increase together with light intensity (Dorais, Ehret, and Papadopoulos, 2008). The analysis of CV showed that Porpora was not able to maintain its high typical AA content in fruits when grafted. Although it is not possible to know exactly to what extent it was contributory, in Porpora the high decrease in the AA content of the fruits was correlated with an increase in total leaf area, that could have strongly reduced the amount of light reaching the fruits.

## Conclusions

Grafting influenced biomass production and partitioning, as well as yield and quality of cherry tomato, however, the results have been often dependent on the specific scion-rootstock combinations.

The analysis of the SS explained by each factor and by their interaction allowed to identify some variables in which the role of the scion (e.g. fruit shape, firmness and TA) or the rootstock (e.g. AA content) prevailed in determining the final characteristics of the grafted plant and its product yield and quality.

Moreover, the analysis of the coefficients of variation (CVs), compared to the absolute values observed in each grafting combination, provided information regarding the ability of each scion to impose its typical characteristics for the different examined variables and on its potential benefits.

The rootstock seems not to act independently on various characteristics of the plant and of the fruit quality and a complex reciprocal effect of the scion seems to be confirmed. However, new insight on the relationship between scion productive vigour and grafting onto different hybrids have been provided for cherry tomato, together with an approach that aimed to highlight scion active role in quality traits maintenance in grafted plants and that could be implemented in the following researches on this topic that still remain necessary.

**Table 1** – Scions and rootstocks used grouped by their productive vigour and their botanical classification respectively.

| Scion | Productive vigour | Company |
|---|---|---|
| **Porpora** | High | Esasem |
| **Creativo** | Mean | Clause |
| **5525** | Mean | Axia seeds |
| **Dreamer** | Mean | Nuhems |
| **Eletta** | Mean | Top seeds |
| **Beka** | Low | Top seeds |
| **Caprice** | Low | Top seeds |
| | | |
| **Rootstock** | **Hybrid** | **Company** |
| **Optifort** | *S. lycopersicum* × *S. habrochaites* | Monsanto |
| **Kaiser** | *S. lycopersicum* × *S. habrochaites* | Rijk Zwaan |
| **Mozart** | *S. lycopersicum* × *S. habrochaites* | Royal seeds |
| **Interpro** | *S. lycopersicum* × *S. habrochaites* | Vilmorin |
| **Bental** | *S. lycopersicum* × *S. peruvianum* | Top seeds |
| **Pittam** | *S. lycopersicum* × *S. peruvianum* | Top seeds |
| **Dynafort** | *S. lycopersicum* × *S. pimpinellifolium* | Seminis |
| **8614** | *S. lycopersicum* × *S. lycopersicum* | Top seeds |

**Table 2** – Main effects on epigeous dry biomass production and partitioning, harvest index and leaf area

| | Total epigeous biomass (g plant$^{-1}$) | | Vegetative fraction (g plant$^{-1}$) | | Reproductive fraction (g plant$^{-1}$) | | Harvest index (%) | | Total leaf area (×1000 cm$^2$ plant$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Scion** | | | | | | | | | | |
| Porpora | 1108.9 | a | 772.6 | a | 336.2 | a | 30.41 | d | 59.7 | a |
| Creativo | 957.1 | c | 629.0 | c | 328.0 | a | 34.59 | a | 43.1 | d |
| 5525 | 826.9 | f | 542.3 | e | 284.6 | c | 34.48 | a | 39.5 | e |
| Dreamer | 901.7 | e | 599.1 | d | 302.6 | b | 33.67 | ab | 46.7 | c |
| Eletta | 1006.4 | b | 706.9 | b | 299.4 | b | 30.04 | d | 56.6 | b |
| Beka | 933.3 | d | 638.9 | c | 294.3 | bc | 31.78 | c | 48.1 | c |
| Caprice | 899.2 | e | 599.9 | d | 299.3 | bc | 33.26 | b | 47.0 | c |
| **Rootstock** | | | | | | | | | | |
| Optifort | 1004.5 | a | 709.9 | a | 294.6 | d | 29.33 | d | 58.3 | a |
| Kaiser | 1007.2 | a | 709.0 | a | 298.2 | cd | 29.95 | cd | 54.2 | b |
| Interpro | 990.1 | a | 687.8 | b | 302.3 | cd | 30.71 | c | 50.7 | c |
| Mozart | 955.3 | b | 664.5 | c | 290.7 | d | 30.67 | c | 50.1 | c |
| Bental | 939.2 | bc | 628.5 | d | 310.6 | bc | 33.29 | b | 47.8 | d |
| Pittam | 955.4 | b | 640.9 | d | 314.5 | abc | 33.19 | b | 48.2 | d |
| Dynafort | 866.8 | e | 574.4 | ef | 292.4 | d | 33.81 | b | 47.2 | d |
| 8614 | 917.7 | c | 589.8 | e | 327.9 | a | 36.05 | a | 41.5 | e |
| Un-grafted | 892.5 | d | 566.4 | f | 326.1 | ab | 36.43 | a | 39.9 | e |
| *Significance* | | | | | | | | | | |
| *Scion* (*S*) | *** | | *** | | *** | | *** | | *** | |
| *Rootstock* (*R*) | *** | | *** | | *** | | *** | | *** | |
| *S* × *R* | *** | | *** | | *** | | *** | | *** | |

*P <0.05, **P <0.01 or ***P <0.001, respectively. n.s., not significant. For each column and experimental factor, mean values labelled with the same lowercase letter did not differ statistically according to the Student–Newman–Keuls (P<0.05).

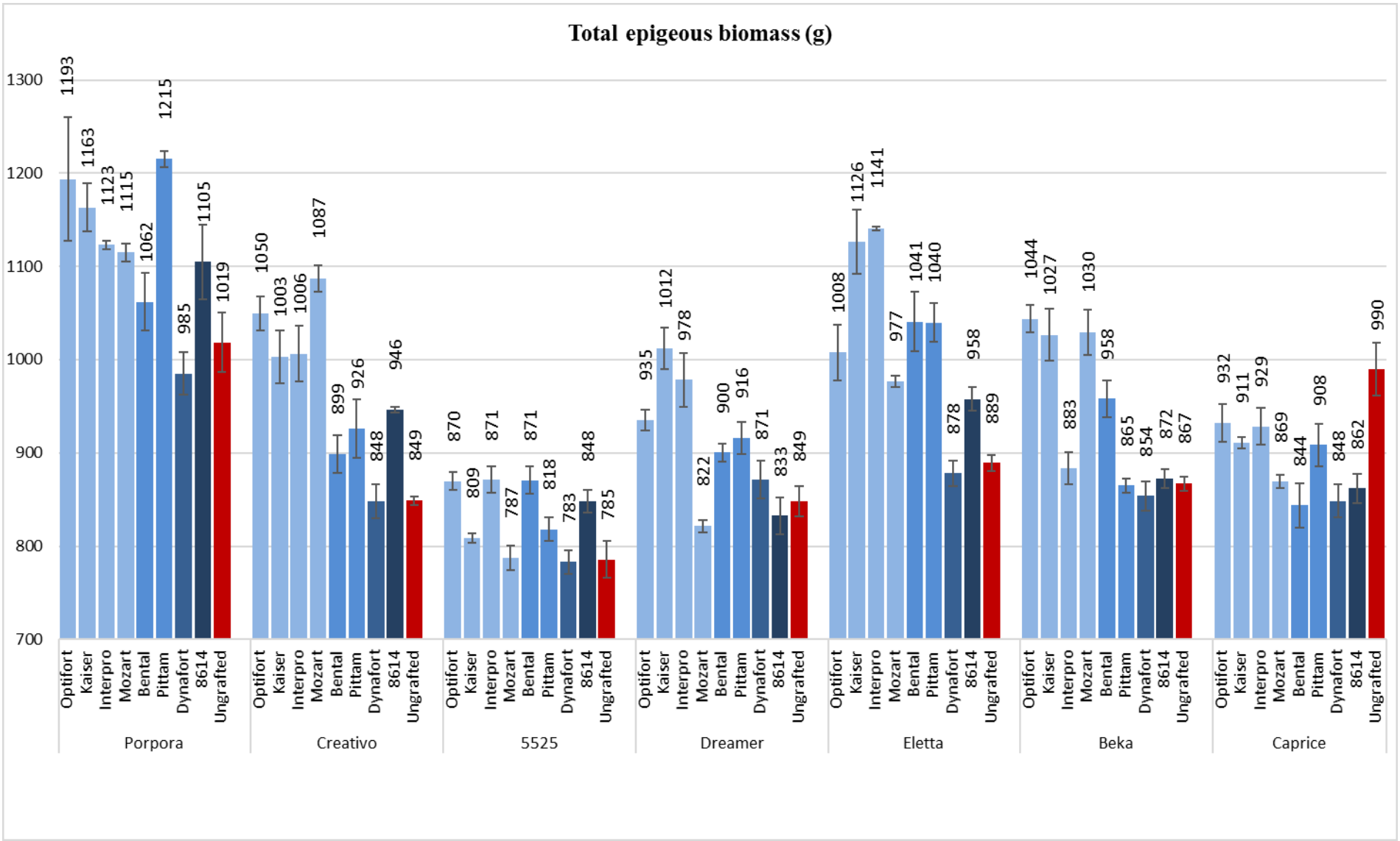



**Figure 1** – Total epigeous biomass production in the different grafting combinations (means ± SE).

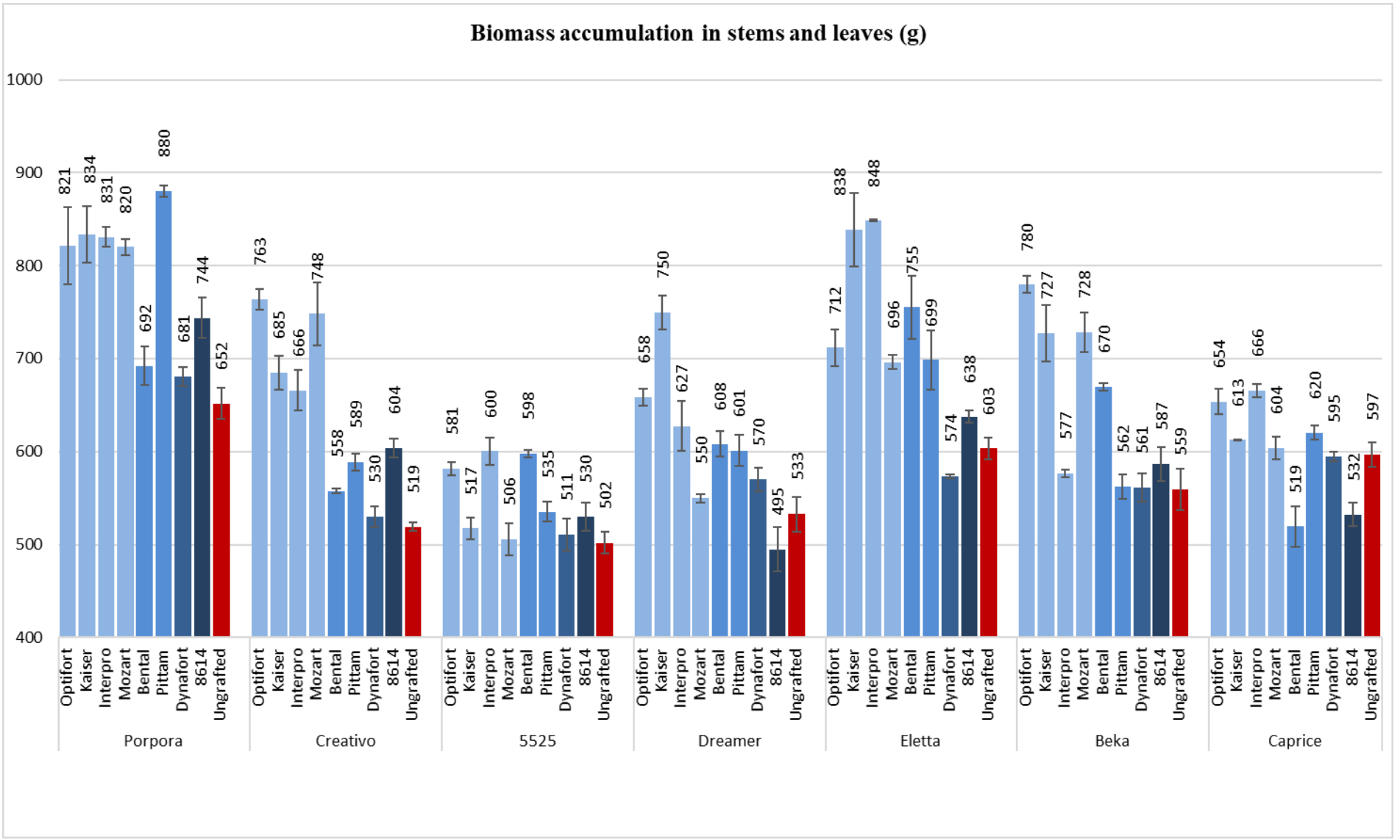



**Figure 2** – Biomass accumulation in stem and leaves in the different grafting combinations (means ± SE).

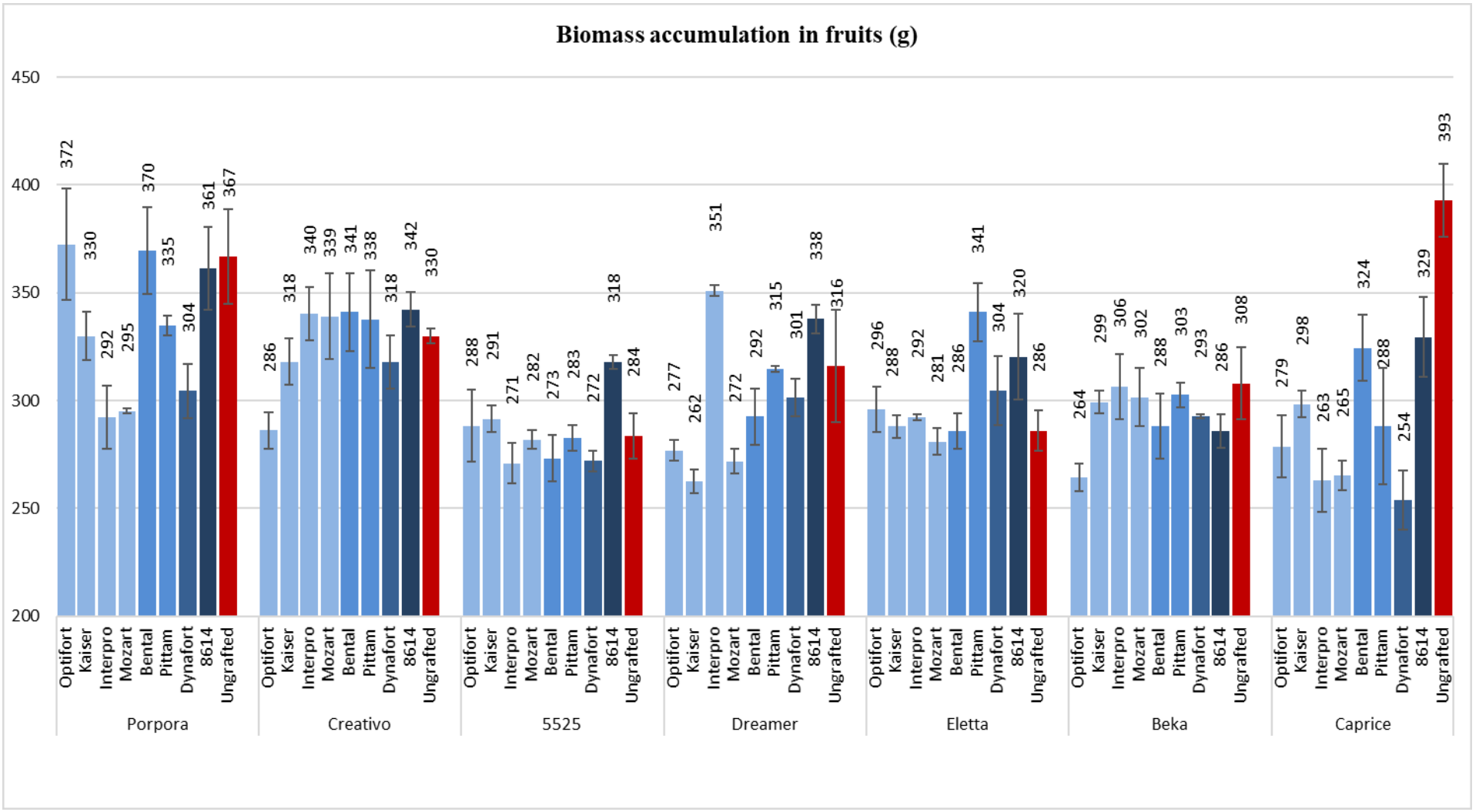



**Figure 3** – Biomass accumulation in the fruits in the different grafting combinations (means ± SE).

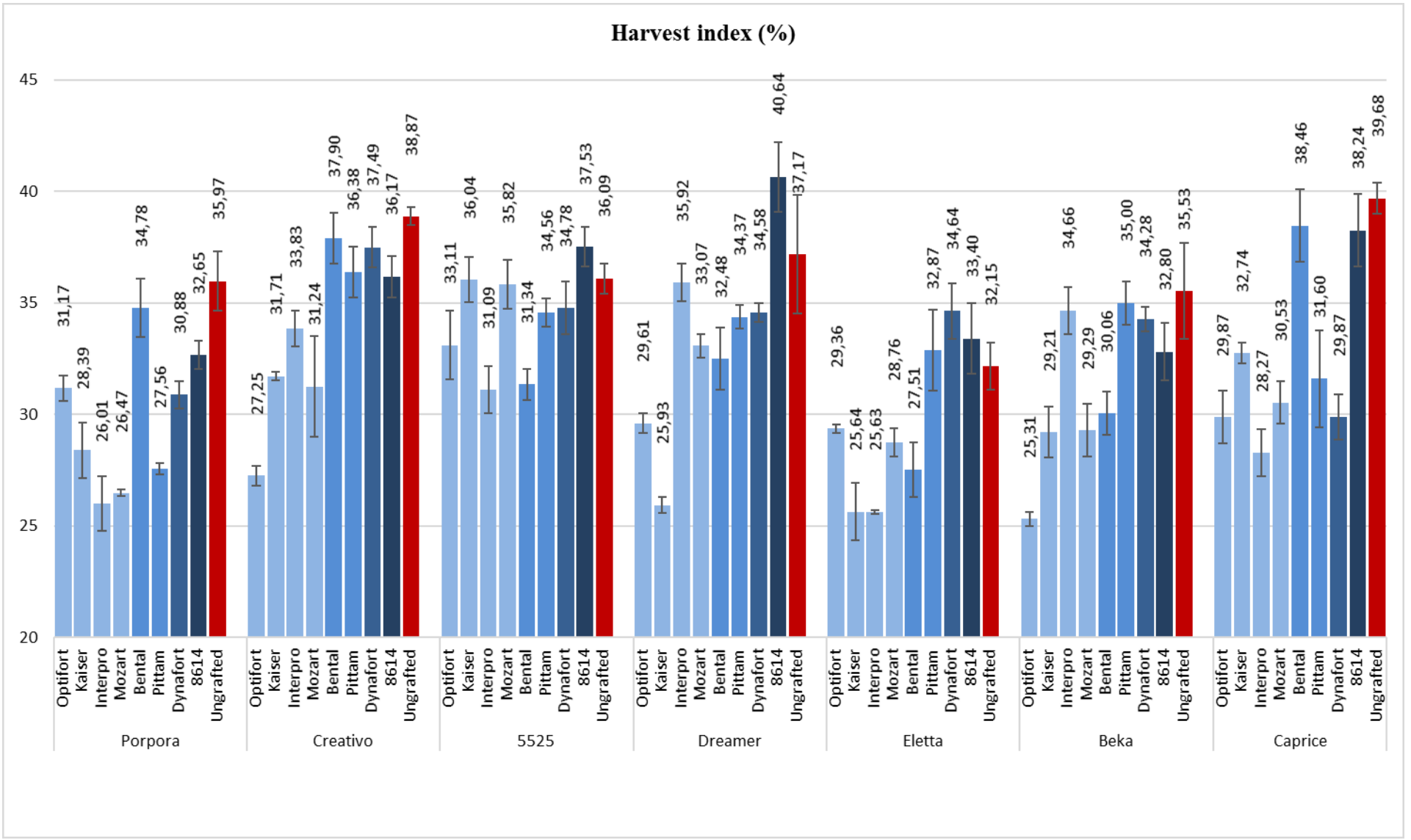



**Figure 4** – Harvest index of the different grafting combinations (means ± SE).

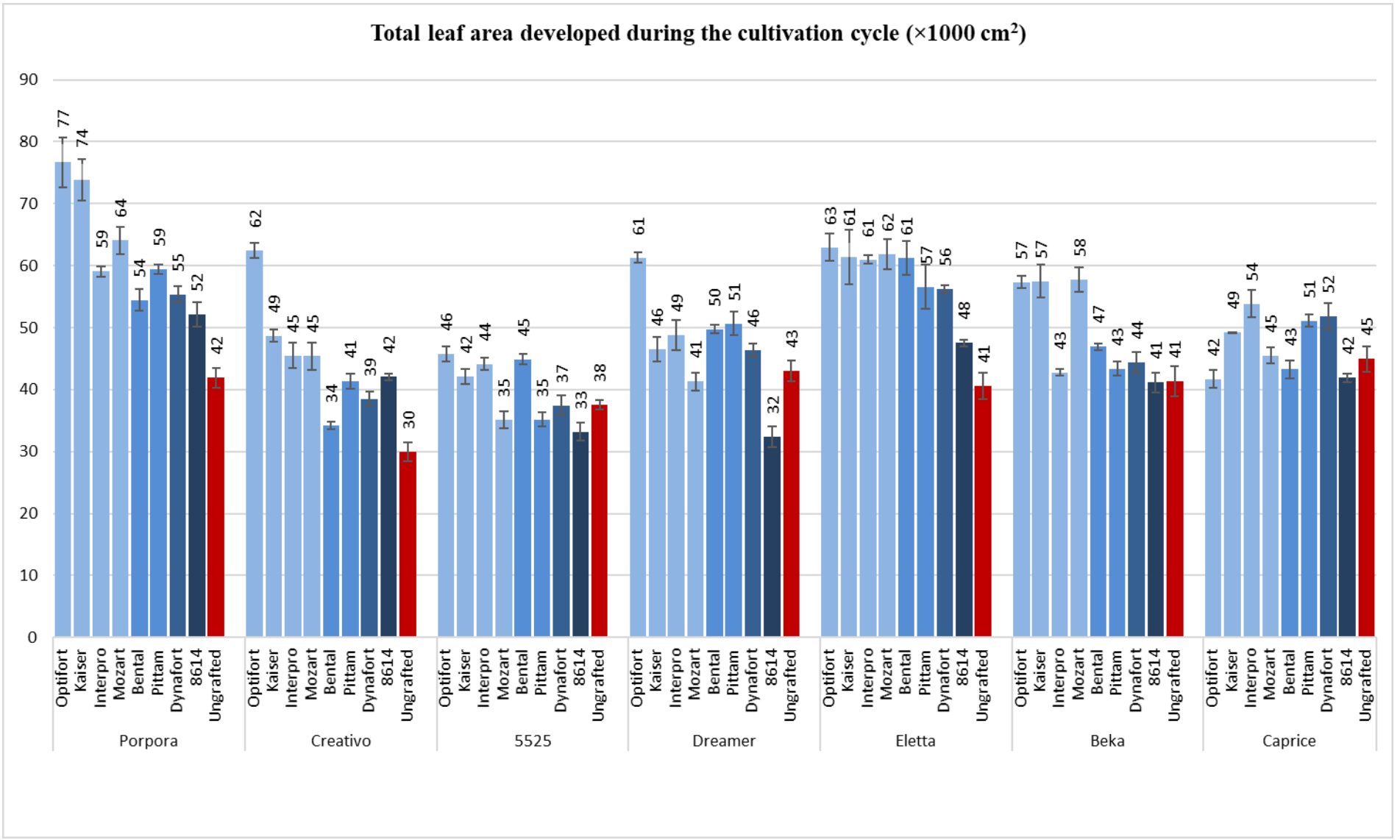



**Figure 5** – Total leaf area developed throughout the cultivation cycle in the different grafting combinations (means ± SE).

**Table 3** – Main effects of scion and rootstock on plant yield and on its components.

| | Yield | | Number of fruits | | Fruit fresh weight | |
|---|---|---|---|---|---|---|
| | (Mg ha$^{-1}$) | | (n) | | (g) | |
| **Scion** | | | | | | |
| Porpora | 145.9 | a | 227.7 | d | 25.3 | a |
| Creativo | 135.2 | b | 256.1 | c | 20.9 | b |
| 5525 | 123.3 | d | 258.8 | c | 18.7 | c |
| Dreamer | 130.9 | c | 272.2 | b | 18.9 | c |
| Eletta | 124.7 | d | 273.4 | b | 18.2 | c |
| Beka | 103.6 | e | 376.0 | a | 11.0 | d |
| Caprice | 101.8 | e | 368.1 | a | 11.0 | d |
| **Rootstock** | | | | | | |
| Optifort | 128.3 | a | 287.7 | ab | 18.2 | a |
| Kaiser | 125.5 | abc | 293.4 | ab | 18.0 | a |
| Interpro | 121.7 | c | 288.8 | ab | 17.7 | a |
| Mozart | 122.8 | bc | 282.1 | ab | 17.8 | a |
| Bental | 125.8 | abc | 290.1 | ab | 17.9 | a |
| Pittam | 126.7 | ab | 297.9 | a | 17.9 | a |
| Dynafort | 116.2 | d | 275.4 | b | 17.6 | a |
| 8614 | 127.6 | ab | 297.1 | a | 17.8 | a |
| Un-grafted | 117.9 | d | 300.4 | a | 16.5 | b |
| *Significance* | | | | | | |
| *Scion* (*S*) | *** | | *** | | *** | |
| *Rootstock* (*R*) | *** | | ** | | ** | |
| *S* × *R* | *** | | ** | | ** | |

*P <0.05, **P <0.01 or ***P <0.001, respectively. n.s., not significant. For each column and experimental factor, mean values labelled with the same lowercase letter did not differ statistically according to the Student–Newman–Keuls (P<0.05).

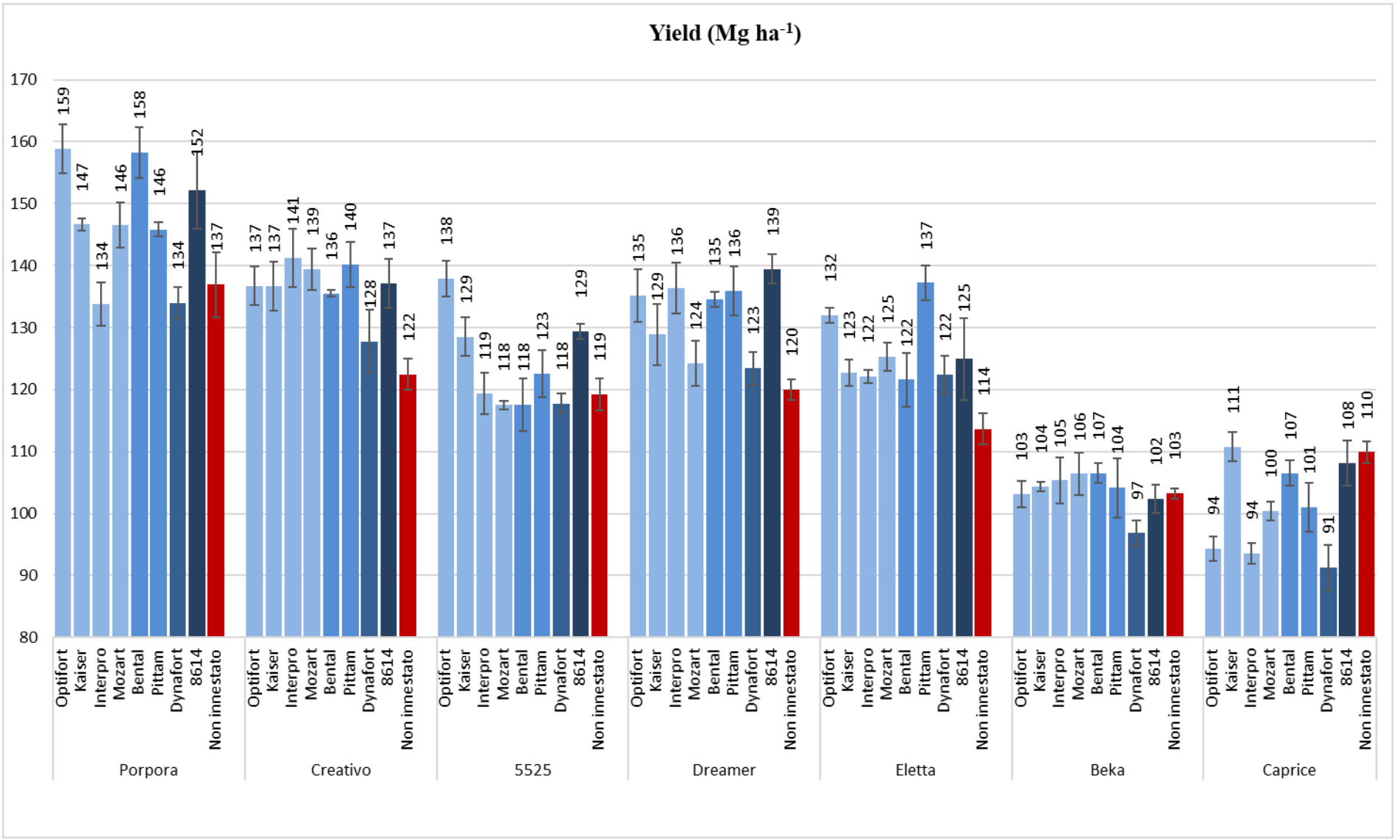



**Figure 6** – Total yield of the different grafting combinations (means ± SE).

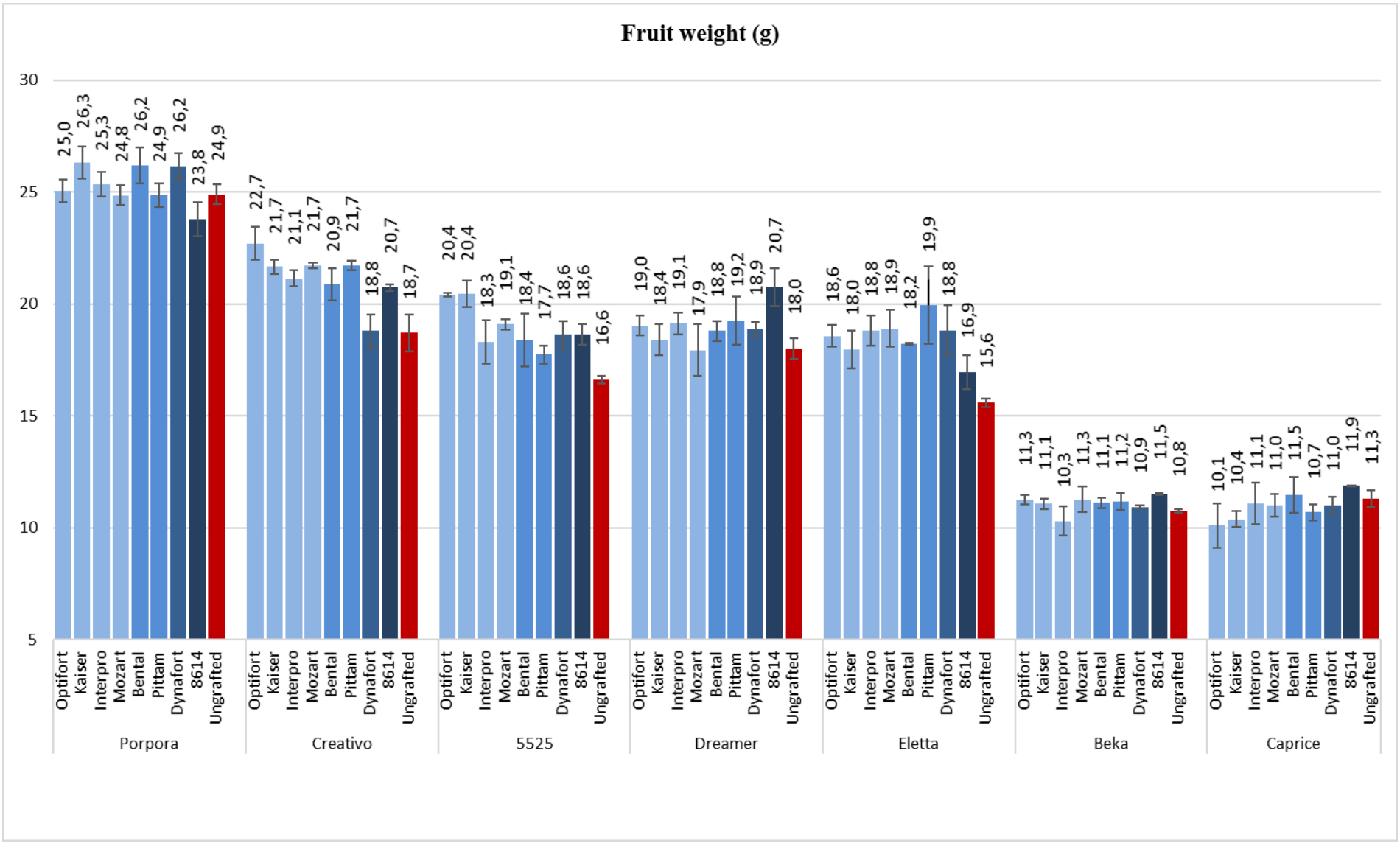



**Figure 7** – Average fruit weight among the different grafting combinations (means ± SE).

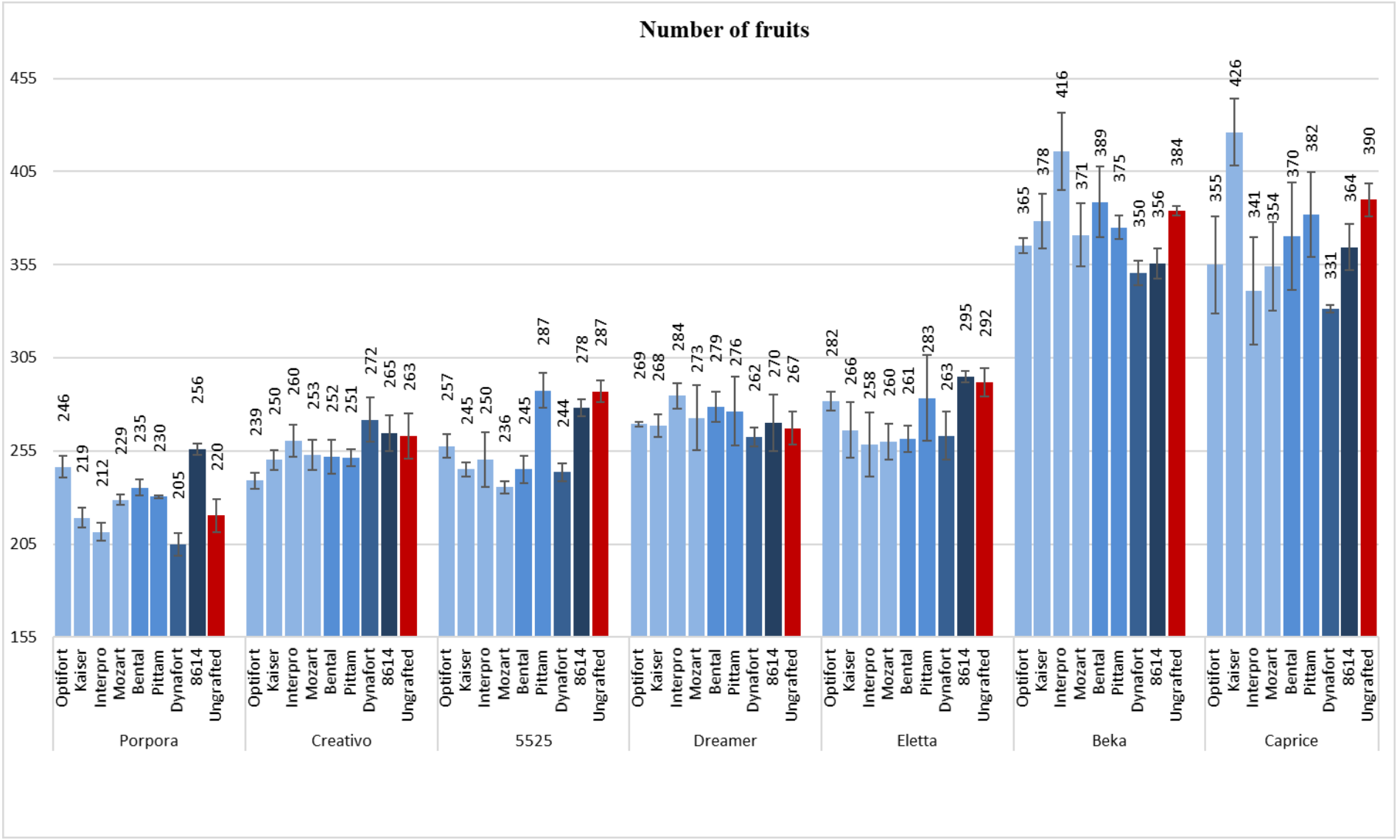



**Figure 8** – Total number of fruits per plant in the different grafting combinations (means ± SE).

**Table 4** – Shape index and quality traits of the fruits as affected by scion and rootstock.

| Main factors | Shape index | Firmness (g) | a* | b* | Lightness | TSS (%) | TA (mg citric acid kg$^{-1}$ FW) | TSS / TA | Ascorbic acid (mg kg$^{-1}$ FW) |
|---|---|---|---|---|---|---|---|---|---|
| **Scion** | | | | | | | | | |
| Porpora | 1.15 bc | 835 bc | 12.0 c | 18.3 ab | 38.3 c | 4.94 d | 3.97 c | 1.25 c | 196 b |
| Creativo | 1.16 b | 1127 a | 11.0 d | 18.5 ab | 39.1 bc | 5.33 c | 4.10 c | 1.31 bc | 171 c |
| 5525 | 1.21 a | 1232 a | 12.3 c | 19.2 a | 40.7 a | 4.83 d | 4.54 b | 1.08 d | 194 b |
| Dreamer | 1.14 c | 855 bc | 10.3 d | 19.1 a | 39.7 b | 5.01 d | 3.71 d | 1.36 b | 174 c |
| Eletta | 1.16 b | 830 bc | 13.5 b | 18.0 b | 38.4 c | 5.27 c | 3.07 e | 1.73 a | 227 a |
| Beka | 1.21 a | 910 b | 15.0 a | 18.4 ab | 38.6 c | 5.98 b | 4.82 a | 1.25 c | 211 b |
| Caprice | 1.20 a | 764 c | 14.9 a | 18.3 ab | 38.4 c | 6.25 a | 4.86 a | 1.30 bc | 206 b |
| **Rootstock** | | | | | | | | | |
| Optifort | 1.18 | 934 ab | 12.2 bcd | 18.5 | 39.1 | 4.98 e | 3.89 | 1.31 ab | 159 e |
| Kaiser | 1.18 | 903 b | 12.4 bcd | 18.3 | 38.9 | 4.98 e | 4.05 | 1.27 b | 167 de |
| Interpro | 1.17 | 896 b | 13.0 abcd | 18.3 | 38.7 | 5.47 abc | 4.33 | 1.29 b | 178 cde |
| Mozart | 1.17 | 942 ab | 11.8 d | 18.9 | 39.7 | 5.10 de | 4.13 | 1.28 b | 189 cd |
| Bental | 1.17 | 894 b | 12.3 bcd | 18.4 | 38.8 | 5.33 cde | 4.21 | 1.29 b | 187 cd |
| Pittam | 1.18 | 959 ab | 12.1 cd | 18.6 | 39.4 | 5.41 bcd | 4.15 | 1.33 ab | 196 c |
| Dynafort | 1.17 | 915 b | 13.2 abc | 18.7 | 38.7 | 5.50 abc | 4.21 | 1.35 ab | 194 c |
| 8614 | 1.18 | 952 ab | 14.1 a | 18.5 | 39.1 | 5.81 a | 4.23 | 1.42 a | 239 b |
| Un-grafted | 1.17 | 1030 a | 13.5 ab | 18.6 | 38.8 | 5.76 ab | 4.18 | 1.42 a | 266 a |
| *Significance* | | | | | | | | | |
| *Scion* (*S*) | *** | *** | *** | ** | ** | *** | *** | *** | *** |
| *Rootstock* (*R*) | *ns* | * | *** | *ns* | ns | *** | *ns* | *** | *** |
| *S* × *R* | *ns* | * | ** | *ns* | *ns* | *ns* | * | *ns* | *** |

*P <0.05, **P <0.01 or ***P <0.001, respectively. n.s., not significant. For each column and experimental factor, mean values labelled with the same lowercase letter did not differ statistically according to the Student–Newman–Keuls (P<0.05).

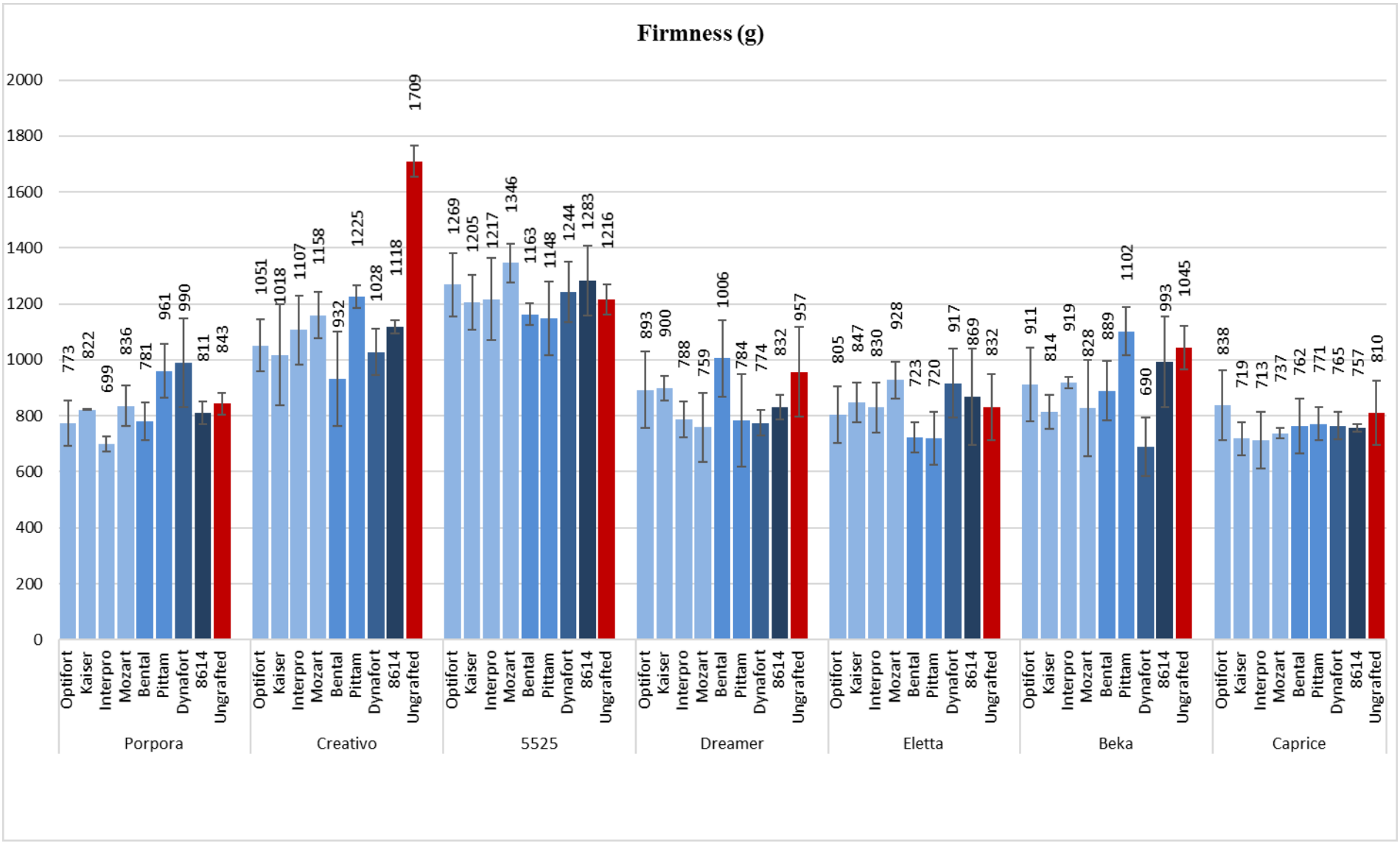



**Figure 9** – Firmness of the fruits in the different grafting combinations (means ± SE).

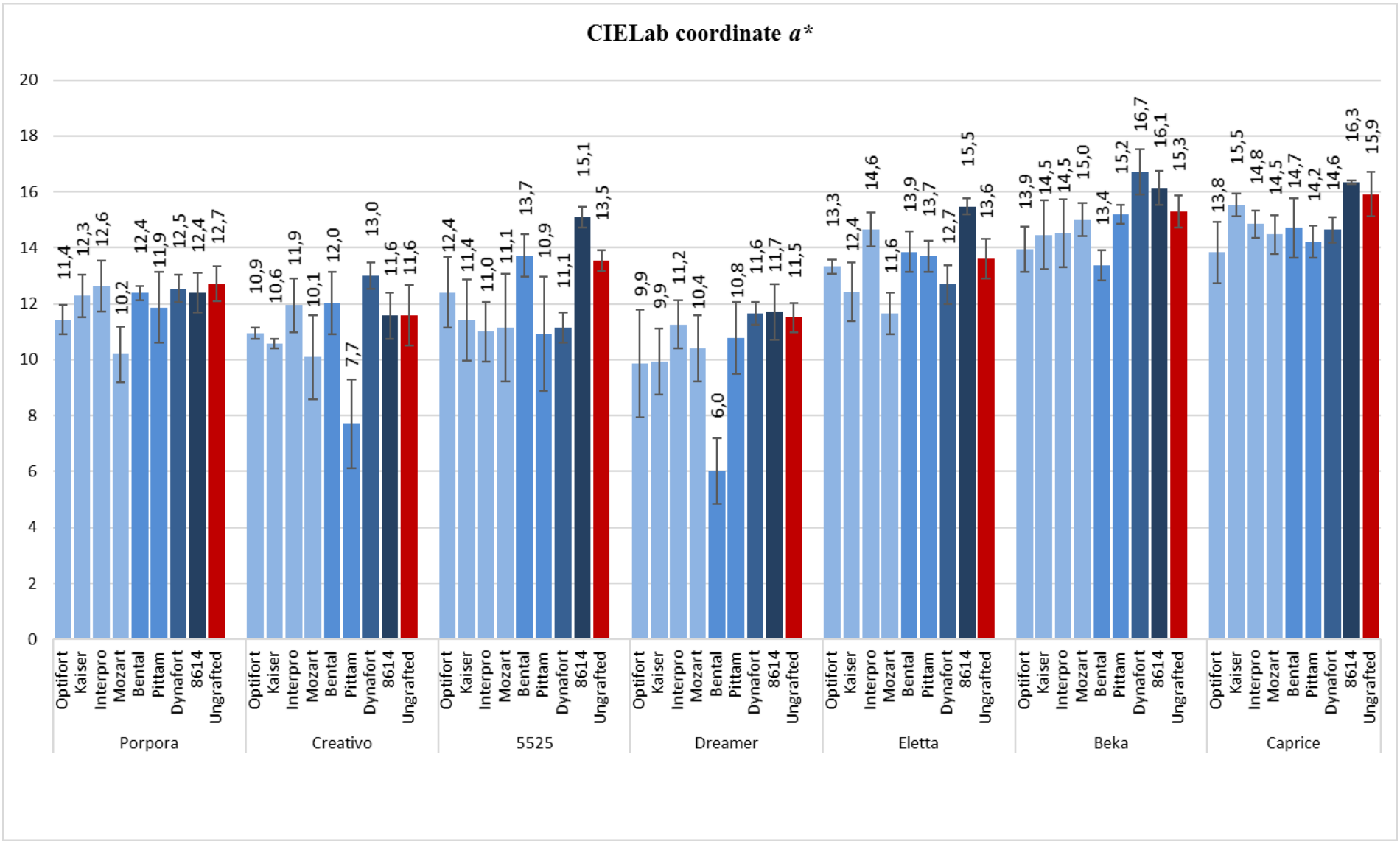



**Figure 10** – CIELab coordinate a* of the fruits of different grafting combinations (means ± SE).

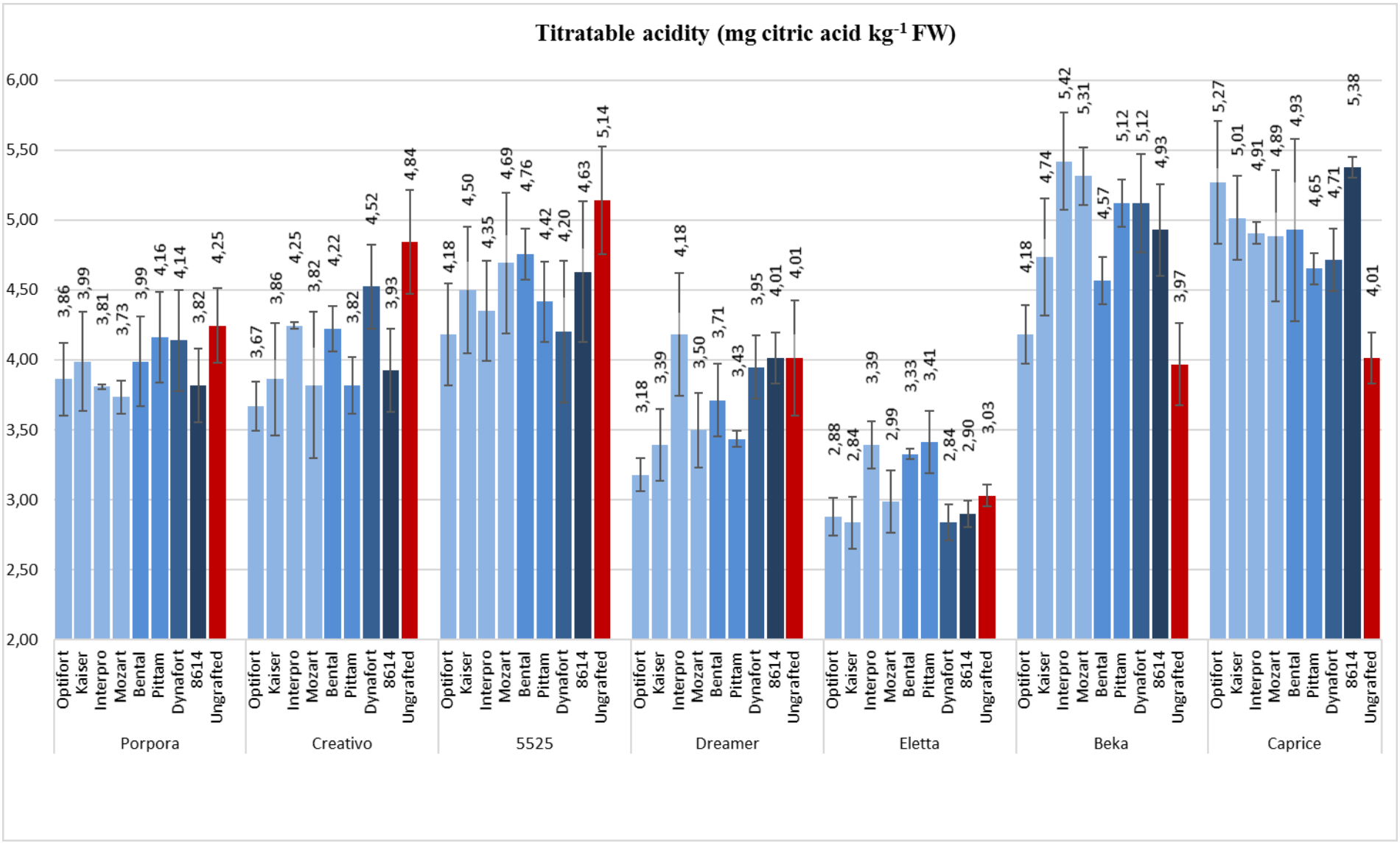



**Figure 11** – Titratable acidity of the fruits of different grafting combinations (means ± SE).

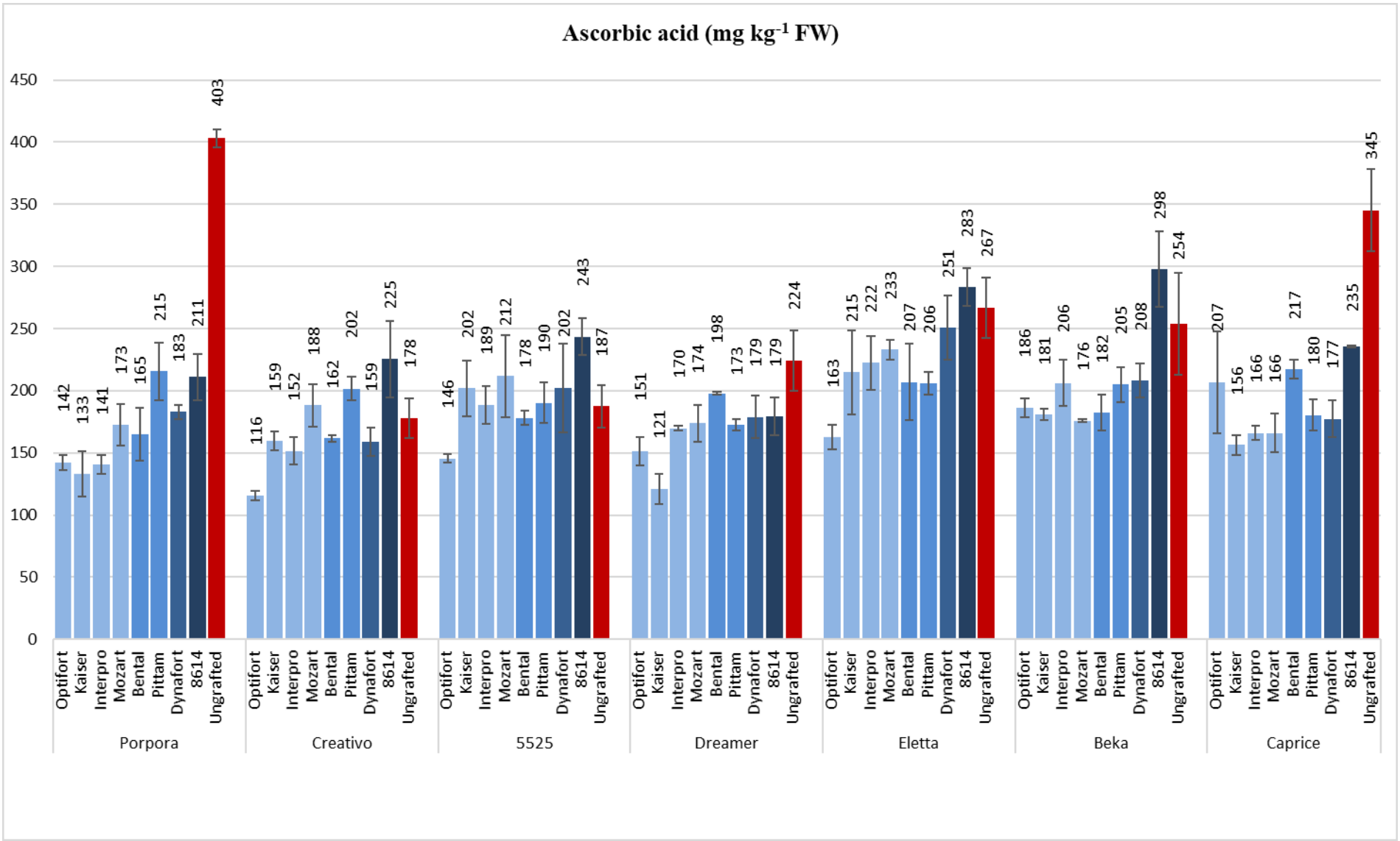



**Figure 12** – Ascorbic acid content in the fruits of different grafting combinations (means ± SE).

**Table 5** - Percentage (%) of variation (sum of squares - SS) explained by scion, rootstock and their interaction.

| | Total epigeous biomass | Vegetative fraction | Reproductive fraction | Harvest index | Total leaf area | Yield | Number of fruits plant$^{-1}$ | Fruit fresh weight |
|---|---|---|---|---|---|---|---|---|
| ***Scion (S)*** | 55.9 *** | 45.8 *** | 23.8 *** | 17.4 *** | 42.6 *** | 77,6 *** | 83.1 *** | 94.0 *** |
| ***Rootstock (R)*** | 17.2 *** | 25.9 *** | 14.2 *** | 33.7 *** | 27.7 *** | 5,7 *** | 1.9 ** | 0.6 ** |
| ***S × R*** | 19.8 *** | 22.7 *** | 36.4 *** | 34.7 *** | 23.4 *** | 9,1 *** | 6.4 ** | 2.2 ** |

| | Shape index | Firmness | a* | b* | L | SSC | Acidity | Sugar / acids | Ascorbic acid |
|---|---|---|---|---|---|---|---|---|---|
| ***Scion (S)*** | 51.9 *** | 45.9 *** | 47.0 *** | 9.4 ** | 24.3 *** | 45.1 *** | 55.3 *** | 58.4 *** | 13.6 *** |
| ***Rootstock (R)*** | 1.1 ns | 3.8 * | 8.3 ** | 1.9 ns | 3.6 ns | 14.5 *** | 2.1 ns | 5.8 *** | 34.5 *** |
| ***S × R*** | 8.1 ns | 16.2 * | 16.0 ** | 22.2 ns | 14.7 ns | 8.1 ns | 14.5 * | 10.4 ns | 27.7 *** |

*P <0.05, **P <0.01 or ***P <0.001, respectively. n.s., not significant.

Table 6 - Percentage (%) of variation (sum of squares - SS) explained by scion, rootstock and their interaction, considering the intermediate vigour scions only.

| | Total epigeous biomass | Vegetative fraction | Reproductive fraction | Harvest index | Total leaf area | Yield | Number of fruits plant$^{-1}$ | Fruit fresh weight |
|---|---|---|---|---|---|---|---|---|
| ***Scion (S)*** | 44,8 *** | 37,3 *** | 26,8 *** | 23,9 *** | 41,6 *** | 27,1 *** | 13,7 *** | 31,2 *** |
| ***Rootstock (R)*** | 28,0 *** | 33,9 *** | 20,1 *** | 42,3 *** | 33,7 *** | 29,6 *** | 15,1 ** | 19,5 *** |
| ***S × R*** | 20,2 *** | 22,4 *** | 25,1 *** | 32,5 *** | 18,5 *** | 17,5 * | 23,2 ns | 19,2 * |

| | Shape index | Firmness | a* | b* | L | TSS | TA | TSS / TA | Ascorbic acid |
|---|---|---|---|---|---|---|---|---|---|
| ***Scion (S)*** | 43,4 *** | 45,0 *** | 25,9 *** | 14,6 ** | 21,8 *** | 13,3 *** | 51,8 *** | 69,4 *** | 23,9 *** |
| ***Rootstock (R)*** | 4,1 ns | 5,7 ns | 12,4 *** | 3,5 ns | 6,3 ns | 29,3 *** | 8,2 * | 2,8 ns | 30,2 *** |
| ***S × R*** | 6,2 ns | 16,1 * | 23,4 *** | 11,4 ns | 9,9 ns | 11,1 ns | 8,1 ns | 4,6 ns | 16,0 * |

*P <0.05, **P <0.01 or ***P <0.001, respectively. n.s., not significant.

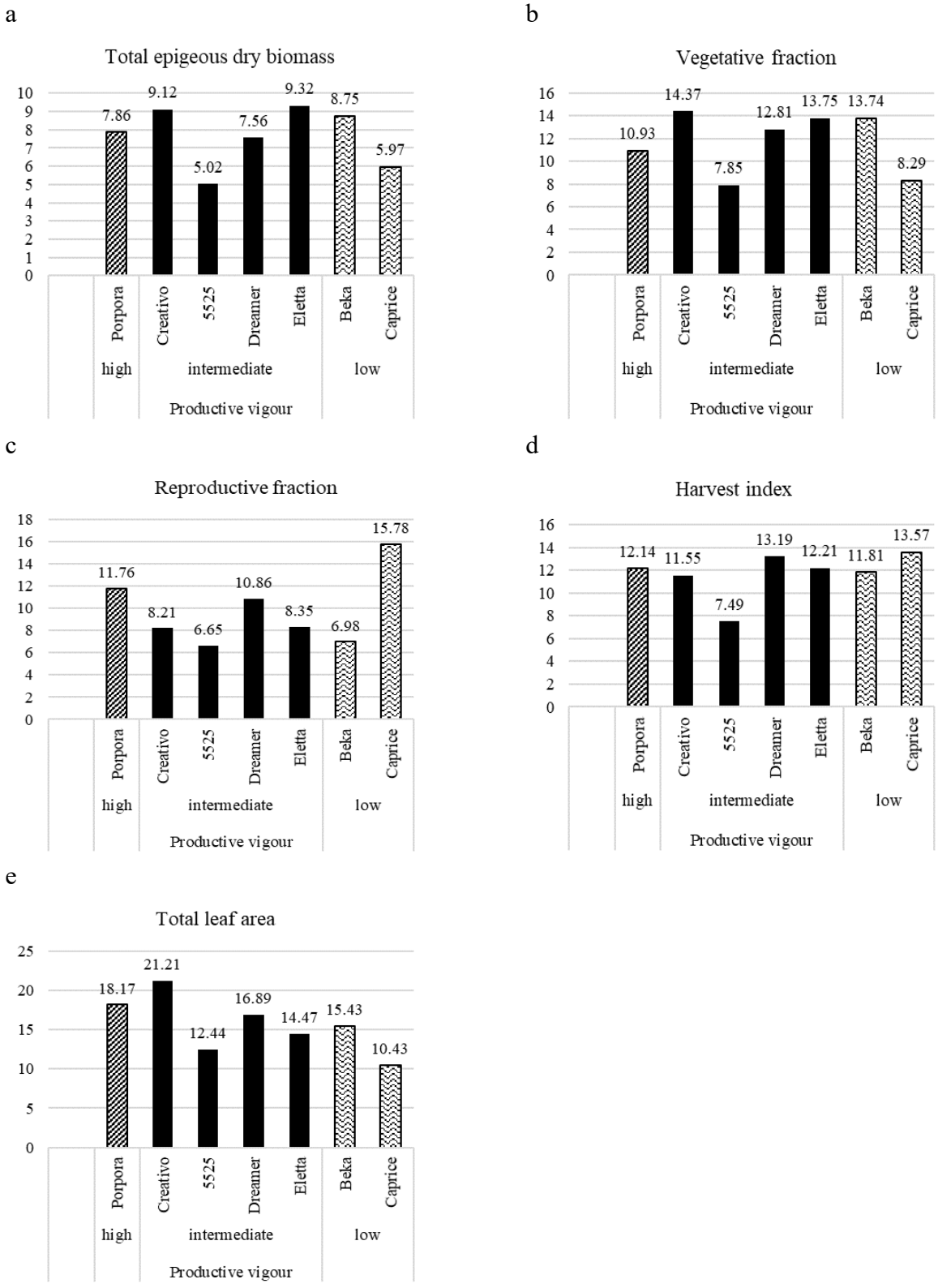



**Figure 13** – Coefficients of variation (%) of epigeous biomass production (a) and partitioning (b;c), harvest index (d) and total leaf area (e) of the different scion cultivars.

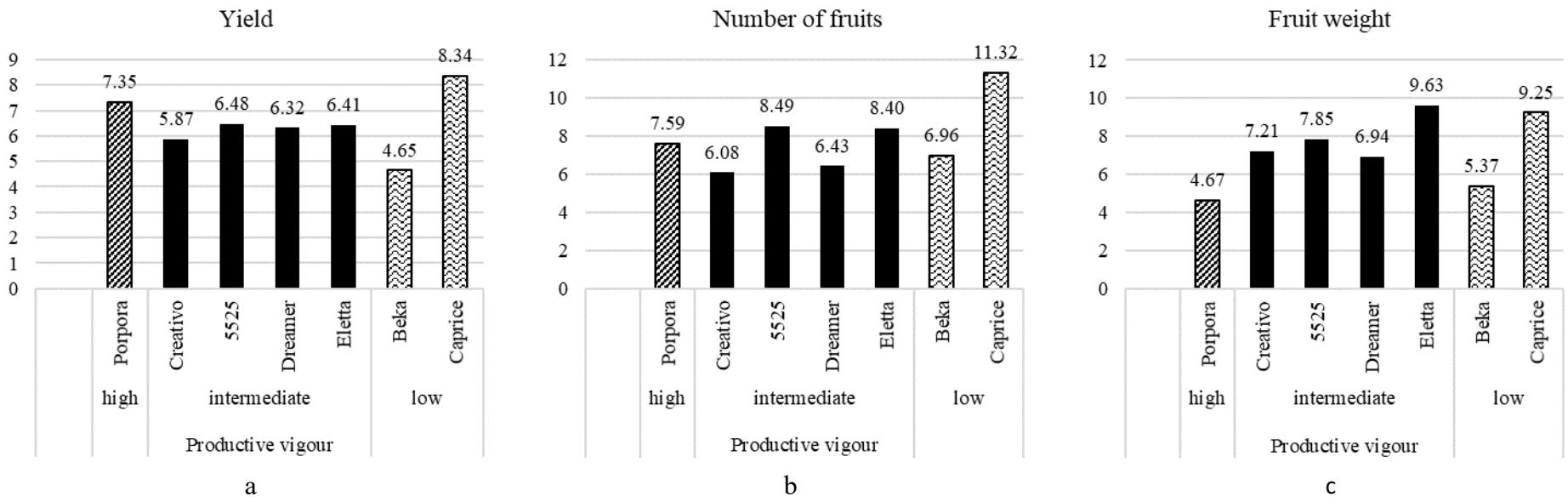



**Figure 14** – Coefficients of variation (%) of yield (a) and its components (b;c) of the different scion cultivars.

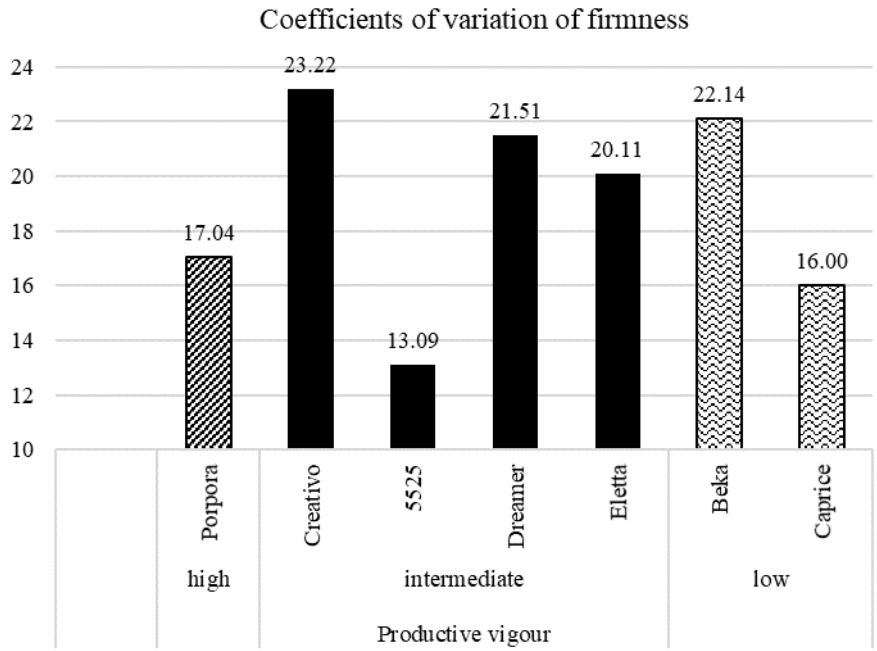



**Figure 15** - Coefficients of variation (%) of firmness of the fruits in the different scion cultivars.

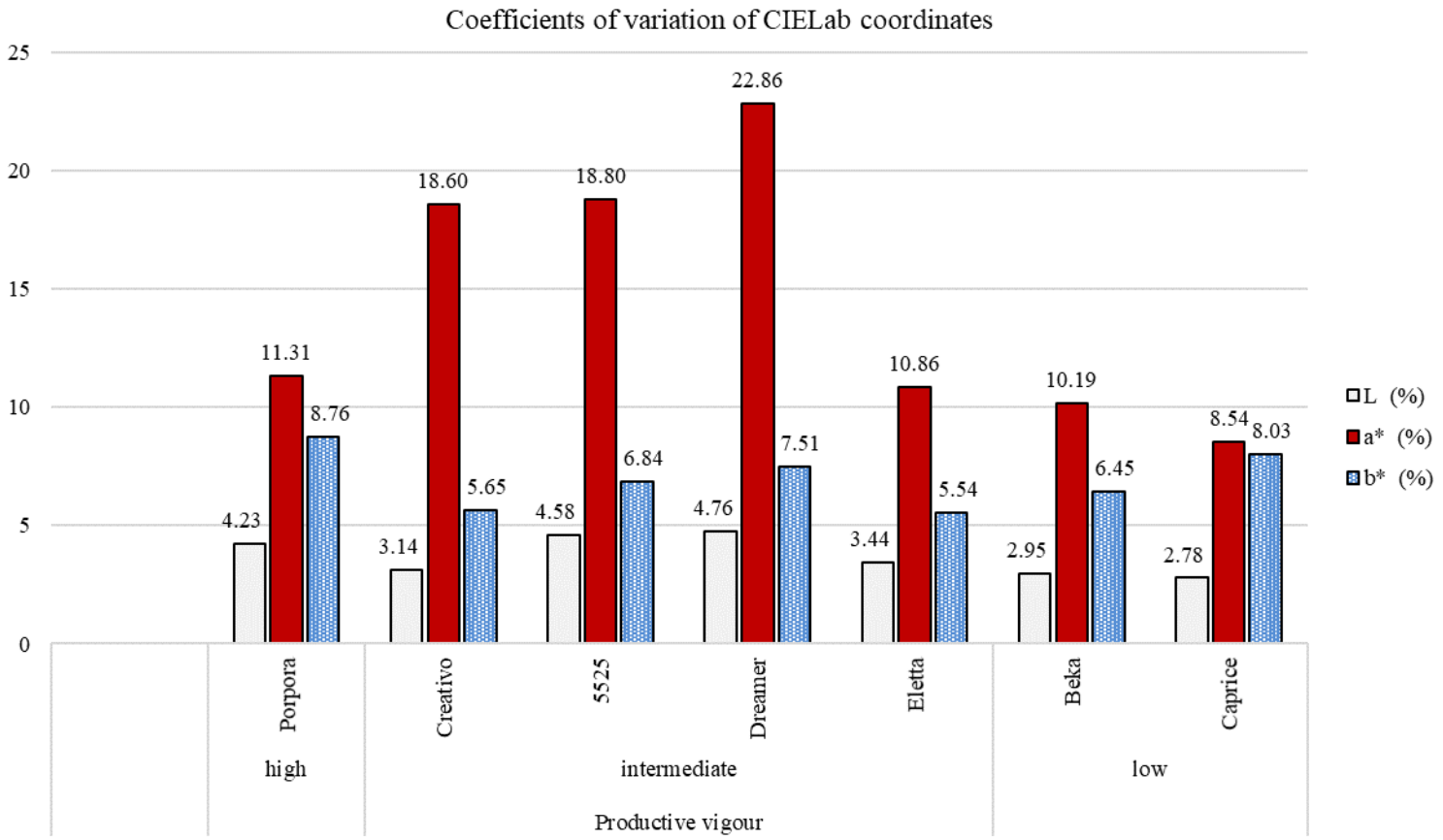



**Figure 16** - Coefficients of variation (%) of the CIELab coordinates of the fruits in the different scion cultivars.

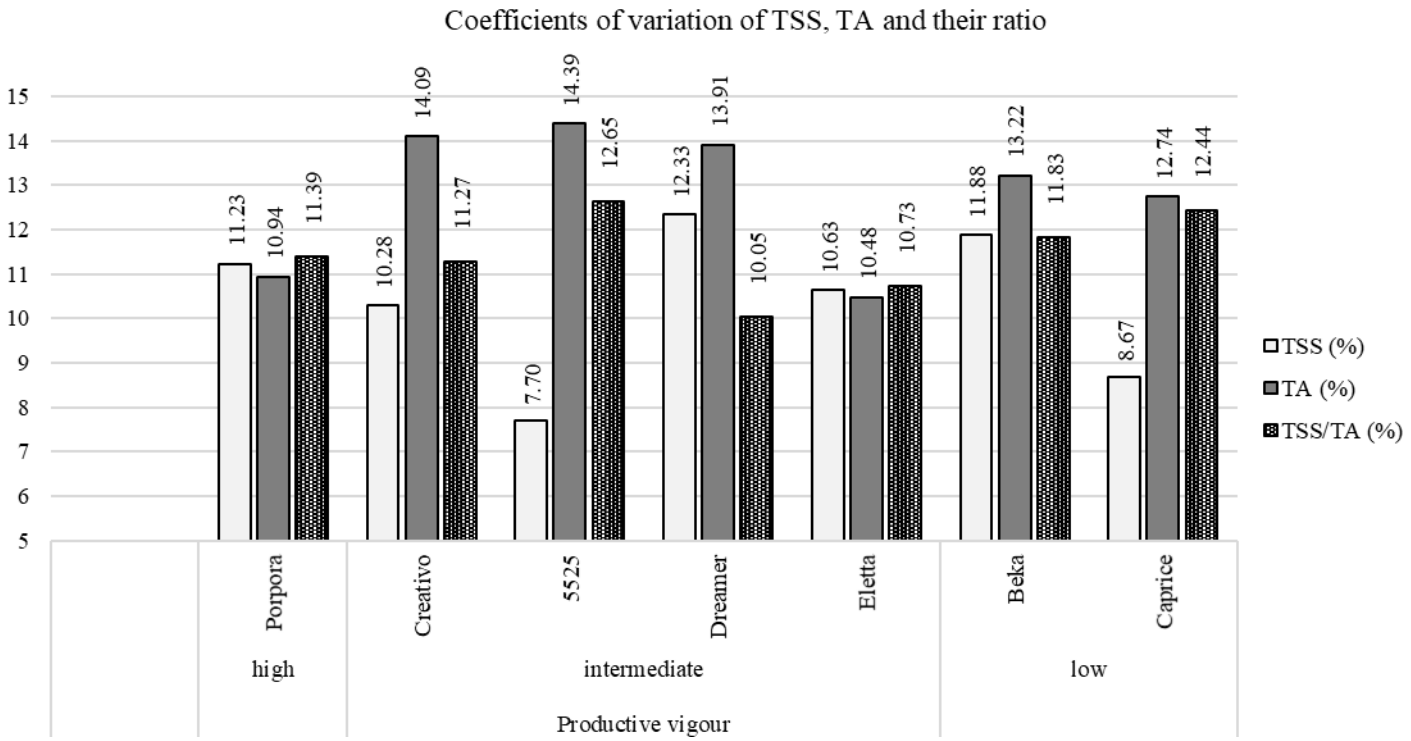



**Figure 17** - Coefficients of variation (%) of total soluble solids (TSS), titratable acidity (TA) and their ratio in the different scion cultivars.

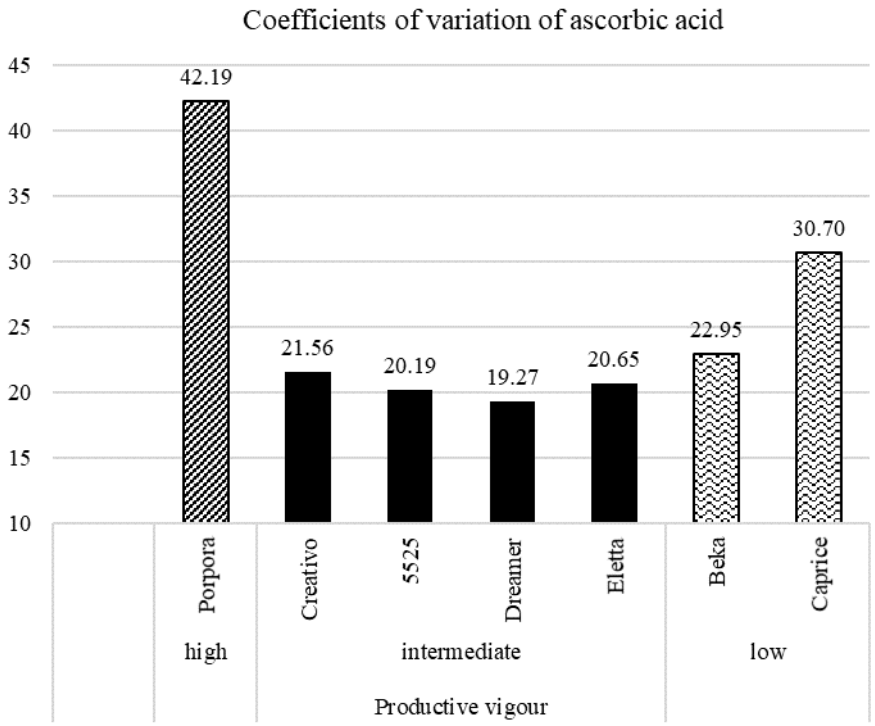



**Figure 18** - Coefficients of variation (%) of the ascorbic acid content of the fruits in the different scion cultivars.

## Main conclusions

The present research provided both interesting knowledge and potential technology transfers, together with additional suggestions for further studies.

One of the most important goal achieved was the identification, within the experiments of the first line of research, of *C. baccatum* var *baccatum* and *C. pubescens* accessions that, besides exhibit higher tolerance to low temperatures, showed optimal grafting compatibility with a widespread pepper cultivar and, when used as rootstocks were able to alleviate chilling stress better than the commercial rootstock used as a control. However, the good results obtained under limiting conditions are not always confirmed to last when the stress period ceases; for that reason, the fact that two of these new rootstocks showed the same performances of the commercial rootstock in terms of yield and fruit quality during a long-terms experiment under optimal conditions was even more satisfying. Since nothing is known about the tolerance of these two accessions to nematodes or soil pathogens, further studies could provide information on this regard, allowing the relative technology transfer in reasonable time.

Throughout the second line of research experiment, the inhibition of biomass production under root hypoxia was lower than expected, but strong alterations were induced by this stress condition in un-grafted tomato. Grafted tomato plants exhibited better photosynthetic performances under conditions of oxygen stress as a consequence of reduced impairment of the photosynthetic machinery deriving from both stomatal and non-stomatal limitations. These better performances were likely linked to a better functionality of the roots under conditions of oxygen deprivation, a condition that was mirrored also in the greater extension and functionality of the assimilating leaf apparatus which, in turn, derived from a better carbohydrate partitioning inside the plant. However, to ascertain the origin of this better functionality of the root system and sink-source arrangement inside the plant, further investigations are needed, considering histological (development of aerenchymas) and hormonal cues.

Finally, the third line of research provided a deeper knowledge of grafting effect on fruit yield and quality of cherry tomato.

The analysis of the variability explained by each factor and by their interaction allowed to identify some variables, like fruit shape, firmness and titratable acidity, in which the role of the scion prevailed in determining the final characteristics of the fruits of grafted plant, and others, like ascorbic acid content, in which rootstock contribution was predominant.

The analysis of the coefficients of variation, compared to the absolute values observed in each grafting combination, provided information regarding the ability of each scion to impose its typical characteristics for the different examined variables and on its potential benefits. This approach aimed for the first time to highlight scion active role in quality traits maintenance in grafted cherry tomato plants and could be implemented in the following researches on this topic that still remain necessary.

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