

Article

Effects of a Local Tomato Rootstock on the Agronomic, Functional and Sensory Quality of the Fruit of a Recovered Local Tomato (*Solanum lycopersicum* L.) Named “Tomate Limachino Antiguo”

Juan Pablo Martínez ^{1,*}, Raúl Fuentes ², Karen Fariás ¹, Nelson Loyola ³, Alejandra Freixas ⁴, Claudia Stange ⁵, Boris Sagredo ⁶, Muriel Quinet ⁷ and Stanley Lutts ⁷

¹ Instituto de Investigaciones Agropecuarias (INIA—La Cruz), Chorrillos 86, La Cruz 2280454, Chile

² Departamento de Industrias, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2390123, Chile

³ Escuela de Agronomía, Facultad de Ciencias Agrarias y Forestal, Universidad Católica del Maule, km 6, Camino a Los Niches, Curicó 5253545, Chile

⁴ Escuela de Medicina, Universidad Católica del Norte, Larrondo 128, Coquimbo 1780000, Chile

⁵ Center of Molecular Biology in Plants, Department of Biology, Faculty of Science, University of Chile, Las Palmeras 3425, Nuñoa, Santiago 7800003, Chile

⁶ Instituto de Investigaciones Agropecuarias (INIA—Rayentué), Av. Salamanca s/n, Sector Los Choapinos, Rengo 2941530, Chile

⁷ Groupe de Recherche en Physiologie Végétale, Earth and Life Institute, Agronomy (ELI-A) Université Catholique de Louvain, Croix du Sud 5 (Bte L7.07.13), 1348 Louvain-la-Neuve, Belgium

* Correspondence: jpmartinez@inia.cl; Tel.: +56-33-2321780



Citation: Martínez, J.P.; Fuentes, R.; Fariás, K.; Loyola, N.; Freixas, A.; Stange, C.; Sagredo, B.; Quinet, M.; Lutts, S. Effects of a Local Tomato Rootstock on the Agronomic, Functional and Sensory Quality of the Fruit of a Recovered Local Tomato (*Solanum lycopersicum* L.) Named “Tomate Limachino Antiguo”. *Agronomy* **2022**, *12*, 2178. <https://doi.org/10.3390/agronomy12092178>

Received: 18 August 2022

Accepted: 8 September 2022

Published: 14 September 2022

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The Old Limachino Tomato is a valuable fruit with exceptional nutritional values and organoleptic sensory properties. However, it suffers from a short shelf-life, compromising post-harvest behavior. As an attempt to improve the fruit’s qualities, Limachino (L) scion was grafted onto rootstock from the rustic landrace Poncho Negro (R). Fruits produced in this graft combination were compared with fruits produced by self-grafted plants (L/L) and from a long-shelf-life cultivar Seminis (LSL). The trials were carried out for 146 days during summer of two consecutive years. Poncho Negro rootstock increased the total number of fruits produced by Limachino scion (L/R). It did not affect the fresh weight of individual fruits but reduced their water content. It has no impact on the Limachino fruit form (quality), a typical characteristic well appreciated by consumers. Fruits produced by LSL exhibited a higher firmness but a lower titratable acidity and antioxidant capacity than L/R and L/L fruits. Panels of 104 untrained final consumers and a trained panel of 13 experts attributed the highest value to L/R fruits and the lowest one to LSL. It was concluded that Poncho Negro rootstock contributes to increasing preferences and the level of acceptability towards Limachino fruits. Further research is needed to develop local technologies in order to expand the production of local tomatoes that are highly valued by consumers.

Keywords: fruit-quality; local-rootstock; landrace tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit crops in the world (FAOSTAT 2020). It contains a wide range of health-promoting compounds including vitamins, phenolic compounds and carotenoids and thereby contributes to the prevention of numerous chronic diseases [1]. Hence, besides the quantitative aspects of tomato production, which are crucial for a producer’s incomes, the qualitative aspects of the fruit are of paramount importance for the consumer’s health. Functional attributes and sensory properties (taste, aroma, texture, etc.) are key determinants for consumers’ acceptance [2–4].

Unfortunately, modern high-yielding cultivars rarely fulfill the requirement of consumers who often complain of a lack of flavor and aroma [5].

Old cultivars and landraces that have been neglected during the past decades may present interesting properties for both fruit nutritional values and sensory properties [1,6,7]. In the central zone of Chile, an old iconic local cultivar “Limachino” was rediscovered in 2014 as a result of an initiative launched by Chilean authorities to rescue the country’s agricultural heritage. Limachino has a high nutritional value and exhibits an exquisite flavour and seductive aroma. Since the beginning of the 20th century, this cultivar was only cultivated in few specific geographical areas of Chile (mainly in the communes of Olmué and Limache ($32^{\circ}57'35.85''$ S– $72^{\circ}20'48.37''$ W and $33^{\circ}03'10.82''$ S– $71^{\circ}06'28.32''$ W) [8]. Because of its fascinating qualitative properties, this cultivar recently became a protected geographical indication (PGI) and was renamed as Tomate Limachino Antiguo (in English, Old Limachino Tomato) to differentiate it from other cultivars cultivated in the same area. Unfortunately, the fruits from this cultivar also present a major drawback in relation to their weak post-harvest qualities due to their low firmness and shelf-life after being harvested. Serious mechanical damages often occur when harvesting and transporting the fruit, which also has a very short storage capacity. Such weaknesses explain why this cultivar was progressively neglected and then almost disappeared from the market around 1980–1981 until its recent rediscovery.

Improving the post-harvest behavior of Limachino Antiguo thus constitutes an important challenge. This goal may be achieved by classical breeding with other cultivars, but this appears as a long and risky task considering the genetic complexity of traits related to fruit sensory properties and post-harvest quality [1,9]. In tomato, grafting of high value varieties onto appropriate rootstocks is a simple and cheap alternative to long-lasting breeding programs. It constitutes a standard practice commonly used to increase yield-related parameters in terms of fruit number or fruit size, as well as tolerance towards abiotic and biotic constraints [10,11]. Commercial rootstocks frequently consist in hybrids between the cultivated species (*Solanum lycopersicum*) and wild relatives (*S. pennelli*, *S. habrochaites* and *S. peruvianum*) displaying a high level of tolerance to abiotic factors such as salinity, drought or low temperatures [12–14]. In contrast to the quantitative aspects of tomato production, data regarding the impact of rootstocks on fruit qualitative properties remain scarce [15]. Some studies, however, demonstrated that commercially available rootstocks are not necessarily suitable for quality improvement. In 2011, Turhan et al. [16] using the well-known rootstocks ‘Beaufort’ and ‘Arnold’ already noticed that fruit quality measured in terms of dry matter, concentration of soluble solids, total sugar and vitamin C content was lower in grafted plants than in non-grafted ones. More recently, Ellenberger et al. [13] reported a decrease in lycopene content using ‘Beaufort’ while Mauro et al. [12] observed a decrease in ascorbic acid and other nutraceutical traits for fruits produced by scion grafted on ‘Optifort’ or ‘Dynafort’. Grieneisen et al. [10], Lang et al. [17] and Fu et al. [18] considered that even the best rootstocks for yield increase had no significant impact on fruit quality.

Scion-rootstock compatibility depends on the grafting procedure and both pre- and post-grafting environmental conditions but is also genetically controlled [19]. The rootstock and scion exchange a wide range of substances, including small organic molecules, essential nutrients and signaling molecules among which phytohormone play a crucial role. The root-to-shoot translocation of phytohormones may indeed assume key functions in scion adaptation to environmental constraints such as salinity and water deficit [19–21]. On a long-term basis, compounds such as abscisic acid, aminocyclopropane carboxylic acid (the immediate precursor of senescing hormone ethylene), auxin and cytokinins are involved in fruit development and maturation processes and may, thus, influence both qualitative parameters and post-harvest storage capacities [1]. According to Zhou et al. [15] numerous mobile proteins, protein-coding mRNAs and non-coding small RNAs may also be found in the xylem sap of grafted plants and some of them may have a strong influence on fruit metabolism and properties. Špika et al. [5] recently demonstrated that the modification

of the sensory profile and volatile aroma compounds of tomato is a direct function of interactive specific effects between scion and rootstocks.

Since the use of commercial rootstocks does not constitute a guarantee of fruit quality improvement, some local landraces well adapted to a target environment might constitute an alternative [6]. Fullana-Pericàs et al. [22] demonstrated that the traditional landrace ‘de Ramellet’ used as a rootstock allowed an increase in the shelf-life of produced fruits while the commercial rootstock ‘Maxifort’ did not. In the North part of Chile, tomato producers have to struggle with ion toxicities consisting in salinity and boron excess. The traditional landrace Poncho Negro displays a fascinating ability to cope with the simultaneous occurrence of NaCl and B excess ([7,23–25]. The Poncho Negro—Limachino Antiguo as a rootstock-scion combination is efficient since Alfaro [26] recently demonstrated that Poncho Negro rootstock improved Limachino Antiguo resistance against *Pseudomonas syringae*. This prompted us to study its impact on the fruit quality produced by the Limachino Antiguo scion. Consumers’ acceptance in relation to fruit quality may be apprehended through quantitative estimation of measurable properties. However, other criteria such as taste or flavour remain subjective but can be assessed using a panel of experts but also by untrained consumers [27].

2. Materials and Methods

2.1. Plant Material, Grafting and Growing Conditions

Seeds of two accessions of *Solanum lycopersicum* L., namely, Poncho Negro (R, accession SLY001) and Old Limachino Antiguo Tomato (L., accession SLY074), were provided by the INIA-La Platina seed bank. An additional commercial cultivar Seminis (DRW7742) was used as a control since this cultivar exhibits an exceptional long-shelf-life. It will be thereafter designated as LSL. Following Martínez et al. [28], seeds were sown on a perlite-peat (1:1, v/v) substrate for initial germination, and seedlings were then grown in growth chambers in four outdoor containers filled with a perlite-peat (1:2 v/v), moistened regularly with half-strength modified Hoagland nutrient solution, which contained the following chemical concentrations: 5 mM KNO₃, 1 mM NH₄H₂PO₄, 0.5 mM MgSO₄, 5.5 mM Ca(NO₃)₂, 25 µM KCl, 10 µM H₃BO₃, 1 µM MnSO₄, 1 µM ZnSO₄, 0.25 µM CuSO₄, 10 µM Na₂MoO₄ and 1.87 mg L⁻¹ Fe-EDDHA. Twenty-one days after germination, in September (spring in the Southern hemisphere), the plants were grafted and moved on soil to a greenhouse located at the Regional Centre of INIA-La Cruz, Valparaíso Region (32°59′ LS, 71°10′ LW, 105 m.a.s.l.). Splice grafting was performed when plants reached three pairs of fully expanded leaves and a stem diameter of about 2 mm, according to Bhatt et al. [29] with slight modifications. The Limachino tomato plant was grafted on the Poncho Negro plant (L/R treatment) and on itself (L/L treatment) using a silicone clip. Preliminary studies [26] demonstrated that grafting process had no impact on Limachino behavior and that self-grafted plants presented similar physiological, phenological and agronomical properties as non-grafted plants. Plant-to-plant distance within each row was 0.6 m and, in a row, was 0.4 m. Therefore, the planting density for all treatments was 4.16 plants per m². In relation to this value, it is pertinent to point out that the Old Limachino Tomato is a local non-hybrid accession with less vigor than commercial varieties. This difference means that the plants associated with the commercial varieties require a greater separation between them, which means that the planting density is much lower in relation to the planting density of Limachino accessions. Thus, the usual density at which the Old Limachino Tomato has been grown for almost a century has ranged, on average, between 3.8 and 4.2 plants m⁻², while the value for commercial varieties ranges, on average, between 2.3 and 3 plants m⁻². The study covered the 2018–2019 and 2019–2020 seasons. The type of grafting (L/L, L/R or no grafting for LSL) will hereafter be considered as “treatments”.

Plants were then grown in greenhouse under a polyethylene roof with 80% transmittance with a natural light intensity at the top of the canopy around 1200 µmol m⁻² s⁻¹ (photosynthetic photon flux density, PPFD) at noon (Figure 1). The greenhouse cover

material comprised 3 mm-thick 3-layer thermal polyethylene with a two-season duration (UV 2T). At that time, the architecture and construction of the greenhouse considered a hood-type design with lateral, front, rear, and zenithal ventilation, which were open for most of the day and during the entire experimental period (spring-summer) to avoid heat excesses. The mean air temperature outside the greenhouse ranged from 8.8 °C to 26.3 °C during the cultivation period, and the average relative humidity outside the greenhouse was 65.0 ± 7.3 (Figure 1). As indicated in Figure 2, the photoperiod was 14 h light/10 h dark (spring-summer) and 11 h light/13 h dark (autumn). The experiment lasted 146 days.

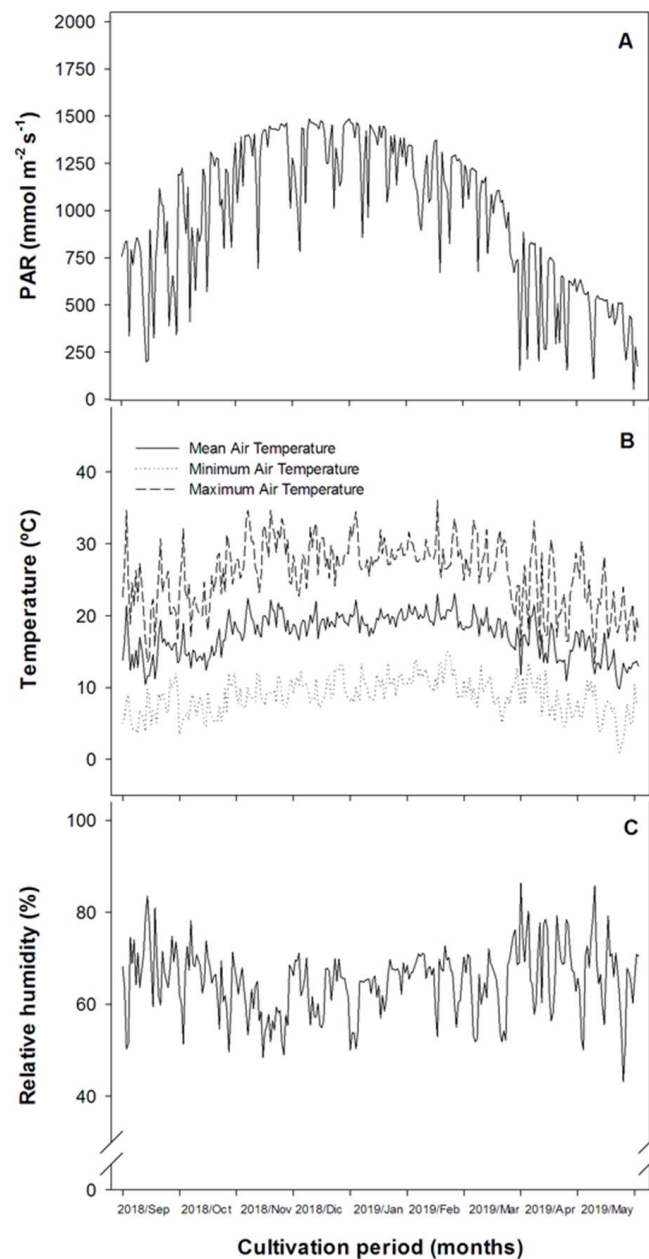


Figure 1. Photosynthetic active radiation-PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (A); variation in minimum, mean and maximum air temperatures ($^{\circ}\text{C}$) (B); relative humidity (%) (C) recorded in La Cruz-Chile outside the greenhouse during the 2018–2019 cultivation period. Each figure reports daily monthly averages. The database used can be accessed by request.

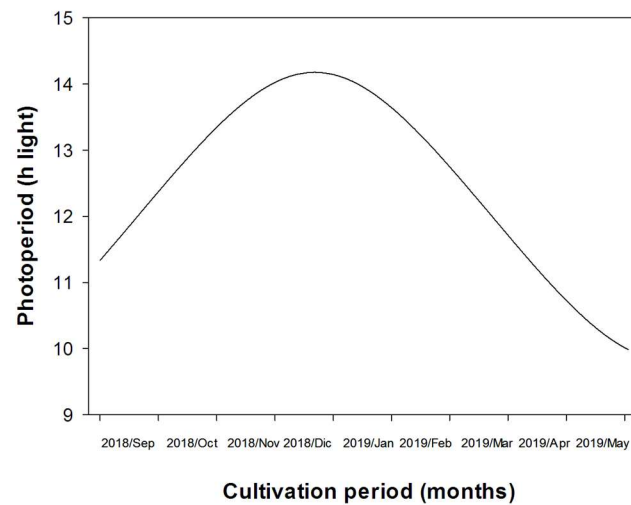


Figure 2. Photoperiod (h light) recorded in La Cruz-Chile during the 2018–2019 cultivation season. The database can be accessed by request.

Irrigation was supplied using an automatic pressurized drip system with a flow rate of 1.20 L/emitter h⁻¹ and a 0.4 m emitter spacing. The surface flow rate was 2 L m⁻² h⁻¹. Irrigation was carried out 3 times per week during the 146 days of cultivation with periods of time defined in accordance to the developmental stages of the plant. Fertilization was supplied through fertigation management following the plants' acclimatization, starting two weeks after transplanting. The fertilization was applied at four developmental stages with 275 Kg ha⁻¹ N, 100 Kg ha⁻¹ P₂O₅, 500 Kg ha⁻¹ K₂O, 151 Kg ha⁻¹ CaO, 100 Kg ha⁻¹ MgO and 75 Kg ha⁻¹ S. The pH and the EC of the nutrient solution were set to 6.65 and 0.88 dS.m⁻¹, respectively. An orange-colored plastic mulch was used for weed control.

The experiment was carried out in a one-way design with 12 random blocks (replicate) that contained the three treatments (6 plants per treatment). As a result of this design, a total of 216 plants were involved. Except for the fruit quality analysis, one plant (considered as a "unit") per block was randomly selected to measure each variable according to the type of treatment; i.e., each variable was measured 12 times for each season (2018–2019 and 2019–2020). Regarding fruit quality analyses, a sample of 3 fruits (cluster) was taken per plant for each replicate (or block) and treatment, leading to a total number of 108 fruits for each season.

2.2. Shoot Biomass and Yield

Fresh weight (FW) and dry weight (DW) of stems, leaves and fruits were determined 146 days after transplanting (DAT) using twelve replicates per treatment. The entire plant was harvested at the end of the cycle, the aerial organs were separated, and the FW was weighed on a balance (Intell-lab, mod. WTB2000). Subsequently, and after the samples were kept at 80 °C for 48 to 72 h in an oven in the case of leaves and stems, DW was estimated. In the case of fruits, samples were kept for 4 to 5 days in the oven due to their high-water content. Productivity was assessed by considering the total weight of fresh and dry fruit from the 1st to the 5th bunch at maturity expressed in kg m⁻² (stage of maturity 5–6 at the color scale according to US standards, Grierson and Kader [30] and USDA [31]). The total fruit yield (TFY) per plant and the number of fruits per plant were recorded at each harvest and were measured excluding fruit that presented defects and/or damage such as cracking or rot. For this purpose, 6 replicates per treatment were carried out at harvest time.

2.3. Fruit Agronomic Quality Attributes

To measure each agronomic and functional variable, three fruits of uniform size and red color (stage 5–6) per plant and per replicate were harvested manually during the morning. For agronomic analysis, some variables were measured immediately after harvest

(fresh fruit weight, firmness, soluble solids, color and size) while variables such as titratable acidity, FRAP and polyphenol content were measured with fruit stored at $-80\text{ }^{\circ}\text{C}$.

In the case of agronomic attributes and following Martínez et al. [32,33], single fruit fresh weight (FFW), dry weight (FDW), water content on a fresh weight basis (WC_{FW} , %), dry weight basis (WC_{DW} , g g^{-1}) and fresh to dry weight (FW/DW) were determined on three fruits (one cluster) per plant and replicate. Total soluble solids (TSS) and titratable acidity (TA) were estimated using the seminal methodology developed by Almasoum [34] and TSS/TA was calculated. Fruit size was determined by the equatorial and polar diameters of the fruits using vernier calipers $150 \times 0.02\text{ mm}$ ($6 \times 1/1000\text{ IN}$) and a model caliper (foot meter). Firmness was carried out using a Silverado model FHT 803 digital pressure gauge with an 11 mm diameter plunger. Visual colour measurements were carried out following US standards Grierson and Kader [30] and USDA [31], which established quality by classifying six color stages as illustrated in Figure A1 (Appendix A). Color changes were quantified by color parameters, L^* , which were measured at the polar area on each tomato after being tested with a CM-5 Spectrophotometer (Konica Minolta, Tokyo, Japan) [2,35], using an 8 mm lens aperture. Subsequently, redness values of the tomatoes were computed as a^*/b^* [2] and used to compare the colors of the control and treated plants.

2.4. Fruit Functional Attributes

Frozen samples were used to measure functional attributes of the fruit. Twelve replicates per treatment were performed. The fruit antioxidant capacity was measured by FRAP's method first proposed by Benzie and Strain [36] with some modifications made by Martínez et al. [33]. The total phenolic compounds (TP) were determined according to Singleton and Rossi [37], and subsequent slight modifications were made by Galati et al. [38]. The total phenolics content was expressed as a μg of gallic acid equivalents per g FW and all analyses were performed in triplicate by replicate using an a Spectrophotometer-UV MINI 1240 Shimadzu.

2.5. Fruit Sensorial Attributes

Sensory evaluation was performed by Regional Centre for Studies in Healthy Foods (CREAS), Curauma, Region of Valparaíso, and by the laboratory of the Catholic University of Maule, San Isidro Campus, Maule Region. While CREAS conducted a sensory analysis based on 104 untrained consumers (final consumers), the University conducted an analysis based on an expert panel of 13 people.

The group conducted by CREAS was made up of men and women, workers, students and housewives between 19 and 70 years of age, with an average age of 27 years, who provided verbal consent for participation following Stone and Sidel [27]. A structured 7-point hedonic scale was used for the application of acceptability test, with categories ranging from "I like it very much" to "I dislike it very much". The panelists were asked to evaluate 3 tomato samples, corresponding to the 3 treatments already mentioned and duly coded, indicating on this scale the level of liking of each sample, respecting the order of the presentation. On the other hand, they were also asked to evaluate the samples in the same order they were introduced (that is, from left to right), marking the sample of their preference with an X as well as adding comments if desired.

As mentioned, the group (panel) conducted by the University consisted of 13 trained panelists ranging in age from 20 to 27 years with experience in sensory evaluations of this type [39]. The evaluation procedure was carried out in line with the methodologies developed by Wittig [40] and Stone and Sidel [27]. The panelists first evaluated the appearance and acceptability on a scale of 1 to 9, where 1 corresponded to "extremely disliked" and 9 corresponded to "extremely liked". Then, they evaluated the perception of the organoleptic attributes of the fruits such as color, sweetness, flavor, acidity, aroma, floury, texture and blandness with a line from 1 to 13 cm in a spiderweb diagram. The evaluations were always carried out in the same environment, where the panelists were positioned in such a way that they did not interact with each other and were given instructions on how to carry

out the evaluation. Three treatments with six replications each were evaluated, producing a total of 18 samples.

2.6. Statistical Analysis

Data on productivity, functional and sensorial fruit quality were analyzed using a one-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$. The model was defined as a single design with 12 free random blocks (replicate) and 18 plants per replicate. As previously mentioned, one cluster involved three fruits per plant. The main source of variation is the type of grafting chosen corresponding to the three treatments already described. When the ANOVA was significant at $p \leq 0.05$, the Duncan Multiple Range Test was used to compare the means of each variable under study. All analyses were conducted in RStudio version 3.0.2 (R Development Core Team, 2012; available online <http://www.R-project.org> (accessed on 17 August 2022); the software was sourced in La Cruz, Chile). We also performed principal component analyses (PCA) using the 'FactoMineR' package to compare the agronomic and functional quality attributes of the fruits of the three treatments.

3. Results

3.1. Shoot Biomass and Yield

Figure 3A,B show the total fresh and dry biomass (kg m^{-2}) assessed in the three treatments mentioned above: (1) long-shelf-lived Seminis (LSL) tomato, (2) self-grafted Old Limachino tomato (L/L) and (3) grafted Old Limachino tomato on Poncho Negro rootstock (L/R). Total fresh biomass did not show significant differences depending on the used treatment (Figure 3A), but the total dry biomass (Figure 3B) was the highest for LSL plants (1.136 kg m^{-2}) compared to the other two treatments, with L/L showing the lowest value (0.949 kg m^{-2}).

Figure 3C,D display the total fruit productivity on fresh weight (FW) and dry weight (DW) evaluated in the three treatments. Fruit productivity on fresh weight basis (kg of total fresh fruit per m^2) of L/R plants was not significantly higher (16.0 kg m^{-2}) compared to L/L (15.5 kg m^{-2}) and LSL (14.9 kg m^{-2}) plants. However, when fruit productivity was estimated on a dry weight basis (kg of total dry weight of fruits per m^2), the L/R plant presented the highest average value (0.826 kg m^{-2}) compared to the other two treatments while the productivity of L/L plants was the lowest.

LSL presented the highest total leaf fresh weight per m^2 (1.91 kg m^{-2}) compared to the other two treatments, and L/R presented the lowest one (Figure 3E). The total leaf dry weight showed a similar behavior (Figure 3F). Likewise, the total stem fresh and dry weight were both higher in the LSL treatment compared to the other two types of treatment (Figure 3G,H), reaching mean values of 0.85 kg m^{-2} for the fresh weight and 0.12 kg m^{-2} for the dry weight. The L/R treatment showed the lowest mean value for these variables: 0.51 and 0.078 kg m^{-2} , respectively.

Another productivity indicator is the number of fruits per square meter or per plant. Figure 4 illustrates that the L/R treatment reached higher values comparatively to other treatments (LSL and L/L). It can indeed be observed that 134.96 fruits per square meter were obtained for L/R treatment, while values are 106.73 and 121.97 for LSL and L/L treatments, respectively. Using a plantation density of $4.1 \text{ plants m}^{-2}$ and following the same order for treatments, the number of fruits per plant for each treatment was 32.91 (L/R), 29.75 (L/L) and 26.02 (LSL).

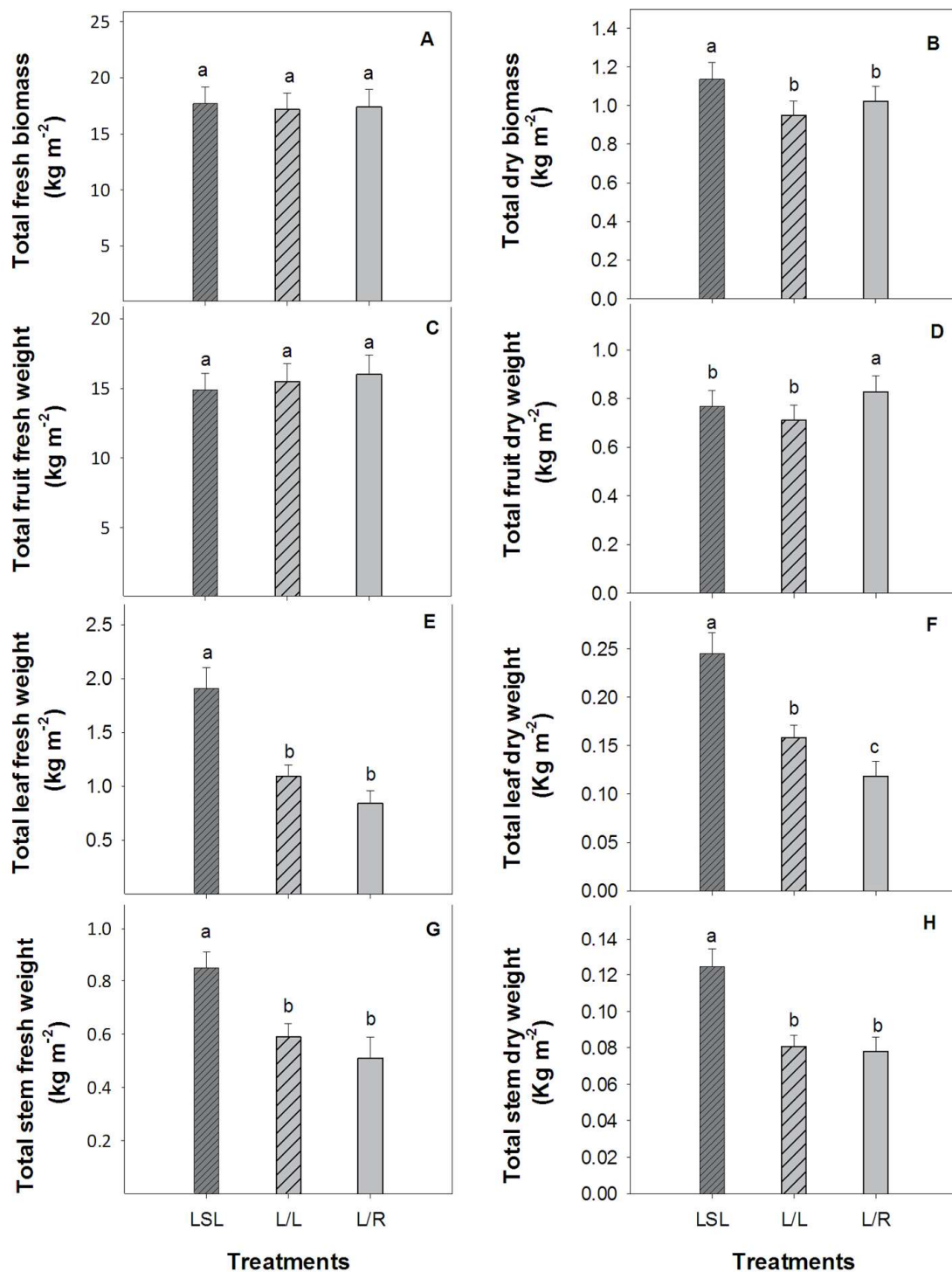


Figure 3. Total fresh biomass (A), total dry biomass (B), total fresh fruit weight (C), total dry fruit weight (D), total leaf fresh weight (E), total leaf dry weight (F), total fresh stem weight (G), and total dry stem weight (H) (kg m^{-2}) for plants corresponding to treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock). All parameters were expressed in kg m^{-2} under greenhouse conditions. Each value represents the mean \pm SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

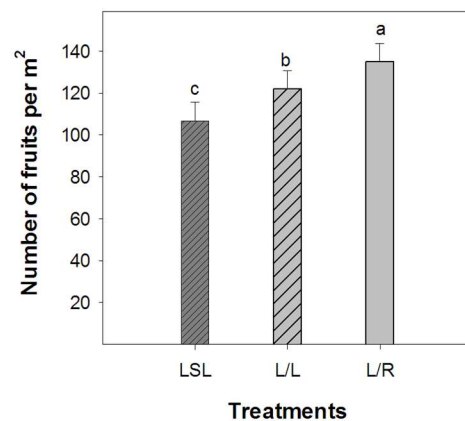


Figure 4. Number of fruits per m² produced by plants from the treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean ± SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

3.2. Fruit Agronomic Quality Attributes

The fresh and dry weight of individual fruit obtained from the LSL treatment were 10 and 21% higher, respectively, compared to the L/L and L/R treatments (Figure 5A,B). The equatorial diameter had a mean value of 6.4 cm and was not influenced by the treatment (Figure 5C). In contrast, the polar diameter was higher for LSL than for the two other treatments: L/L and L/R presented the same polar diameter (Figure 5D).

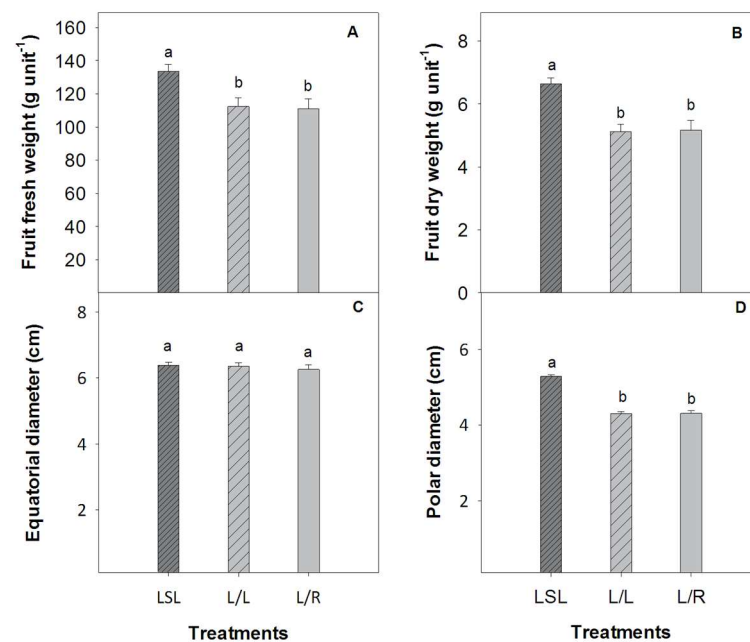


Figure 5. Fruit fresh weight (A) (g unit⁻¹), fruit dry weight (B) (g unit⁻¹), equatorial diameter (C) (cm) and polar diameter (D) (cm) by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean ± SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

Figure 6 shows the visual color of the fruit for the three treatments either according to the USDA scale or according to the photometric spectrum. According to the USDA scale, significant differences between treatments were observed, especially in treatments in which the L accession was included. Indeed, fruits associated with the L/R and L/L

treatments exhibited a mean score of 5.9 and 5.8, respectively, while the mean value for the LSL treatment was only 5.6 (Figure 6A). In contrast, according to the second method, the color parameters L^* was lower in L/L and L/R fruits than in LSL fruits (Figure 6B). Regarding the ratio a^*/b^* (degree of redness), higher mean values were observed for L/R and L/L fruits than for LSL fruits (Figure 6C).

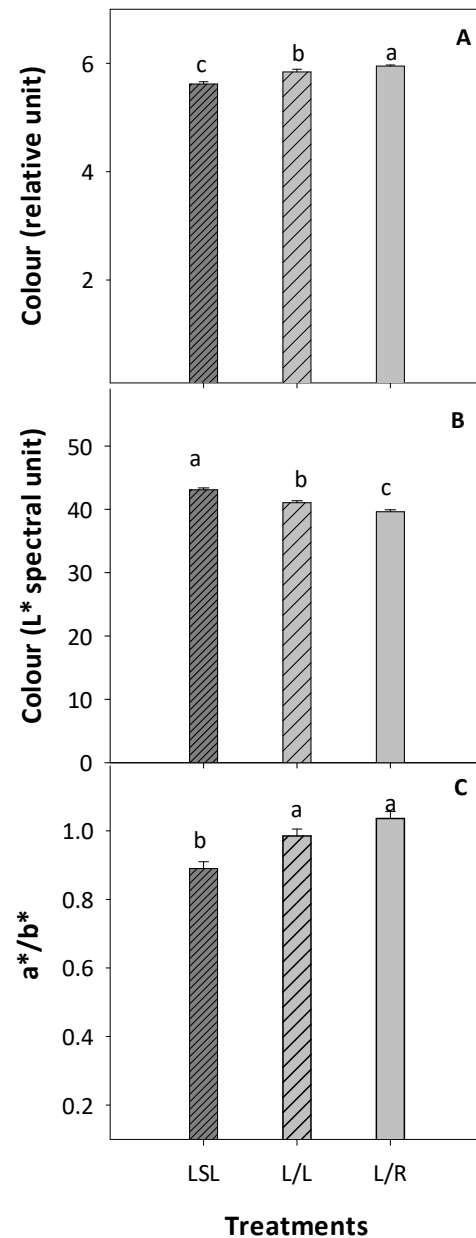


Figure 6. Fruit visual color (A) (method USDA units), fruit spectral color parameters L^* (B) (spectral units) and fruit level of redness a^*/b^* (C) by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean \pm SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

Water content on a fresh (WC_{FW} , %) and dry (WC_{DW} , $g\ g^{-1}$) weight basis is shown in Figure 7A,B. The WC_{FW} (%) mean value was higher in the fruit of the L/L plants (95.3%) compared to similar mean values recorded for L/R and LSL treatments (94.8%) (Figure 7A). A similar response was observed in the WC_{DW} mean value, where the fruit of the L/L plants presented a higher mean value ($WC_{DW} = 22.49\ g\ g^{-1}$) compared to the other two treatments (Figure 7B). The FW/DW ratio of the fruit produced by the L/L treatment

(23.49) showed a significantly higher mean value compared to the two other treatments (Figure 7C).

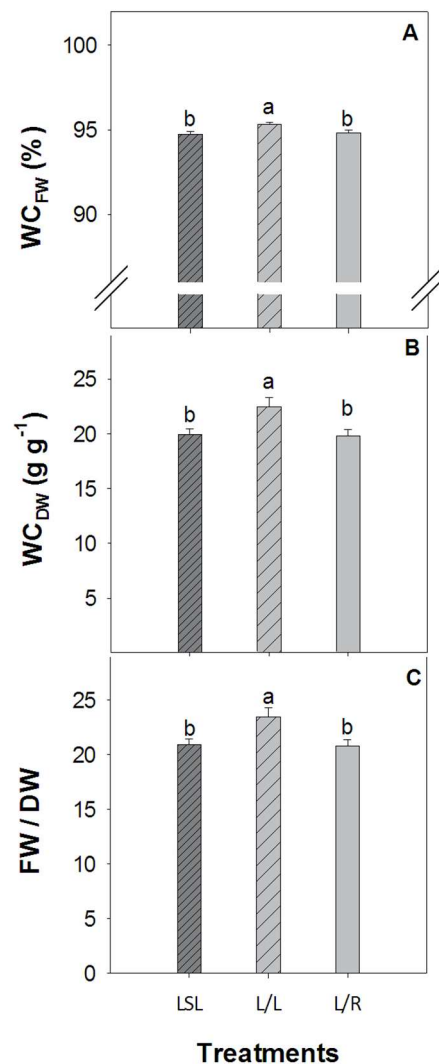


Figure 7. Water content on a fresh (WC_{FW} , %) weight basis (A), water content on a dry (WC_{DW} , $g g^{-1}$) weight basis (B) and ratio fresh weight to dry weight (FW/DW) of the fruit (C) by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean \pm SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

The fruit produced by LSL showed the highest mean fruit flesh firmness (1.6 Newton) compared to the other two treatments, which showed mean values below 0.6 Newton (Figure 8). As a conclusion, fruits from LSL plants are stiffer than the those coming from the L/L and L/R treatments, and no difference was recorded between treatments involving Limachino fruits.

In relation to the chemical quality of the fruits, Figure 9 shows the content of total soluble solids (TSS, $^{\circ}$ Brix), titratable acidity (TA, mg citric acid L^{-1}) and the ratio of total soluble solids to titratable acidity (TSS/TA) for each considered treatment. The treatment had no impact on total soluble solids content, and a mean value close to 4.2 $^{\circ}$ Brix was obtained in all cases (Figure 9A). The titratable acidity of LSL fruits (Figure 9B) showed a lower value (2.7 g citric acid L^{-1}) compared to the other two treatments: L/L and L/R had similar values of c.a. 3.5 mg citric acid L^{-1} . From this difference, the ratio of total soluble solids to titratable acidity (TSS/TA) was higher in the LSL fruits (1.6) compared to the L/L and L/R fruits (Figure 9C).

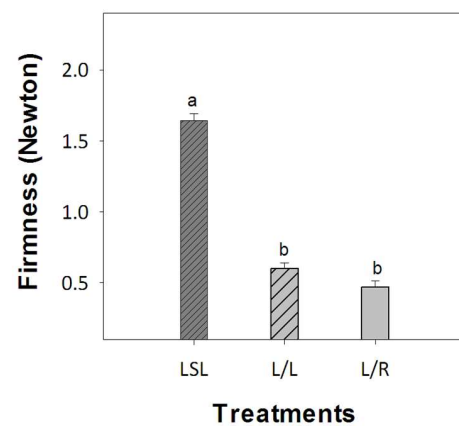


Figure 8. Pulp firmness (Newton) of the fruit produced by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean \pm SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

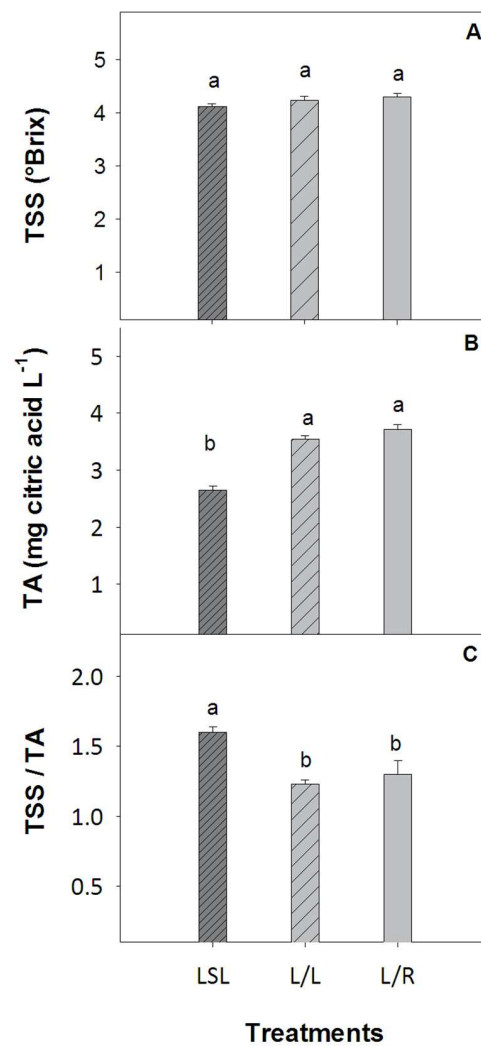


Figure 9. Total soluble solids (TSS, °Brix) (A), titratable acidity (TA, mg citric acid L⁻¹) (B) and ratio TSS/TA of the fruit (C) by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean \pm SE ($n = 12$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) among treatments.

3.3. Fruit Functional Attributes

The FRAP antioxidant capacity (Figure 10A) and total poly-phenol content (Figure 10B) were significantly higher in fruits Limachino, (L/L and L/R treatments). In relative terms, the FRAP mean value was 50% higher in these two treatments compared to the LSL one, while the polyphenol content was 40% higher in the L/L and L/R treatments compared to LSL.

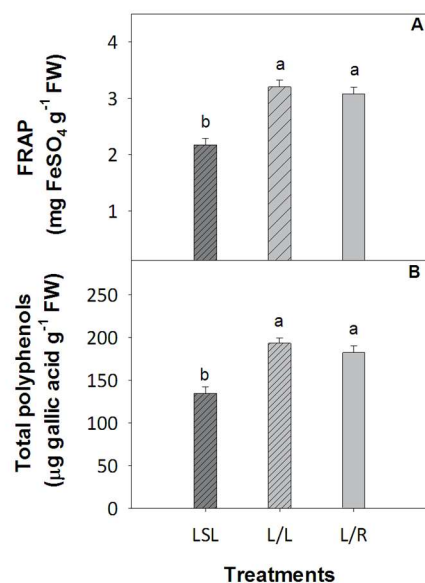


Figure 10. FRAP: Ferric-reducing antioxidant power (mg FeSO₄ g⁻¹ FW) (A) and total phenolic compounds (TP, µg of gallic acid g⁻¹ FW) (B) of the fruit produced by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions. Each value represents the mean ± SE (*n* = 12). Lower-case letters indicate differences (*p* ≤ 0.05, Duncan test) among treatments.

3.4. PCA Analysis of the Agronomic and Functional Fruit Quality

A PCA analysis was conducted to make the effect of Poncho Negro (R) on the agronomic and functional attributes of the Old Limachino Tomato (L) precise. The analysis confirms that R did influence these attributes. Principal component 1 (PC1) represented 38.32% of the variance (Figure 11A), and it was mainly explained by the following variables: ferric-reducing antioxidant power (FRAP), total phenolic compounds (TP), titratable acidity (TA), fruit visual color (COL) and fruit level of redness (a*/b*), fruit fresh weight (FFW), fruit dry weight (FDW) and polar diameter (PDIA). In other words, PC1 is explained by variables related to antioxidant capacity, acidity and color. Principal Component 2 (PC2) accounted for 24.69% of the variance and was mainly explained by the variable Equatorial diameter (EDIA) and components related to the fruit water content such as water content on fresh weight basis (WC_{FW}), water content on a dry weight basis (WC_{DW}) and the ratio of fresh weight to dry weight FW/DW. On the other hand, Figure 11B clearly shows a first and large separation between the LSL treatment (mainly the lower right quadrant) and the L/L and L/R treatments (upper and lower left quadrants). Additionally, a second separation can be noted between the L/L and L/R treatments. Indeed, there is a shared zone between the two, but at the same time, the presence of a differentiated response of the L/R treatment was clearly noted, which tends to cover the lower left quadrant of the graph.

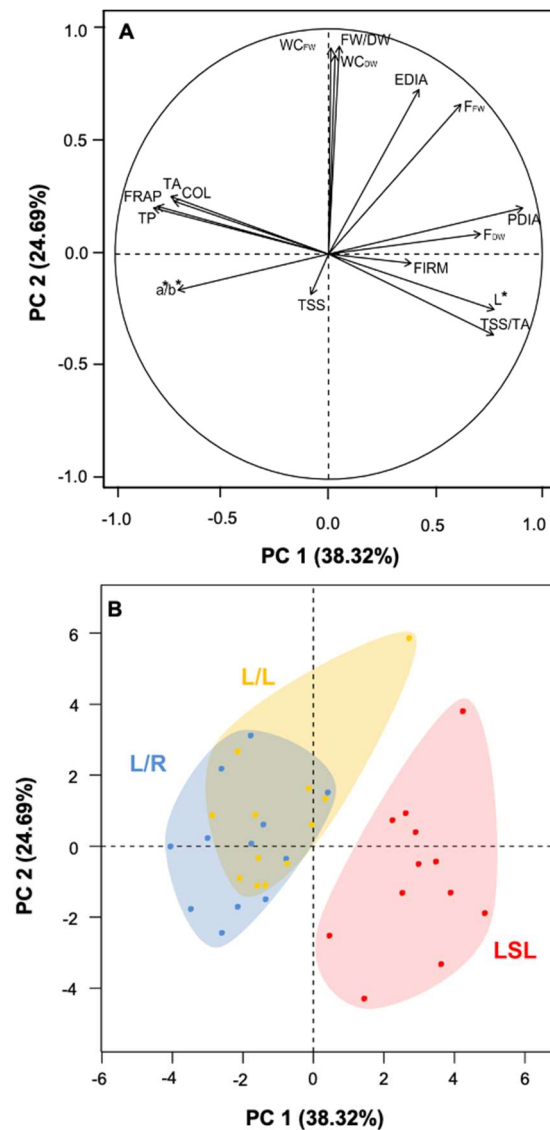


Figure 11. Principal components analysis (PCA) of the agronomic and functional fruit quality for the three treatments (A,B). Individual graphs showing the mean values per each replicate. Abbreviations are explained as follows: a*/b*: Fruit level of redness; COL: fruit visual color; EDIA: equatorial diameter; F_{DW}: fruit dry weight; F_{FW}: fruit fresh weight; FW/DW: fresh weight to dry weight ratio; FRAP: ferric-reducing antioxidant power of the fruit; FIRM: pulp firmness; L*: fruit spectral color parameters; PDIA: polar diameter; TA: titratable acidity of the fruit; TP: total phenolic compounds of the fruit; TSS: total soluble solids of the fruit; TSS/TA: sweetness to acidity ratio; WC_{FW}: water content on a fresh weight basis; WC_{DW}: water content on a dry weight basis.

3.5. Fruit Sensorial Attributes

From a more subjective point of view, Table 1 shows the preferences and the degree of acceptability for the fruits corresponding to the three treatments reported by the untrained sensory panel (final consumers). Fruits from the L/R treatment showed the highest number of preferences followed by L/L fruits, while fruits from the LSL treatment clearly exhibited the lowest level of preference. In relation to acceptability ranges, it is observed that, for categories 5 to 7 (Table 1), there was greater acceptability by the panel towards fruits from the L/L and L/R who exhibited almost similar values than for LSL.

Table 1. Preferences and categories of acceptability perceived by the panelists for the fruits belonging to each treatment: long-shelf-life (LSL), Old Limachino Tomato autografted (L/L) and Old Limachino Tomato grafted on Poncho Negro rootstock (L/R).

Treatment	Preferences (Number of People)	Acceptability (Number of People)		
		Level of Liking (Range)		
		1–3	4	5–7
LSL	24	46	22	36
L/L	34	22	21	61
L/R	46	25	16	63

On the other hand, Figure 12 shows the sensory attributes of the fruit such as color, sweetness, flavor, acidity, aroma, floury, texture and blandness (to the chewing and touch), as perceived by the panelists of the trained panel. The level of visual color perceived by the panelists was significantly higher in L/L and L/R fruits compared to LSL. In contrast, the sweetness perceived by the panelists was higher in LSL than in the other two treatments L/L and L/R. In terms of flavors, the fruits associated with treatments L/L and L/R were ranked higher by the panelists, with values of 7.5 and 6.9, respectively. The fruits from these plants (L/L and L/R) showed a higher level of acidity compared to the fruit associated with the LSL treatment, reaching a level of 4 on the evaluation scale. The level of aroma was lower in the fruit of the LSL plants (4.7) compared to the fruit of the L/L (6.8) and L/R (6.3) plants. The level of floury was lower in fruits issued from L/L treatments with a value of 1 on the evaluation scale compared to those fruits associated with L/R and LSL treatments, which showed values of 5 and 7, respectively. Fruit texture was significantly higher in LSL fruits (9.2) compared to the other two fruits. Finally, the L/L and L/R fruits showed a level of blandness significantly higher than LSL fruits, with values of 5 and 9, respectively, far from the near-zero value reported for the fruit associated with this last treatment.

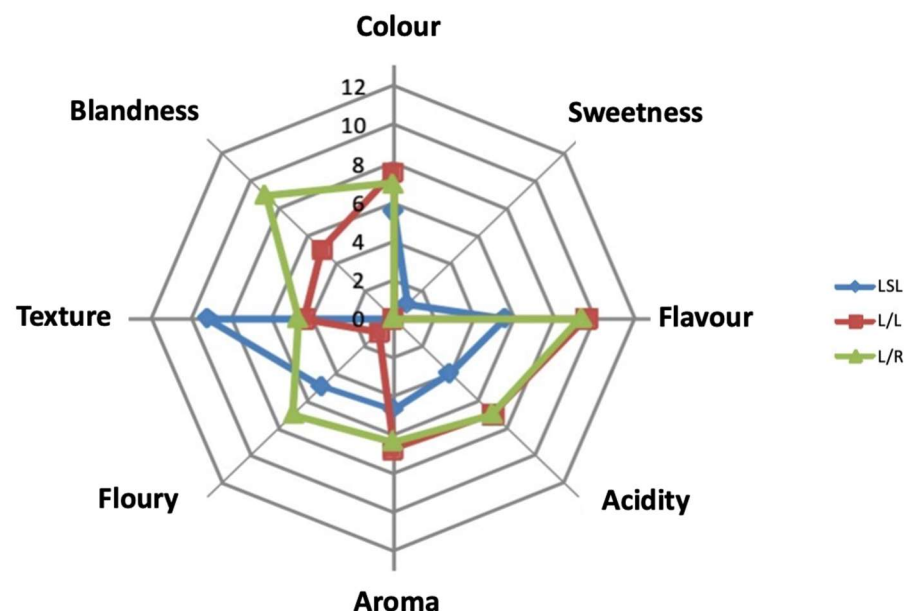


Figure 12. Sensory attributes of fruit: colour, sweetness, flavor, acidity, aroma, floury, texture and blandness as perceived by the expert panel by treatments LSL (long-shelf-life tomato), L/L (self-grafted Old Limachino tomato) and L/R (grafted Old Limachino tomato on Poncho Negro rootstock) under greenhouse conditions.

4. Discussion

About 40 commercial tomato rootstocks are available in the Chilean market, and the most common ones include Arazi, Armstrong, Arnold, Beaufort, Brigeot, Emperor, King Kong, Optifort, Maxifort, Multifort and Unifort. All of them have a foreign origin and usually correspond to inter-specific hybrids that have been selected on the basis of their resistance to common diseases (viruses such as ToMV, among others, fungi present in the soil such as *Fusarium oxysporum f sp lycopersci*, *Verticillium alboatrum* and *Verticillium dahlia*, among others) and nematodes (*Meloidogyne arenaria* and *Meloidogyne javanica*) [26]. To the best of our knowledge, the present study is the first attempt to use local Chilean landrace (Poncho Negro; R) as a rootstock for another landrace (Old Limachino Tomato; L) in relation to fruit properties in the absence of stress.

The present study reports positive effects of the use of R on yield and some agronomic, functional and sensory quality attributes of L fruit relative to fruit produced by self-grafted (L/L) plants on the one-hand and fruits produced by a well-known long-shelf-life tomato (LSL) plants Seminis on the other hand. Total biomass expressed on a dry weight basis was higher for LSL while the total weight of produced fruits expressed on the same basis was higher for L/R (Figure 3). Since the leaf total fresh weight was the lowest for L/R and stem weight was similar in L/R and L/L, we concluded that the harvest index was the highest in L/R (0.80) and the lowest in LSL (0.67), while L/L had an intermediate value (0.74), thus confirming that L/R was the best combination allowing photosynthate distribution towards harvestable parts of the plant.

The highest fruit production of L/R combination in terms of weight per surface could be related to its higher number of fruits (Figure 4). Fullana-Pericàs et al. [22] already demonstrated that the use of local landrace as rootstock improves the fruit's number in a more efficient manner than using a commercial rootstock such as Maxifort. The long-shelf-life (LSL) produced a lower number of fruits, but individual fruits had the highest weight (Figure 5A and 5B). This suggests that the photosynthates produced are allocated to a low number of sink organs, thus sustaining the growth of individual fruits. It must be mentioned that L/R produced a higher number of fruits than L/L, but that individual fruit dry weights were similar in the two types of plant (Figure 5B). This suggests that photoassimilate production might have been higher in source leaves of L/R than in the source leaves of L/L and/or that photoassimilates were more efficiently translocated from source to sink organs in the former than in the latter. From an agronomic point of view, fruit-thinning management has been suggested to improve the weight and the size of fruits associated with local accessions: It can remove smaller or weaker fruits from the plant or even the floret (Figure 13B), a large yellow flower that is a strong center of photoassimilate consumption but causes deformed fruits ("rosca") (Figure 13C). Thus, by using this manual management (blossom and fruit thinning), photosynthetic assimilates (DM) will be concentrated in a few fruits, thereby increasing their size and individual weight, as observed by Ucan-Chan et al. [41] who carried out fruit thinning in tomato, thus increasing the weight of the fruits that remained in the bunch.



Figure 13. Different outcomes of Old Limachino Tomato plants: (A) fruit, (B) floret and (C) deformed fruit (or "Rosca").

When comparing LSL on the one hand and L/L or L/R on the other hand, it is interesting to note that size and form differences are related to the polar diameter side

and not to the equatorial side (Figure 5C,D). The visible effect of this phenomenon is the typically flattened shape of the L accessions, a characteristic of the fruit that is important since it is well recognized by consumers [8]. Hopefully, the use of the local rootstock did not alter this geometry (Figure 13A). In this regard, the literature concerning the study devoted to the effect of rootstock on tomato geometry is scarce and not consensual. On the one hand, Qaryouti et al. [42] and, more recently, Mauro et al. [43] reported a null effect of different rootstocks on several commercial tomato varieties. These findings suggest that fruit geometry is controlled by the scion genotype [44]. On the other hand, Turhan et al. [16] and Casals et al. [4] did report changes in the geometry of local tomato fruits grafted and grown under conventional, high-tunnel cultivation, observing differences between graft combinations under outdoor and organic cultivation. These findings suggest that certain scion–rootstock combinations and environmental conditions may have played some role in the geometric changes detected.

The use of the local Poncho Negro rootstock also allowed obtaining fleshier fruit with less water, thus reinforcing its interest to improve the quality of fruit produced by the L scion (Figure 7). A positive impact of some commercial interspecific hybrids rootstocks on fruit fresh weight has often been attributed to an increase in the fruit's water content [10,16,17], leading to a decrease in fruit quality and nutritive value. This, obviously, did not occur with the Poncho Negro rootstock and could be at least partly related to the fact that Poncho Negro is issued from a dry area and behaves as a water saver with high water-use efficiency in relation to efficient aquaporin functionality and regulation [23].

Fruit color is known to be one of the quality factors in fresh tomato for consumer preference [2]. According to our data, this physical attribute would be a deciding factor of higher preference and acceptability of L/L and L/R fruits by consumers comparatively to LSL. Minolta color a^*/b^* values (Figure 6C) were closely associated with USDA color status values (Figure 6A) as found by Batu [2]. According to this scale, local rootstock R did have a positive effect on the level (or intensity) of the red color of the scion L fruit. Shade tests measured by Minolta color L^* (Figure 6B) detected the presence of white and yellow shades in our fruit material, but these shades were significantly less intense in fruit grafted with the local rootstock. Hence, the use of the local rootstock Poncho Negro does contribute to improving the physical color quality of the Old Limachino Tomato and, therefore, its level of preference and acceptability by consumers. According to Goisser et al. [45], the values of Minolta color L and a^*/b^* are exponentially correlated with lycopene content with a correlation coefficient (r^2) of 0.94 and 0.90. This leads us to hypothesize that both L/L and L/R fruits contained more lycopene than fruits from LSL, although this compound was not quantified in the present study. This hypothesis is supported by the fact that fruits from L/L and L/R exhibited a higher FRAP index than those from LSL, with lycopene being a strong and efficient antioxidant in tomato fruits [1]. The fact that Poncho Negro rootstocks had no deleterious impact on the fruit's lycopene content contrasts with the recent observation of Ellenberger et al. [13], who reported a decrease in lycopene content of the fruit for scions grafted on commercial hybrid rootstocks.

Polyphenols also play a major role as an antioxidant in tomato fruits. Both L/L and L/R fruits had higher total polyphenols concentrations than LSL (Figure 10B) (about 36% higher than that observed in the long-shelf-life tomato). Martínez et al. [28] and Fuentes et al. [46] observed a similar association between FRAP antioxidant capacity and total polyphenol content in *Solanum chilense* fruits and landrace berries, respectively. The antioxidant properties and their potential positive health effects attributable to polyphenols attracted great interest in studies on tomatoes [47–50]. The Poncho Negro rootstock did not increase the polyphenol content of Old Limachino fruits. This is not necessarily a negative point since polyphenols are also associated with sensory properties such as astringency and bitterness, which may generate unpleasant sensations to the consumer in some cases [51,52].

As expected, the firmness of the LSL fruits was about 3 times higher than that measured in L fruits. The local rootstock Poncho Negro was unable to increase fruit firmness of the scion fruits and, thus, could not contribute to improving postharvest quality of

Limachino (extension of shelf life, less sensitivity to physical or mechanical damage, etc.). In high-yielding commercial varieties, firmness is commonly related to modifications in the hormonal status and more specifically to a decrease in ethylene synthesis, which is involved in fruit softening and acts as transcriptional regulators of cell-wall hydrolase. It is well established that rootstocks and scion exchange numerous hormonal products [19,20,53], and it appears here that Poncho Negro was not able to transfer ethylene-counteracting molecules in order to improve Limachino's fruit shelf life. Hence, a selection within the Limachino accession may be an alternative to reduce ethylene synthesis, but this constitutes a risky task inasmuch as ethylene is involved in the regulation of a plethora of metabolic pathways, which contribute to the taste and flavor of Limachino [1,8]. Our sensory panels exhibited a high preference and acceptability for Limachino fruits compared to the commercial fruit in spite of their low level of firmness. This suggests that, at the final consumer level, the firmness attribute seems to be less relevant than other attributes such as color, flavor or aroma. Nevertheless, improvements in firmness of Limachino still remain a priority for producers.

Similarly, the Poncho Negro rootstock did not influence the titratable acidity of the fruits produced by the scion Limachino (Figure 9). According to Aslam et al. [54], fruit acidity could comprise ascorbic acid (vitamin C) content and the higher TA values of Limachino fruit could be regarded as a positive property. Mauro et al. [12] reported a decrease in the ascorbic content in fruits in response to grafting on interspecific commercial rootstocks but this was not observed in the present case. The L fruits produced by L/L or L/R combination were 40% more acidic than the long-shelf-life LSL variety used in the trials (Figure 9B). Although it has an important role for human nutrition, ascorbic acid is only a minor contributor to acidity comparatively to citric and malic acid [1] and grafting was reported to alter organic acid metabolism in some rootstock/scion combinations [10]. According to Huang et al. [55], phosphoenolpyruvate carboxykinase is often stimulated during the ripening process of commercial cultivars and acts in the dissimilation of malate/citrate to provide sugar through the neoglucogenesis pathway. If this is valid in LSL fruits, it might explain the lower TA values recorded in this material. TSS was the same for the three considered treatments and it is well known that TSS in tomato fruit is mainly due to soluble sugars. By combining the values obtained for TSS (Figure 9A) and TA (Figure 9B) from our experiments, the results showed a lower and very similar value of the TSS/TA ratio for the local L/L and L/R fruits (Figure 9C). Since the TSS/TA ratio is generally associated with fruit flavour, i.e., the balance between sweetness and acidity, it is plausible to claim that acidity contained in tomato fruits is a determinant for consumers' preferences and acceptability for and towards the fruit, similarly to what the sensory analysis reveals. In terms of acceptability, it has been documented that consumer's acceptability of tomato fruits should be high in aroma intensity and sweetness but of intermediate acidity [3,56,57].

The putative interest of Poncho Negro rootstocks for improvements in L fruit perception by consumers is confirmed by the sensory panels analysis (Table 1). In terms of preference, L/R fruits displayed a score of 46 while it was only 34 for L/L. The acceptability levels were clearly higher for L/L and L/R than for LSL. Still, from a subjective perspective, it is plausible to argue that the decision to choose mostly the L/R fruit may be associated with its higher level of perception of attributes such as flavor, color, aroma and acidity detected by the sensory panel. However, when comparing the two fruits from the local accessions (L/L and L/R), no significant changes in acidity level were observed. In other words, it would not be the higher acidity attribute that would be the sustaining or defining preference and acceptability of the L/R fruit by consumers. Moreover, the fact that blandness was two times higher for L/R fruits than for L/L constitutes puzzling information. In addition to sugars and acids, volatile compounds are major determinants of organoleptic fruit properties and trigger a response to the olfactory system contributing to overall taste sensation. Volatiles are metabolically derived from aliphatic amino acids, terpenoids, phenolic compounds and fatty acids [44,58]. According to Zhou et al. [15] and to Špika et al. [5], rootstocks may have an important impact on volatiles profile. We

may, thus, hypothesize that this complex class of compounds was modified in L/R fruits comparatively to L/L ones and contributes to the positive effect recorded by consumers' acceptability and preference.

5. Conclusions

The present work confirms that the local tomato landrace Poncho Negro may be adequately used as a convenient rootstock for the Old Limachino tomato cultivar. Indeed, Poncho Negro rootstock allowed an increase in the total number of fruits produced by Limachino scion without negative impacts on individual fruit weight or typical fruit form. Poncho Negro rootstock, however, remained unable to increase the fruit firmness of Limachino scion fruits compared to the fruit of the long-self-life Seminis cultivar. Untrained and trained experts panels attributed the highest qualitative value for organoleptic attributes to fruits produced by the L/R combination.

Our study was performed under optimal conditions while it is well established that adapted rootstock mainly provides advantages under stress conditions [13,14,18,19,21,22,34]. Since the rustic tomato landrace Poncho Negro is highly resistant to a wide range of constraints [7,23–26], additional studies are currently in progress in our laboratories make precise the interest on the Poncho Negro rootstock with respect to Old Limachino fruits produced under drought and salt conditions.

Author Contributions: Conceptualization, J.P.M. and R.F.; methodology, K.F., N.L., A.F. and J.P.M.; experiments validation, J.P.M., B.S., K.F., A.F. and N.L.; statistical analysis, J.P.M., B.S., R.F., A.F. and M.Q.; investigation, J.P.M., R.F., C.S. and S.L.; resources, J.P.M., M.Q., and S.L.; data curation, K.F., A.F. and J.P.M.; writing—original draft preparation, J.P.M., M.Q., S.L. and R.F.; writing—review and editing, J.P.M., S.L. and R.F.; supervision, J.P.M. and R.F.; project administration, J.P.M.; funding acquisition, J.P.M., M.Q., C.S. and S.L. All authors have contributed substantially to the work reported. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Development and Research National Agency (ANID) of the Ministry of Science and Technology of Chile (FONDECYT projects No 1180958 and No 220909), the PIA Project ACT No 192073 “Plant Abiotic Stress for a Sustainable Agriculture” (PASSA), the Agriculture Ministry project No 220090-70 and the Bilateral Cooperation project between Chile (AGCI) and Belgium (WBI-2019) No 17.

Acknowledgments: Juan Pablo Martínez and Boris Sagredo acknowledge the contribution of the FONDECYT projects No 1180958 and No 1220909 funded by the Development and Research National Agency (ANID) of the Ministry of Science and Technology of Chile. Juan Pablo Martínez acknowledges the support of the Agriculture Ministry project No 220090-70. Juan Pablo Martínez and Claudia Stange acknowledge the support of the PIA Project ACT No 192073 “Plant Abiotic Stress for a Sustainable Agriculture” (PASSA) funded by the Development and Research National Agency (ANID) of the Ministry of Science and Technology of Chile. Juan Pablo Martínez, Raúl Fuentes, Muriel Quinet and Stanley Lutts also acknowledge the support of the Bilateral Cooperation project between Chile (AGCI) and Belgium (WBI-2019) No 17. Juan Pablo Martínez and Raúl Fuentes would like to thank Juan Felipe Alfaro and Luis Morales for their valuable technical assistance. The authors are grateful for the invaluable comments and suggestions made by two anonymous referees are grateful for the invaluable comments and suggestions made by three anonymous referees. Juan Pablo Martínez and Raúl Fuentes thank the Cooperativa Agrícola de Limache (COALIM Ltd.a.) for its constant willingness to exchange ideas on how to grow the Old Limachino Tomato.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A



Figure A1. Colour classification requirements in Old Limachino Tomato based on USDA (1976): (1) Green, (2) Breakers, (3) Turning, (4) Pink, (5) Light Red and (6) Red.

References

- Quinet, M.; Angosto, T.; Yuste-Lisbona, F.J.; Blanchard-Gros, R.; Bigot, S.; Martínez, J.P.; Lutts, S. Tomato fruit development and metabolism. *Front. Plant Sci.* **2019**, *10*, 1554. [[CrossRef](#)] [[PubMed](#)]
- Batu, A. Determination of acceptable firmness and colour values of tomatoes. *J. Food Eng.* **2004**, *61*, 471–475. [[CrossRef](#)]
- Causse, M.; Friguier, C.; Coiret, C.; Lépiciier, M.; Navez, B.; Lee, M.; Holthuysen, N.; Sinesio, F.; Moneta, E.; Grandillo, S. Consumer Preferences for Fresh Tomato at the European Scale: A Common Segmentation on Taste and Firmness. *J. Food Sci.* **2010**, *75*, 531–541. [[CrossRef](#)]
- Casals, J.; Rull, A.; Bernal, M.; González, R.; Romero del Castillo, R.; Simó, J. Impact of grafting on sensory profile of tomato landraces in conventional and organic management systems. *Hortic. Environ. Biotechnol.* **2018**, *59*, 597–606. [[CrossRef](#)]
- Špika, M.J.; Dumičić, G.; Bubola, K.B.; Soldo, B.; Ban, S.G.; Selak, G.V.; Ljubenković, I.; Mandušić, M.; Žanić, K. Modification of the sensory profile and volatile aroma compounds of tomato fruits by the scion x rootstock interactive effect. *Front. Plant Sci.* **2021**, *11*, 616431. [[CrossRef](#)]
- Klein, D.; Gkisaki, V.; Krumbein, A.; Livieratos, I.; Köpke, U. Old and endangered tomato cultivars under organic greenhouse production: Effect of harvest time on flavour profile and consumer acceptance. *Int. J. Food Sci. Technol.* **2010**, *45*, 2250–2257. [[CrossRef](#)]
- Angel, Y.; Esteban, W.; Bustos, R.; Pacheco, P.; Hurtado, E.; Bastías, E. Tomato “Poncho negro”. History and redemption of culture forgotten. *Idesia* **2016**, *34*, 65–69.
- Martínez, J.P.; Jana, C.; Muenza, V.; Salazar, E.; Rico, J.J.; Calabrese, N.; Hernández, J.; Lutts, S.; Fuentes, R. The Recovery of the Old Limachino Tomato: History, Findings, Lessons, Challenges and Perspectives. In *Agriculture Value Chain—Challenges and Trends in Academia and Industry. Studies in Systems, Decision and Control*; Hernández, J., Kacprzyk, J., Eds.; Springer: Cham, Switzerland, 2021; Volume 280, pp. 104–119. [[CrossRef](#)]
- Natalini, A.; Acciarri, N.; Cardi, T. Breeding for nutritional and organoleptic quality in vegetable crops: The case of tomato and cauliflower. *Agriculture* **2021**, *11*, 606. [[CrossRef](#)]
- Grieneisen, M.L.; Aegerter, B.J.; Stoddard, C.S.; Zhang, M. Yield and fruit quality of grafted tomatoes, and their potential for soil fumigant use reduction. A meta-analysis. *Agron. Sustain. Dev.* **2018**, *38*, 29. [[CrossRef](#)]
- Zeist, A.R.; Villela de Resende, J.T.; Zanin, D.S.; Ribeiro da Silva, A.L.B.; Perrud, A.C.; Bueno, G.A.; Arantes, J.H.V.; de Lima, D.P. Effect of acclimation environments, grafting methods and rootstock RVT-66 on the seedling development and production of tomato. *Sci. Hort.* **2020**, *271*, 109496. [[CrossRef](#)]
- Mauro, R.P.; Agnello, M.; Onofri, A.; Leonardi, C.; Giuffrida, F. Scion and rootstock differently influence growth, yield and quality characteristics of cherry tomato. *Plants* **2020**, *9*, 1725. [[CrossRef](#)] [[PubMed](#)]
- Ellenberger, J.; Bulut, A.; Blömeke, P.; Röhlen-Schmittgen, S. Novel *S. pennellii* x *S. lycopersicum* hybrid rootstocks for tomato production with reduced water and nutrient supply. *Horticulturae* **2021**, *7*, 355. [[CrossRef](#)]
- Khapte, P.S.; Kumar, P.; Walkchaure, G.C.; Jangid, K.K.; Colla, G.; Cardarelli, M.; Rane, J. Application of phenomics to elucidate the influence of rootstocks on drought response of tomato. *Agronomy* **2022**, *12*, 1529. [[CrossRef](#)]
- Zhou, Z.; Yuan, Y.; Wang, K.; Wang, H.; Huang, J.; Yu, H.; Cui, X. Rootstock scion interactions affect fruit flavor in grafted tomato. *Hortic. Plant J.* **2022**, *8*, 499–510. [[CrossRef](#)]
- Turhan, A.; Ozmen, N.; Serbeci, M.S.; Seniz, V. Effects of grafting on different rootstocks on tomato fruit yield and quality. *Hortic. Sci.* **2011**, *38*, 142–149. [[CrossRef](#)]
- Lang, K.M.; Nair, A.; Moore, K.J. The impact of eight hybrid tomato rootstocks on BHN 589 scion yield, fruit quality, and plant growth traits in a Midwest high tunnel production system. *HortScience* **2020**, *55*, 936–944. [[CrossRef](#)]
- Fu, S.; Chen, J.; Wu, X.; Gao, H.; Lü, G. Comprehensive evaluation of low temperature and salt tolerance in grafted and rootstock seedlings combined with yield and quality of grafted tomato. *Horticulturae* **2022**, *8*, 595. [[CrossRef](#)]

19. Asins, M.J.; Albacete, A.; Martínez-Andújar, C.; Celiktopus, E.; Solmaz, I.; Sari, N.; Pérez-Alfocea, F.; Dodd, I.C.; Carbonell, E.A.; Topcu, S. Genetic analysis of root-to-shoot signaling and rootstock-mediated tolerance to water deficit in tomato. *Genes* **2021**, *12*, 10. [CrossRef]
20. Ghanem, M.E.; Hichri, I.; Smigocki, A.C.; Albacete, A.; Fauconnier, M.L.; Diatloff, E.; Martínez-Andujar, C.; Lutts, S.; Dodd, I.C.; Pérez-Alfocea, F. Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. *Plant Cell Rep.* **2011**, *30*, 807–823. [CrossRef]
21. Martínez-Andújar, C.; Martínez-Pérez, A.; Albacete, A.; Martínez-Melgarejo, P.A.; Dodd, I.C.; Thompson, A.J.; Mohareb, F.; Estelles-Lopez, L.; Kevei, Z.; Ferrández-Ayela, A.; et al. Overproduction of ABA in rootstocks alleviates salinity stress in tomato shoots. *Plant Cell Environ.* **2021**, *44*, 2966–2986. [CrossRef]
22. Fullana-Pericàs, M.; Conesa, M.À.; Ribas-Carbó, M.; Galmés, J. The use of a tomato landrace as rootstock improves the response of commercial tomato under water deficit conditions. *Agronomy* **2020**, *10*, 748. [CrossRef]
23. Contreras, C.; Montoya, A.; Pacheco, P.; Martínez-Ballesta, M.C.; Carvajal, M.; Bastías, E. The effects of the combination of salinity and excess boron on the water relations of tolerant tomato (*Solanum lycopersicum* L.) cv. Poncho Negro, in relation to aquaporin functionality. *Span. J. Agric. Res.* **2011**, *9*, 494–503. [CrossRef]
24. Díaz, M.; Bastías, E.; Pacheco, P.; Tapia, L.; Martínez-Ballesta, M.C.; Carvajal, M. Characterization of the physiological response of the highly-tolerant tomato cv. Poncho Negro to salinity and excess boron. *J. Plant Nutr.* **2011**, *34*, 1254–1267. [CrossRef]
25. Esteban, W.; Pacheco, P.; Tapia, L.; Bastías, E. Remediation of salt and boron-affected soil by addition of organic matter: An investigation into improving tomato plant productivity. *Idesia* **2019**, *34*, 25–32. [CrossRef]
26. Alfaro, J.F. Efecto del Portainjerto INIA (*Solanum lycopersicum* var. Poncho Negro) Sobre el Mecanismo de Defensa de la Planta Injertada Tomate var. Limachino Inducido por el Sulfuro de Hidrógeno (H₂S) Frente al Fitopatógeno *Pseudomonas syringae* pv *tomato*. Ph.D. Thesis, Pontifical Catholic University of Valparaíso and Federico Santa María Technical University, Valparaíso, Chile, 2018.
27. Stone, H.; Sidel, J.L. *Sensory Evaluation Practices*, 3rd ed.; Elsevier Academic Press: Cambridge, MA, USA, 1993; pp. 1–377.
28. Martínez, J.P.; Fuentes, R.; Farias, K.; Lizana, C.; Alfaro, J.F.; Fuentes, L.; Calabrese, N.; Bigot, S.; Quinet, M.; Lutts, S. Effects of saline stress on fruit antioxidant capacity of wild (*Solanum chilense*) and domesticated (*Solanum lycopersicum* var. *cerasiforme*) tomatoes. *Agronomy* **2020**, *10*, 1481. [CrossRef]
29. Bhatt, R.M.; Upreti, K.K.; Divya, M.H.; Bhat, S.; Pavithra, C.B.; Sadashiva, A.T. Interspecific grafting to enhance physiological resilience to flooding stress in tomato (*Solanum lycopersicum* L.). *Sci. Hortic.* **2015**, *182*, 8–17. [CrossRef]
30. Grierson, A.; Kader, A.A. Fruit ripening and quality. In *Tomato Crop*; Atherton, J.G., Rudich, J., Eds.; Chapman and Hall Ltd.: New York, NY, USA, 1986; pp. 241–280.
31. USDA. *United States Standards for Grade of Fresh Tomatoes*; USDA Marketing Services: Washington, DC, USA, 1976; p. 10.
32. Martínez, J.P.; Antúnez, A.; Pertuzé, R.; Acosta, M.P.; Palma, X.; Fuentes, L.; Ayala, A.; Araya, H.; Lutts, S. Effects of saline water on water status, yield and fruit quality of wild (*Solanum chilense*) and domesticated (*Solanum lycopersicum* var. *cerasiforme*) tomatoes. *Exp. Agric.* **2012**, *48*, 573–586. [CrossRef]
33. Martínez, J.P.; Antúnez, A.; Araya, H.; Pertuzé, R.; Acosta, M.D.P.; Fuentes, L.; Lizana, C.; Lutts, S. Salt stress differently affects growth, water status and antioxidant enzyme activities in *Solanum lycopersicum* L. and its wild-relative *Solanum chilense* Dun. *Aust. J. Bot.* **2014**, *62*, 359–368. [CrossRef]
34. Almasoum, A.A. Effect of planting depth on growth and productivity of tomatoes using drip irrigation with semi-saline water. *Acta Hort.* **2000**, *573*, 773–778. [CrossRef]
35. Ordóñez-Santos, L.E.; Arbones-Macifeira, E.; Fernández-Perejón, J.; Lombardero-Fernández, M.; Vázquez-Odériz, L.; Romero-Rodríguez, A. Comparison of physicochemical, microscopic and sensory characteristics of ecologically and conventionally grown crops of two cultivars of tomato (*Lycopersicon esculentum* Mill.). *J. Sci. Food Agric.* **2009**, *89*, 743–749. [CrossRef]
36. Benzie, I.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
37. Singleton, V.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–148.
38. Galati, E.; Mondello, M.R.; Giuffrida, D.; Dugo, G.; Miceli, N.; Pergolizzi, S.; Taviano, M.F. Chemical characterization and biological effects of Sicilian *Opuntia ficus indica* (L.) Mill. Fruit juice: Antioxidant and antiulcerogenic activity. *J. Agric. Food Chem.* **2003**, *51*, 4903–4908. [CrossRef] [PubMed]
39. Arias-Carmona, M.D.; Romero-Rodríguez, M.A.; Muñoz-Ferreiro, N.; Vázquez-Odériz, M.L. Sensory Analysis of Protected Geographical Indication Products: An Example with Turnip Greens and Tops. *J. Sens. Stud.* **2012**, *27*, 482–489. [CrossRef]
40. Wittig, E. Evaluación Sensorial. Una Metodología Actual para Tecnología de Alimentos. 2001. Available online: <https://repositorio.uchile.cl/handle/2250/121431> (accessed on 30 April 2017).
41. Ucan-Chan, I.; Sánchez-Del Castillo, F.; Contreras-Magaña, F.; Corona-Sáez, T. Effect of plant density and fruit thinning on tomato yield and fruit size. *Rev. Fitotec. Mex.* **2005**, *28*, 33–38.
42. Qaryouti, M.; Qawasmi, W.; Hamdan, H.; Edwan, M. Tomato fruit yield and quality as affected by grafting and growing system. *Acta Hort.* **2007**, *741*, 199–206. [CrossRef]

43. Mauro, R.P.; Rizzo, V.; Leonardi, C.; Mazzaglia, A.; Muratore, G.; Distefano, M.; Sabatino, L.; Giuffrida, F. Influence of harvest stage and rootstock genotype on compositional and sensory profile of the elongated tomato cv. "Sir Elyan". *Agriculture* **2020**, *10*, 82. [[CrossRef](#)]
44. Bertin, N.; Genard, M. Tomato quality as influenced by preharvest factors. *Sci. Hortic.* **2018**, *233*, 264–276. [[CrossRef](#)]
45. Goisser, S.; Wittmann, S.; Fernandes, M.; Mempel, H.; Ulrichs, C. Comparison of colorimeter and different portable food-scanners for nondestructive prediction of lycopene content in tomato fruit. *Postharvest Biol. Technol.* **2020**, *167*, 111232. [[CrossRef](#)]
46. Fuentes, L.; Valdenegro, M.; Gómez, A.G.; Ayala-Raso, A.; Quiroga, E.; Martínez, J.P.; Vinet, R.; Caballero, E.; Figueroa, C.R. Characterization of fruit development and potential health benefits of arrayan (*Luma apiculata*), a native berry of South America. *Food Chem.* **2016**, *196*, 1239–1247. [[CrossRef](#)]
47. Caris-Veyrat, C.; Amiot, M.J.; Tyssandier, V.; Grasselly, D.; Buret, M.; Mikolajczak, M.; Guillard, J.-C.; Bouteloup-Demange, C.; Patrick Borel, P. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J. Agric. Food Chem.* **2004**, *52*, 6503–6509. [[CrossRef](#)] [[PubMed](#)]
48. Slimestad, R.; Verheul, M. Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* mill.) cultivars. *J. Sci. Food Agric.* **2009**, *89*, 1255–1270. [[CrossRef](#)]
49. del Río, D.; Borges, G.; Crozier, A. Berry flavonoids and phenolics: Bioavailability and evidence of protective effects. *Br. J. Nutr.* **2010**, *104*, S67–S90. [[CrossRef](#)]
50. Hanhineva, K.; Törrönen, R.; Bondia-Pons, I.; Pekkinen, J.; Kolehmainen, M.; Mykkänen, H.; Poutanen, K. Impact of dietary polyphenols on carbohydrate metabolism. *Int. J. Mol. Sci.* **2010**, *11*, 1365–1402. [[CrossRef](#)]
51. Ozawa, T.; Lilley, T.H.; Haslam, E. Polyphenol interactions: Astringency and the loss of astringency in ripening fruit. *Phytochemistry* **1987**, *26*, 2937–2942. [[CrossRef](#)]
52. Soares, S.; Brandão, E.; Mateus, N.; De Freitas, V. Sensorial Properties of Red Wine Polyphenols: Astringency and Bitterness. *Crit. Rev. Food Sci. Nutr.* **2015**, *57*, 937–948. [[CrossRef](#)] [[PubMed](#)]
53. Cantero-Navarro, E.; Romero-Aranda, R.; Fernández-Muñoz, R. Improving agronomic water use efficiency in tomato by rootstock-mediated hormonal regulation of leaf biomass. *Plant Sci.* **2016**, *251*, 90–100. [[CrossRef](#)]
54. Aslam, W.; Noor, R.S.; Hussain, F.; Ameen, M.; Ullah, S.; Chen, H. Evaluating morphological growth, yield, and postharvest fruit quality of cucumber (*Cucumis sativus* L.) grafted on cucurbitaceous rootstocks. *Agriculture* **2020**, *10*, 101. [[CrossRef](#)]
55. Huang, Y.X.; Goto, Y.; Nonaka, S.; Fukuda, N.; Ezura, H.; Matsukura, C. Overexpression of the phosphoenolpyruvate carboxykinase gene (SlPEPCK) promotes soluble sugar accumulation in fruit and post-germination growth of tomato (*Solanum lycopersicum* L.). *Plant Biotechnol.* **2015**, *32*, 281–289. [[CrossRef](#)]
56. Casals, J.; Rivera, A.; Sabaté, J.; del Castillo, R.R.; Simó, J. Cherry and fresh market tomatoes: Differences in chemical, morphological, and sensory traits and their implications for consumer acceptance. *Agronomy* **2019**, *9*, 9. [[CrossRef](#)]
57. Baldwin, E.A.; Scott, J.W.; Einstein, M.A.; Malundo, T.M.M.; Carr, B.T.; Shewfelt, R.L.; Tandon, K.S. Relationship between sensory and instrumental analysis for tomato flavor. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 906–915. [[CrossRef](#)]
58. Distefano, M.; Mauro, R.P.; Page, D.; Giuffrida, F.; Bertin, N.; Leonardi, C. Aroma Volatiles in Tomato Fruits: The Role of Genetic, Preharvest and Postharvest Factors. *Agronomy* **2022**, *12*, 376. [[CrossRef](#)]