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Eggplant grafting on a cold tolerant rootstock reduces fruit chilling susceptibility and improves antioxidant stability during storage

Running title: Eggplant grafting improves postharvest chilling tolerance

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Abstract

BACKGROUND: Vegetable grafting has been increasingly evaluated to improve preharvest tolerance to biotic and abiotic stresses. Instead, very few studies have identified rootstock-scion combinations able to improve fruit shelf-life and reduce the susceptibility to postharvest disorders. Herein, a purple eggplant scion (cv. Monarca) was grafted onto a cold tolerant hybrid *Solanum* rootstock (*Java*) and the changes in growth, quality, postharvest chilling tolerance, and antioxidant stability were evaluated.

RESULTS: Eggplant grafting enhanced plant vigor and fruit growth rate, decreasing the time from set to harvest by 10-15%. Grafted eggplants had a thinner shape and lighter pulp color than the control. The rootstock-scion combination tested showed lower respiration (~60 %), dry matter (~15-20%), and phenolic compounds contents (~15-20%) than eggplants from non-grafted plants. Grafting, markedly improved fruit performance during postharvest storage. Remarkably, grafted eggplants showed much higher tolerance to chilling injury than the control, evidenced by a reduction of surface scalds along with decreased softening and pulp browning. The trend in antioxidants found at harvest time was reversed after cold storage due to enhanced stability (20% and 100% for pulp and peel) in fruit from grafted plants.

CONCLUSION: Purple eggplant (cv. Monarca) grafting onto *Java* hybrid rootstock modulated fruit growth, quality at harvest, and increased fruit CI tolerance during storage. Grafting may be a *bona fide* strategy to induce phenotypic traits able to improve vegetable postharvest performance.

Keywords: postharvest; vegetable; chilling injury; aubergine; phenolics; quality.

1. INTRODUCTION

Grafting is an ancient vegetative propagation technique fusing plant segments usually from different individuals[1]. It has been used for a long time in perennial fruit species, mostly to improve plant tolerance to pests and diseases, soil, or climatic limitations, aiding crop management practices[2-9]. Although the technique has been also technically feasible for annual species for a long time, its commercial use was initially restricted due to both economic and scaling up difficulties. The improvements of approximation grafting techniques achieved in the last decade reduced plant production costs, renewing the interest in its use in annual vegetable species[10]. Today grafted plants are being tested under commercial productions of tomato, watermelon, melon cucumber, pepper, and eggplant among others[11].

One of the most studied families is the *Solanaceae,* particularly the tomato[5,6,9,10,12]. Based on this research, some rootstock-scion combinations able to modulate plant vigor, enhance yields, and plant fitness under restrictive conditions have been identified[11,13]. In eggplant, initial grafting studies focused on improving the tolerance to phytopathogens such as *Fusarium, Verticillium,* and nematodes[15,16]. Subsequent work looked for advantageous agronomic traits including yield and precocity[13,17-20]. In terms of fruit quality, the results reported in the literature are widely variable. In some cases, no changes in overall fruit composition have been detected, whereas other studies reported modifications in carpomentric attributes[13,17]color[18,20,21], and sweetness[22]. Moreover, the effects of eggplant rootstock-scion combination on some constituents such as antioxidants differ greatly, ranging from significant increases[13,18] to lack of changes[20] or even reductions[22].

A survey of works conducted so far in grafted vegetable crops points out that few works have relied on grafting as a means of modulating desirable properties during postharvest storage. The only report characterizing the performance of grafting in eggplant during storage at 10 °C did not show promising results. The evaluated rootstock-scion combination reduced consumer acceptance and accelerated vitamin C loss and softening during storage [22]. Given its subtropical origin, eggplant is very sensitive to chilling injuries, both in the field and during postharvest storage[23,25-27]. Interestingly, scion-rootstock compatibility studies[13,12,24] have shown that the species not only yields compatible intraspecific rootstocks but also interspecific combinations with tomato and several hybrids[14,24]. This markedly expands the rootstock-scion combinations to explore. The use of *Solanum torvum* as a rootstock improved the fitness of eggplant plants developing under low temperature[26,28]. The *Solanum* hybrid rootstock *Java* is recommended to improve eggplant crop cold tolerance[29]. Whether or not the use of such cold-tolerant rootstock-scion combinations may improve the responses to chilling of harvested fruit is currently unknown. In this work, we evaluated the influence of eggplant grafting (scion cv Monarca, rootstock cold-tolerant hybrid *Solanum Java*) on the fruit growth rate, metabolic rate, quality along with development, postharvest chilling tolerance, and antioxidant stability. Since most of the research conducted to date have relied on the evaluation of a single maturity stage[13,15-22], analyzed the fruit at harvest at three maturity stages, which is particularly useful in immaturely harvested vegetables like eggplants, having a broad harvest window (from a small baby stage to fully elongated fruit[23]).

2. MATERIALS AND METHODS

2.1. Plant material

Ungrafted American purple eggplants (cv. Monarca, control) or grafted plants (Monarca scion on Java interspecific hybrid rootstock, Takii seed, Japan) were grown in a greenhouse in La Plata (Buenos Aires, Argentina. 34°59′18″ S, 57°56′17″ W) from September 2019 to Feb 2020. Seeds were sown on 4 Aug. in planting trays with 35 cm^3 cells containing a commercial Sphagnum peat moss and vermiculite substrate (Sunshine mix 3®, Sun Gro Horticulture Canada Ltd., New Brunswick, Can.). Greenhouse temperatures were 22–15 ± 1°C (day-night). Fluorescent bulbs (450 lumens) were used to extend natural sunlight to at least 11 h. At the 2-true leaf stage, seedlings were manually transplanted into a vertic-Paleudol soil in double rows on 10 Aug. in La Plata, Argentina, in an arch-type greenhouse (2.5 m height × 10 m width × 40 m length), covered with low-density polyethylene (150 μm thick). The sides were closed with polyethylene (100 μm thick). Passive ventilation was provided by opening the sides of the greenhouse when the air temperature exceeded 25°C. The light was from natural solar radiation with an average day length of 13, and 9 h. While seedlings were being produced the soil was prepared with a rolling cultivator and shaped into raised beds. Plants were spaced 1 m between rows (rows were 40 m long) and 0.8 m between plants (51plants/row). Plants were pruned 2 months after transplanting to four stems. Plants were fertilized with Nitrofoska® (EuroChem Agro, Barcelona, Spain), with 7% N (4% from ammonia and 3% from nitrate) 12% P as P_2O_5, and 40% K as K_2O. The fertilizer was mixed at 0.15 kg L^{-1} and applied after transplanting through an irrigation system daily during spring and summer. Water was supplied for 1 h day^{-1} (6:00 and 18:00, for 30 min each) at an emitter flow rate of 4

$Lh^{-1}m^{-2}$. An anti-aphid screen was located on the greenhouse sides as a physical barrier. The fruit was naturally pollinated.

2.2. Experiment 1. Influence of grafting on the growth rate, shape, color, seed development, respiration, dry matter, and antioxidants fruit

Fruit from control and grafted plants was tagged immediately after the set. Elongation was followed daily to record the time required to reach the baby, (9 cm long), mid-commercial maturity (mid-CM,17 cm long), and late-commercial maturity (late-CM, 19 cm long) stages. Fruit from control and grafted plants were harvested at the three maturity stages indicated above and used to assess fruit maximum diameter, seed number per slice, seed mean size, surface and pulp color, dry matter content, and respiration rate. Samples from peel and pulp tissues from each fruit group and maturity stage were taken, frozen in liquid N_2, and stored at -80 ºC until used for phenolics, anthocyanins, and chlorogenic acid evaluation. Twenty fruits were evaluated for each treatment and harvest and three independent harvests were analyzed throughout the season (Dec-Feb).

2.2.1. Fruit diameter, seed number per slice, and seed area

Fruit equatorial diameter was evaluated with a digital caliper. For seed evaluation, a slice of eggplant was cut transversely to the main axis in the equatorial zone. The samples were held at room temperature for 30 min to favor seed browning in other to enhance image segmentation of seeds from the pulp. Digital images were acquired with a scanner (HP digital scanner 9250c, United States). The raw data files obtained were subsequently analyzed using Image J Software[30] according to **Valerga et al.**[25] to determine the seed number and the mean seed area per slice (mm^2).

2.2.2. Respiration rate

Fruit was placed in an air-tight jar container (3 L) and incubated for 10 min at 20 °C. The concentration of CO_2 in the headspace was determined with an infrared sensor (Compu-Flow, Model 8650, Alnor CA, United States) and used to calculate the respiration rate. Results were expressed as mg CO_2 kg^{-1} h^{-1}. Four replicates were evaluated for each fruit group (control and grafted) and maturity stage (baby, mid-CM, and late-CM).

2.2.3. Dry matter

Two grams of fruit pulp from the equatorial zone were weighed and dried at 105 °C in an oven until reaching constant weight. Dry matter content (DM) was calculated by weight difference according to DM (g kg^{-1}) = ((WI-WF)/WI)/1000, being WI and WF the weight at an initial and final time, respectively. Five measurements were evaluated on each fruit group (control and grafted) and maturity stage (baby, mid-CM, and late-CM).

2.2.4. Peel color and pulp lightness

Fruit external color was evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) obtaining the CIE L^*, a*, and b^* parameters. Pulp color was followed by assessing the L^* lightness values. Three measurements were done on each fruit and averaged.

2.2.5. Total phenolics: Folin-Ciocalteu method

Peel (0.5g) and pulp (1.0 g) samples were frozen in liquid N_2 and ground in a mill (Peabody, PE-MC9100, China). The resulting powder was suspended in 5 mL of ethanol and vortexed for 3 min. The extraction procedure was repeated three times. Samples were

centrifuged at 13,000 × *g* for 10 min at 4 °C (Sorvall ST 16R, United States). Measurements were done spectrophotometrically at 760 nm according to **Singleton et al.**[31]using the Folin-Ciocalteu reagent. Chlorogenic acid was used as a standard. Results were expressed as chlorogenic acid equivalents in milligrams per kilogram on fresh weight basis. Six measurements were done for each fruit group (control and grafted) and maturity stage (baby, mid-CM, and late-CM).

2.2.6. Phenolic compounds: UV method

Peel and pulp samples were extracted as described in section *2.2.5.* The supernatants were mixed and diluted with ethanol (1:25 and 1:10 respectively). Measurements were done according to **Luthria and Mukhopadhyay**[32]. Chlorogenic acid was used as a standard. Results were expressed as chlorogenic acid equivalents in milligram per kilogram on fresh weight basis. Six measurements were done for each fruit group (control and grafted) and maturity stage (baby, mid-CM, and late-CM).

2.2.7. Anthocyanins

Peel anthocyanins were extracted and quantified according to **Zaro et al.**[23] Results were calculated using a molar extinction coefficient of delphinidin-3-glucoside (29,000 $Lmol^{-1}cm^{-1}$) and expressed in milligram per kilogram on fresh weight basis. Six measurements were done for each fruit group (control and grafted) and maturity stage (baby, mid-CM, and late-CM).

2.3. Experiment 2. Influence of grafting on postharvest chilling injury tolerance, pulp browning, weight loss, softening, and antioxidants stability

Eggplants from control and grafted plants were harvested at mid-commercial maturity (mid-CM, 17 cm long) and immediately transported to the laboratory. Fruit was further selected to eliminate fruit that had blemishes or other defects and washed with chlorinated water (150 mg L^{-1}NaClO, pH 6.5). Fruit was subsequently packed in plastic trays and covered with perforated PVC film and stored at 1 °C (±0.5 °C). After 0-, 14-, 21-, and 28-days samples were removed from storage and used to evaluate surface scalds, pulp browning, lightness (*L**), and texture. Pulp and peel tissue samples were taken, frozen in liquid nitrogen, and stored at -80 °C until use for antioxidant evaluation. Twenty fruits were used for each fruit group (control and grafted) and the whole experiment was conducted in triplicate.

2.3.1. Surface scald index

Surface scald development was evaluated according to **Concellón et al.**[33]. A five-point hedonic scale based on the surface area affected was used (1 = 0-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = > 80%). A surface scald index (SSI) was calculated as: SSI = ∑ (damage level ×Nº of fruit in this level)/Total number of fruits. Twenty fruits were used for each group (control and grafted) and storage time.

2.3.2. Pulp browning index and lightness

Internal browning was determined using a visual browning index (BI) and used objectively by a colorimeter. Fruit pulp color was categorized in 5 groups using a hedonic scale (1 = no browning, 2 = incipient browning, 3 = low browning, 4 = moderate browning, 5 = severe browning). The BI was calculated as ∑ (Browning level × Nº of

fruit in this level)/Total number of fruits. Pulp lightness (L*) was evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) immediately after cutting the equatorial zone. Twenty fruits were used for each group (control and grafted) and storage time.

2.3.3. Texture

Fruit texture was evaluated on a TA.XT2 Texture Analyzer (Stable Microsystems, United Kingdom) equipped with a 3 mm diameter flat probe. Samples were compressed to the transversal axis at the equatorial region at a speed of 1 mm s^{-1}. The resistance to compression (RC) was calculated as the initial slope of the curve plot (force vs distance) expressed in N mm^{-1}. Twenty fruits were used for each group (control and grafted) and storage time.

2.3.4. Antioxidant capacity (TEAC)

Peel and pulp samples were extracted in ethanol as described in section *2.2.5*. The antioxidant capacity was evaluated using the cationic radical ABTS$^{+\bullet}$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) according to **Arnao et al.**[34]. Trolox® was used as a standard. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in milligram per kilogram on fresh weight basis. Six measurements were done for each fruit group (control and grafted) and storage time.

2.4. Statistical analysis

Experiments were performed according to a fully randomized design. Data were analyzed by two-way ANOVA using the InfoStat software and means were compared using the Tuckey test at a level of significance P<0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of grafting on eggplant growth, shape, seed development, color, respiration, dry matter, and phenolic antioxidants

We initially determined the effect on the growth of the *Java* rootstock and cv. Monarca scion. The grafted plants showed increased vigor as evidenced by a greater vegetative growth and plant height (**Supplementary Figure 1**). The number of days required until reaching the baby stage showed no significant differences between control and grafted plants (9 days). Afterward, grafted eggplants showed a higher growth rate than the control (fruit elongation rate 0.84 cm day^{-1} and 0.72 cm day^{-1} respectively). As a result, fruit from grafted plants reached both mid-and late-commercial maturity stages 2 and 3 days earlier than the non-grafted control (**Figure1A**). In line with what has been often reported in vegetable crops[13,16,20] including eggplant[13] grafted plants were more vigorous than the control. Traditionally this was attributed to changes in the root system, such as higher mineral nutrients, and water uptake, and hormonal changess[11]. However, today it is accepted that grafting-induced-phenomes are due to changes in whole plant gene expression profiles through far more complex mechanisms than initially envisioned[35].

Grafting induced subtle but significant changes in fruit shape; at the three maturity stages studied the fruit had a lower maximum diameter than the corresponding control (**Figure 1B**). Fruit shape has been previously reported to be affected by grafting operations in Solanaceous vegetables. **Tsaballa et al.**[35] reported heritable shape changes after grafting the round-shaped cultivar, cv. "Mytilini Round" (scion) on the long-shaped cultivar, cv. "Piperaki Long" (rootstock). Given the developmental stages studies the observed effects should be caused by the alteration in the polarity of cell expansion. Such

effects are highly controlled at the hormonal level Nanda et al.,[36] which are in turn known to be altered in response to grafting. Gálvez et al.,[37]. Pulp lightness (L*) was similar in control and grafted plants at the baby and mid-commercial maturity stage. However, at late maturity grafted eggplants maintained lighter color than the control (**Table 1**). This is desirable in terms of quality since white pulp is a valued attribute in eggplants[20]. Regarding peel color, no differences were detected between control and grafted eggplants (**Table 1**). No distinction in seed number was found between control and grafted fruit either. Seed expansion took place throughout development, without differences in seed size between control and grafted fruit (**Table 1**).

Respiration rate decreased during development as would be expected for eggplant fruit given its non-climacteric nature. Remarkably, grafted fruit showed at the three stages tested lower respiration rate (~50-60%) than the control (**Figure 2A**). This is relevant given that fruit respiration is the physiological indicator correlating best with fresh produce perishability[23]. The change in fruit metabolic activity may be caused by different modifications including an overall decrease in cell respiration, changes in cell size or density, or both. Previous work reported that changes in eggplant growth rate may affect tissue packing and modulate whole fruit respiratory activity[25]. Microstructural evaluation of control and grafted eggplants may be useful to provide further insights in the case of grafted plants.

Dry matter content ranged between 5.0 and 6.5% with significant differences ought to both the maturity stage and treatment. A declining trend was detected along with development as was informed in the previous studies[25]. Regarding the outcome of grafting, the dry matter content of grafted plants was, already at the baby stage, lower than in the control. Such differences were also observed at mid-and late-commercial maturities (**Figure 2B**). Previous works in other species have shown that the rate of fruit

growth affects the dry matter content at harvest. Several reports found that fast-growing fruit tends to yield more succulent tissues[25]. This has been related to a faster water uptake compared to photoassimilate incorporation. Also, since fiber material increase in developing eggplants due to fiber xylem vessel and seed coat thickening, and this process is more prominent the longer the fruit remains *in planta*, lower deposition of lignocellulosic material could be speculated in fast-growing grafted eggplants.

Peel total phenolics showed no differences between control and grafted fruit at the baby stage. In contrast, at mid-and late maturities grafted eggplants had lower phenolics (*ca.* 15-20%) than the controls (**Figure 3A)**. Anthocyanin content showed no major variations in control fruit along with development (**Figure 3B)**. Instead, grafted eggplants showed a significant increase between the baby and mid-commercial maturity stages, with no further changes afterward. Most importantly, grafted fruit showed a significantly lower (20%) level of anthocyanins than the control at all three stages evaluated. The changes observed in chlorogenic acid content followed a similar trend to that found for total phenolics; a drop along development leading to lower levels in commercially mature grafted fruit (**Figure 3C).** Pulp phenolics were four times lower than in the peel but the overall trend found during development was similar; a progressive drop from the baby stage to late-commercial maturity. As for peel tissues, lower content of total phenolics was found in fruit from grafted plants (**Figure 4 A& B)**. Previous works analyzing the impact of grafting on antioxidant compounds have shown a quite variable outcome depending on the rootstock-scion combination. Though significant enrichment was reported in a few cases[13,18], the most common outcome has been either a slight decrease or a lack of variation[17,20,21,38]. The results found herein in eggplant, are also in line with the latter group. Work by **Moncada et al.**[18] also showed that grafting may impact to different extent distinct subgroups of phenolics. The authors reported that grafting

reduced total phenolics, but without affecting anthocyanins. In the present work, though grafted fruit showed reductions in both chlorogenic acid and anthocyanins the effect was more intense for non-flavonoid phenolics. The mechanism by which such an impact occurs is not understood. Changes in the specific biosynthetic pathway may be involved[1]. However, the overall similar reduction observed in antioxidants and total dry matter content in fruit picked from grafted plants (~15-20%) suggests that the observed change may be a dilution effect determined by the changing fruit growth rate and water content. Taken together results show that the *Java* hybrid rootstocks modulate purple cv Monarca eggplant scion vigor fruit growth rate, shape, respiration rate, dry matter, and antioxidant content.

3.2. Effect of grafting on eggplant postharvest chilling tolerance, postharvest performance, and antioxidant stability

Control and grafted mid-CM eggplants were subsequently stored at 1 °C for 28 days to evaluate whether the grafting combination used modulated fruit's postharvest resistance to chilling injury. It is worth noting that although the temperature used in the study (1 °C) was very low to exacerbate chilling injuries, eggplants would be chilling damaged already below 10 °C, including at normal home refrigeration, if stored long enough. The surface scald index (SSI) increased during storage in both control and grafted fruit (**Figure 5A**). Until day 14 no differences were observed between control and grafted fruit. After 21 days of storage, a rapid increase in the SSI was found in the control. Contrariwise, grafted fruit showed no change in external chilling injury symptoms. At the last sampling date, grafted fruit also showed higher SSI than the control. Peel chilling injuries are normally accompanied by a reduction in tissue firmness[39]. In line with this, a rapid drop in fruit resistance to compression was detected from day 14, when chilling

injury symptoms start to become evident (**Figure 5B**). Grafted fruit maintained at the end of the storage period higher firmness than the controls. The degree of internal damage induced by cold storage was followed through a hedonic visual browning index and pulp lightness (**Figure 6A & B**). Pulp browning increased along with storage. After 14 days of storage, higher browning was detected in the control fruit. These differences become even greater after 21 and 28 days. The reduced susceptibility to brown fruit picked from grafted plants was also detected by objective lightness (L^*) measurements. The drop in pulp L^* values found in grafted fruit at the end of the storage period was comparable to that observed in the non-grafted control one week earlier.

The antioxidant capacity of fruit from both control and grafted plants showed a decreasing trend during storage. However, the AOX degradation rate was for peel and pulp tissues 100 and 20% lower in grafted eggplants than in the respective control. Interestingly, the trend in antioxidants found at harvest time was reversed after cold storage due to enhanced stability in fruit from grafted plants (**Figure 7A & B**). Since eggplant peel and pulp antioxidant capacity have been shown to highly correlate ($r^2 =$ 0.981 and 0.996 respectively)[27] with total phenolics the rapid loss of AOX was likely a consequence of tissue browning. As of today, the role of antioxidants in CI protection in fruits in general and in eggplant is far from being completely understood. Based on the timing at which massive antioxidant losses occurred (3 to 4 weeks) and with results from previous works by Concellón et al.,[40] , Tsouvaltzis et al.,[41] and Babellahi et al.,[42] reporting strong changes in eggplant integrity even after 3 days of cold storage, it could be speculated that antioxidant degradation is a consequence of tissue damage rather than a protective response. The fact that control fruit having more antioxidants at harvest was much more susceptible to postharvest chilling injury than grafted fruit also shows at least total contents of these compounds either in the peel or pulp tissues cannot *per se* explain

eggplant cold tolerance. However, based on global and bulk measurements of antioxidant metabolites and/or enzymes is not possible to univocally determine where or not the level/changes in these compounds have a protective function against chilling stress, are just a consequence of the loss in tissue compartmentation that initiates a cascade of uncontrolled oxidation or both. Addressing this biologically intriguing question would require a spatial temporal in vivo analysis of both ROS and antioxidants and redox status at the cellular level.

The underlying mechanism of postharvest cold tolerance observed in the harvested fruit is far from being understood[5-8,11]. Early physiological works on grafting tended to associate the changes induced in the scion with modifications in root functionality and hormonal control[43,44]. Recent omic studies have shown that whole plant transcriptional profiles are modified in grafted plants with deep changes being detected already very early. Epigenetic mechanisms such as changes in DNA methylation and translocation of non-coding RNA have been also demonstrated to participate in controlling gene expression in grafted individuals[45-47].

Some studies have related eggplant CI with a redox imbalance initiated by the overproduction of superoxide radical[48] and in several species, fruit postharvest cold tolerance has been correlated with an increased accumulation of antioxidant compounds[43,49,50]. This did not seem to be the case in the present work, where fruit from grafted plants had lower antioxidant compounds at the beginning of the storage period. However, the enzymatic antioxidative defenses may be playing a protective role. Work in tomato in which, a cold-tolerant accession of *Solanum habrochaites* (LA 1777) was used as a rootstock exhibited significantly higher levels of whole plant cold tolerance and greater guaiacol peroxidase and superoxide dismutase activities[49]. Besides that, it is important to highlight that given that we did not use a self-grafted genotype that at this

stage we cannot rule out that grafting "*per se*" could be responsible for the acquired resistance to stresses as reported in some cases[15,16].

Chilling injury is the most common postharvest physiological disorder in eggplant.[33] Consequently, several postharvest treatments have been evaluated to control it. Some of them such as polyamines[51], jasmonic acid[52] or 1-MCP[53], and low temperature conditioning have shown promising results at a laboratory scale[54]. Besides that, none of these treatments have been used commercially at a large scale due to implementation economic and/or market constraints. The use of grafted plants yielding cold-tolerant fruit may be envisioned as a broader approach to address the problem, leaving no residues in the product and not requiring specific facilities of treatment steps within the packinghouse. Such a strategy would also protect against chilling stresses occurring already in the field, which may be common late during the production cycle when the cold season approaches. Altogether the present study shows that purple eggplant (cv. Monarca) grafting on *Java* hybrid rootstock modulated fruit growth, quality at harvest, and enhanced the tolerance to postharvest chilling injury. One limitation of the present work from the physiological perspective is the lack of self-grafted plants. This would have allowed distinguishing the effects induced by the grafting operation from those induced by the rootstock combinations. Such work would be useful to fully understand the response observed herein.

4.   CONCLUSIONS

The present study evaluated the influence of the purple eggplant grafting (*Java* rootstock on Monarca scion) on the growth rate, shape, quality, and stability of antioxidants concentration during fruit growth. Purple eggplant (cv. Monarca) grafting onto *Java* hybrid rootstock modulated fruit growth, quality at harvest, and increase fruit

tolerance to chilling injury during storage. Grafting may be a *bona fide* strategy to induce phenotypic traits able to improve vegetable postharvest performance.

5.   REFERENCES

1.   Rasool A, Mansoor S, Bhat KM, Hassan GI, Baba TR, Alyemeni MN, Ahmad P. Mechanisms underlying graft union formation and rootstock scion interaction in horticultural plants. *Front. Plant Sci*, **11**. (2020).

2.   Wang J, Jiang L, Wu R. Plant grafting: how genetic exchange promotes vascular reconnection. *New Phytol*, **214**(1): 56-65. (2017).

3.   Martínez-Rodríguez MM, Estañ MT, Moyano E, García-Abellan JO, Flores FB, Campos JF, Bolarín MC. The effectiveness of grafting to improve salt tolerance in tomato when an 'excluder' genotype is used as scion. *Environ. Exp. Bot,* **63**(1-3): 392-401. (2008).

4.   He Y, Zhu Z, Yang J, Ni X, Zhu B. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. Exp. Bot,* **66**(2): 270-278. (2009).

5.   Yetisir H, Uygur V. Responses of grafted watermelon onto different gourd species to salinity stress. *J. Plant Nutr,* **33**(3): 315-327. (2010).

6.   Colla G, Rouphael Y, Leonardi C, Bie Z. Role of grafting in vegetable crops grown under saline conditions. *SciHort,* **127**(2): 147-155. (2010).

7.   Rivero RM, Ruiz JM, Sánchez E, Romero L. Does grafting provide tomato plants an advantage against H_2O_2 production under conditions of thermal shock? *Physiol Plant,* **117**(1): 44-50. (2003).

8.   Venema JH, Dijk BE, Bax JM, van Hasselt PR, Elzenga JTM. Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance. *Environ. Exp.Bot,* **63**(1-3): 359-367. (2008).

9.   Schwarz D, Rouphael Y, Colla G, Venema JH. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. *SciHort,***127**(2): 162-171. (2010).

10.   Leonardi C, Giuffrida F. Variation of plant growth and macronutrient uptake in grafted tomatoes and eggplants on three different rootstocks. *Eur. J. Hortic. Sci,* **71**(3): 97. (2006).

11.   Gisbert C, Prohens J, Raigón MD, Stommel JR, Nuez F. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. *SciHort,* **128**(1): 14-22. (2011b).

12.   Lee JM, Kubota C, Tsao SJ, Bie Z, Echevarria PH, Morra L,Oda M. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *SciHort*, **127**(2): 93-105. (2010).

13.   Nawaz M A, Imtiaz M, Kong Q, Cheng F, Ahmed W, Huang Y, Bie Z. Grafting: a technique to modify ion accumulation in horticultural crops. *Front. Plant Sci*, **7**: 1457. (2016).

14.   Fallik E, Ilic Z. Grafted vegetables–the influence of rootstock and scion on postharvest quality. *Folia Hortic,* **26**(2): 79-90. (2014).

15.   Ioannou N. Integrating soil solarization with grafting on resistant rootstocks for management of soil-borne pathogens of eggplant. *J. Hortic. Sci. Biotechnol,* **76**(4): 396-401. (2001).

16.   Bletsos F, Thanassoulopoulos C, Roupakias D. Effect of grafting on growth, yield, and *Verticillium* wilt of eggplant. *HortScience,* **38**(2): 183-186. (2003).

17.   KacjanMaršićN, Mikulič-Petkovšek M,Stampar F. Grafting influences phenolic profile and carpometric traits of fruits of greenhouse-grown eggplant (*Solanum melongena* L.). *J. Agric. Food Chem.,* **62**(43): 10504-10514. (2014).

18.   Moncada A, Miceli A, Vetrano F, Mineo V, PlanetaD,D'Anna F. Effect of grafting on yield and quality of eggplant (*Solanum melongena* L.). *SciHort,* **149**: 108-114. (2013).

19.   Sabatino L, Iapichino G, Maggio A, D'anna E, Bruno M, D'Anna F. Grafting affects yield and phenolic profile of *Solanum melongena* L. landraces. *J. Integr. Agric,* **15**(5): 1017-1024. (2016).

20.   Sabatino L, Iapichino G, D'Anna F, Palazzolo E, MennellaG, Rotino GL. Hybrids and allied species as potential rootstocks for eggplant: Effect of grafting on vigor, yield and overall fruit quality traits. *SciHort,* **228**: 81-90. (2018).

21.   Mozafarian M, Kappel N. Effect of grafting on the quality and appearance of eggplant fruit. *Prog. Agric. Eng. Sci,* **16**(**S2**): 153-161. (2020).

22.   Arvanitoyannis IS, Khah EM, Christakou EC, Bletsos FA. (2005). Effect of grafting and modified atmosphere packaging on eggplant quality parameters during storage. *Int. J. Food Sci.,* **40**(3): 311-322.

23.   Zaro MJ, Keunchkarian S, Chaves AR, Vicente AR, Concellón A. Changes in bioactive compounds and response to postharvest storage conditions in purple eggplants as affected by fruit developmental stage. *Postharvest Biol. Technol.,* **96**: 110-117. (2014).

24.   Gisbert C, Prohens J, Nuez F. Performance of eggplant grafted onto cultivated, wild, and hybrid materials of eggplant and tomato. *Int. J. Plant Prod,* **5**(4): 367-380. (2011a).

25.   Valerga L, Darré M, Zaro MJ, Arambarri A, Vicente AR, Lemoine ML, Concellón A. Micro-structural and quality changes in growing dark-purple eggplant (*Solanum melongena* L.) as affected by the harvest season. *SciHort,* **244**: 22-30. (2019).

26.   Gao Q, Wu Y, Jia S, Huang S, Lu X. Effect of rootstock on the growth, photosynthetic capacity and osmotic adjustment of eggplant seedlings under chilling stress and recovery. *Pak. J. Bot*, **48**(**2**): 461-467.(2016).

27.   Valerga L, Darré M, Zaro MJ, Vicente AR, Lemoine ML, Concellón A. Harvest date affects purple eggplant quality and postharvest life. *Int. J. Vegetable Sci.,* 1-8. (2020)**.**

28.   Okimura M, Matsuo S, Arai K, Okitsu S. Influence of soil temperature on the growth of fruit vegetable grafted on different stocks. Bulletin of the Vegetable and Ornamental Crops Research Station. Series C. Kurume (Japan). https://agris.fao.org/agris-search/search.do?recordID=JP880162888 (1986).

29.   TAKII (2021). In http://www.takii.com/product/java/ Retrieved. May 2021.

30.   Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. ImageJ2: ImageJ for the next generation of scientific image data. BMC bioinformatics, **18**(1), 1-26. (2017).

31.   Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol***299**: 152-178. (1999).

32.   Luthria DL, Mukhopadhyay S. Influence of sample preparation on assay of phenolic acids from eggplant. *J. Agric. Food Chem,* **54**(1): 41-47. (2006).

33.   Concellón A, Añón MC, Chaves AR. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. *Food Chem.,* **88**(1): 17-24. (2004).

34.   Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.,* **73**: 239-244. (2001).

35.   Tsaballa A, Xanthopoulou A, Madesis P, Tsaftaris A, Nianiou-Obeidat I. Vegetable grafting from a molecular point of view; the involvement of epigenetics in rootstock-scion interactions. *Front. Plant Sci.,* **11**: 2129. (2020).

36.   Nanda AK, Melnyk CW. The role of plant hormones during grafting *J. Plant Res.,* **131:** 49–58. (2018).

37.   Gálvez A, Albacete A, Martínez-Andújar C, del Amor FM, López-Marín J. Contrasting rootstock-mediated growth and yield responses in salinized pepper plants (*Capsicum annuum* L.) are associated with changes in the hormonal balance. *Int. J. Mol. Sci.,* **22**: 3297. (2021).

38.   Kyriacou MC, Rouphael Y, Colla G, Zrenner R, Schwarz D. Vegetable grafting: The implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. *Front. Plant Sci.,* **8**: 741. (2017).

39.   Valenzuela JL, Manzano S, PalmaF, Carvajal F, Garrido D, Jamilena M. Oxidative stress associated with chilling injury in immature fruit: postharvest technological and biotechnological solutions. *Int. J. Mol. Sci.,* **18**(7): 1467. (2017).

40.   Concellón, A, Añón MC, Chaves AR. Effect of chilling on ethylene production in eggplant fruit. *Food Chem.,* **92**: 63–69. (2005)

41. Tsouvaltzis, P, Babellahi, F, Amodio, ML, Colelli, G. Early detection of eggplant fruit stored at chilling temperature using different non-destructive optical techniques and supervised classification algorithms. *Postharvest Biol. Technol.,* **159**: 111001. (2020).

42. Babellahi F, Amodio ML, Marini F, Chaudhry MA, de Chiara MLV, Mastrandrea L, Collelli G. Using chemometrics to characterise and unravel the near infra-red spectral changes induced in aubergine fruit by chilling injury as influenced by storage time and temperature. *Biosys. Eng.,* **198**:137-146. (2020)

43. Lo'ay AA, Doaa MH. The potential of vine rootstocks impacts on 'Flame Seedless' bunches behavior under cold storage and antioxidant enzyme activity performance. *SciHort,* **260**: 108844. (2020).

44. Aloni B, Cohen R, Karni L, Aktas H, Edelstein M. Hormonal signaling in rootstock–scion interactions. *SciHort*, **127**(2): 119-126. (2010).

45. Kapazoglou A, Tani E, Avramidou EV, Abraham EM, Gerakari M, Megariti S, Doulis A G. Epigenetic changes and transcriptional reprogramming upon woody plant grafting for crop sustainability in a changing environment. *Front. Plant Sci*, **11**: 2160. (2021).

46. Wu R, Wang X, Lin Y, Ma Y, Liu G, Yu X, Liu B. Inter-species grafting caused extensive and heritable alterations of DNA methylation in *Solanaceae* plants. *PLoS One*, **8**(4): e61995. (2013).

47. Pant BD, Buhtz A, Kehr J, Scheible WR. MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J*, **53**(5): 731-738. (2008).

48. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, **55**: 373-399. (2004).

49. Ntatsi G, Savvas D, Kläring HP, Schwarz D. Growth, yield, and metabolic responses of temperature-stressed tomato to grafting onto rootstocks differing in cold tolerance. *J Am Soc. Hortic. Sc.*, **139**(2): 230-243. (2014).

50. Oustric J, Morillon R, Luro F, Herbette S, Lourkisti R, Giannettini J, Berti L, Santini J. Tetraploid Carrizo citrange rootstock (*Citrus sinensis* Osb.×*Poncirus trifoliata* L. Raf.) enhances natural chilling stress tolerance of common clementine (*Citrus clementina* Hort. ex Tan). *J Plant Physiol*, **214**: 108-115. (2017).

51. del C Rodriguez, López B, Chaves AR. Effect of different treatments on the evolution of polyamines during refrigerated storage of eggplants. *J. Agric. Food Chem*, **49**(10): 4700-4705. (2001).

52. Shi J, Zuo J, Xu D, Gao L, Wang Q. Effect of low-temperature conditioning combined with methyl jasmonate treatment on the chilling resistance of eggplant (*Solanum melongena* L.) fruit. *J. Food Sci. Technol.*, **56**(10): 4658-4666. (2019).

53. Massolo J, Concellón A, Chaves A, Vicente A. 1-Methylcyclopropene (1-MCP) delays senescence, maintains quality and reduces browning of non-climacteric eggplant (*Solanum melongena* L.) fruit. *Postharvest Biol. Technol.*,**59**(1): 10-15. (2011).

54. Shi J, Zuo J, Zhou F, Gao L, Wang Q, Jiang A. Low-temperature conditioning enhances chilling tolerance and reduces damage in cold-stored eggplant (*Solanum melongena* L.) fruit. *Postharvest Biol. Technol.*,**141**: 33-38.(2018).

TABLES

Table 1: Pulp lightness, surface color (L^*, a* and b*), seed number (N°), and seed area of purple eggplant fruit from *Control* and *Grafted* plants harvested at *Baby* stage (9 cm), mid-commercial maturity (*Mid-CM*, 17 cm) and late-commercial maturity (*Late-CM*, 19 cm) stage. Letters within a column indicate differences according to the Tukey test at a level of significance of $P < 0.05$.

		Pulp lightness	Surface Color			Seed N° (N° slice^{-1})	Seed area (mm^2)
			L^*	a^*	b^*		
Baby	*Control*	84,55 **a**	25.46 **a**	3,59 **a**	-0,35 **a**	109 **a**	0.87 **a**
	Grafted	84,96 **a**	25.79 **a**	3,79 **a**	-0,37 **a**	101 **a**	0.79 **b**
Mid-CM	*Control*	83,82 a	24.28 **a**	3,64 **a**	-0,14 **a**	-	1.87 **a**
	Grafted	84,15 **a**	23.88 **b**	4,13 **a**	-0,16 **a**	-	1.87 **a**
Late-CM	*Control*	84,78 **a**	24.59 **a**	4,01 **b**	-0,03 **a**	-	1.99 **a**
	Grafted	83,81 **b**	24,37 **a**	4,69 **a**	-0,03 **a**	-	2.04 **a**

FIGURE LEGENDS

Figure 1: A) Days from set to maturity (DFSM) and **B)** maximum diameter of purple eggplant fruit from control and grafted plants harvested at the baby stage (9 cm), mid-commercial maturity (Mid-CM, 17 cm) or late-commercial maturity (Late-CM, 19 cm). Letters indicate significant differences according to a Tukey test at a significance level of $P < 0.05$.

Figure 2: A) Respiration rate and **B)** dry matter rate of purple eggplant fruit from control and grafted plants harvested at the baby stage, mid-commercial maturity (Mid-CM) or late-commercial maturity (Late-CM). Letters indicate significant differences according to a Tukey test at a significance level of $P < 0.05$.

Figure 3: A) Peel phenolic compounds, **B)** chlorogenic acid, and **C)** anthocyanins in purple eggplant fruit harvested from control and grafted plants at the baby stage, mid-commercial maturity (Mid-CM) and late-commercial maturity (Late-CM). Letters indicate significant differences according to a Tukey test at a significance level of $P < 0.05$.

Figure 4: A) Pulp phenolics and **B)** chlorogenic acid of in purple eggplant fruit from control and grafted plants harvested at the Baby stage, mid-commercial maturity (Mid-CM) or late-commercial maturity (Late-CM). Letters indicate significant differences according to the Tukey test at a significance level of $P < 0.05$.

Figure 5: A) Surface scald index, and **B)** resistance to compression (RC) of purple eggplant fruit from control and grafted plants harvested at mid-commercial maturity and stored at 1 ºC for 0, 14, 21 and 28 days. Letters indicate significant differences according to a Tukey test at a significance level of P <0.05. Inbox: External appearance of control and grafted eggplant fruit harvested at mid-commercial maturity and stored at 1 ºC for 21 days.

Figure 6: A) Browning index, and **B)** pulp lightness of purple eggplant fruit from control and grafted plants harvested at mid-commercial maturity and stored at 1 ºC for 0, 14, 21 and 28 days. Letters indicate significant differences according to a Tukey test at a significance level of P <0.05. Inbox: Internal appearance of control and grafted eggplant fruit harvested at mid-commercial maturity and stored at 1 ºC for 21 days.

Figure 7: A) Peel antioxidant capacity, and **B)** pulp antioxidant capacity of purple eggplant fruit from control and grafted plants harvested at mid-commercial maturity and stored at 1 ºC for 0, 14, 21 and 28 days (d). Letters indicate significant differences according to a Tukey test at a significance level of P <0.05.

Supplementary Fig 1. Vegetative growth and control (cv. Monarca) and grafted eggplant plants (Monarca scion on Java interspecific hybrid rootstock, Takii seed, Japan) grown in a greenhouse in La Plata (Buenos Aires, Argentina. 34°59′18″ S, 57°56′17″ W). Nov 2019.
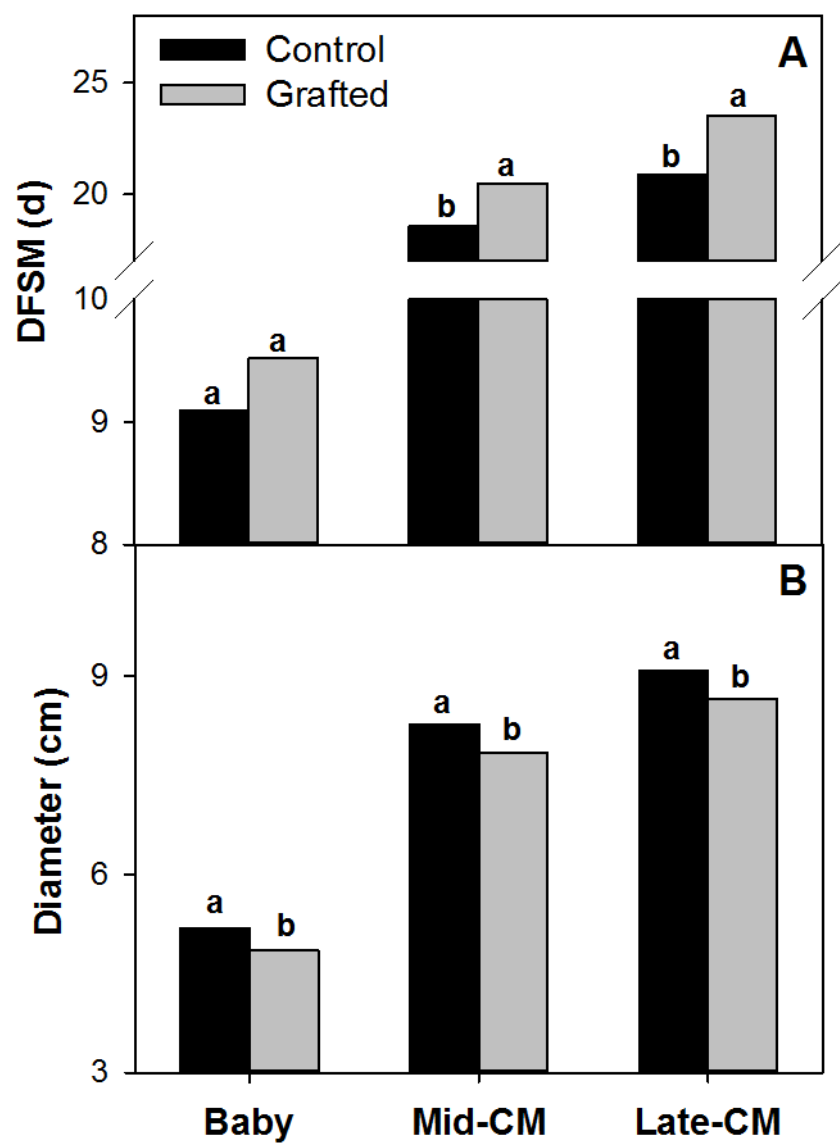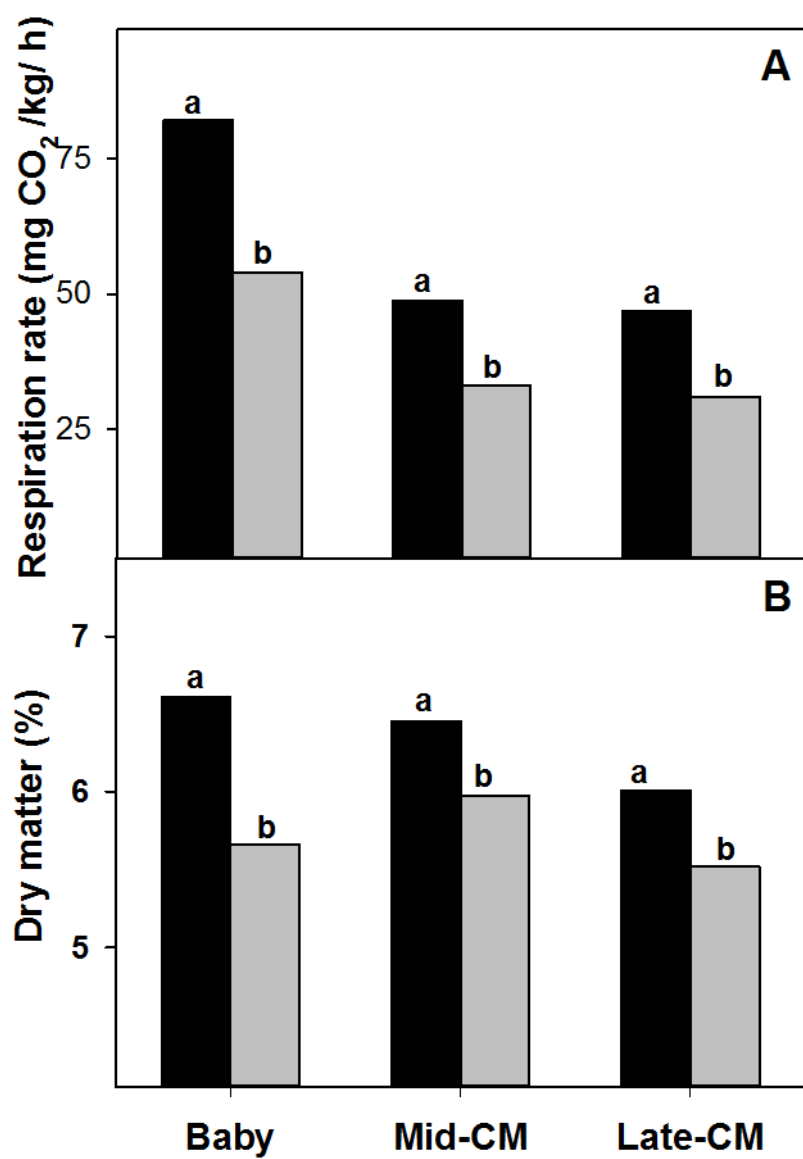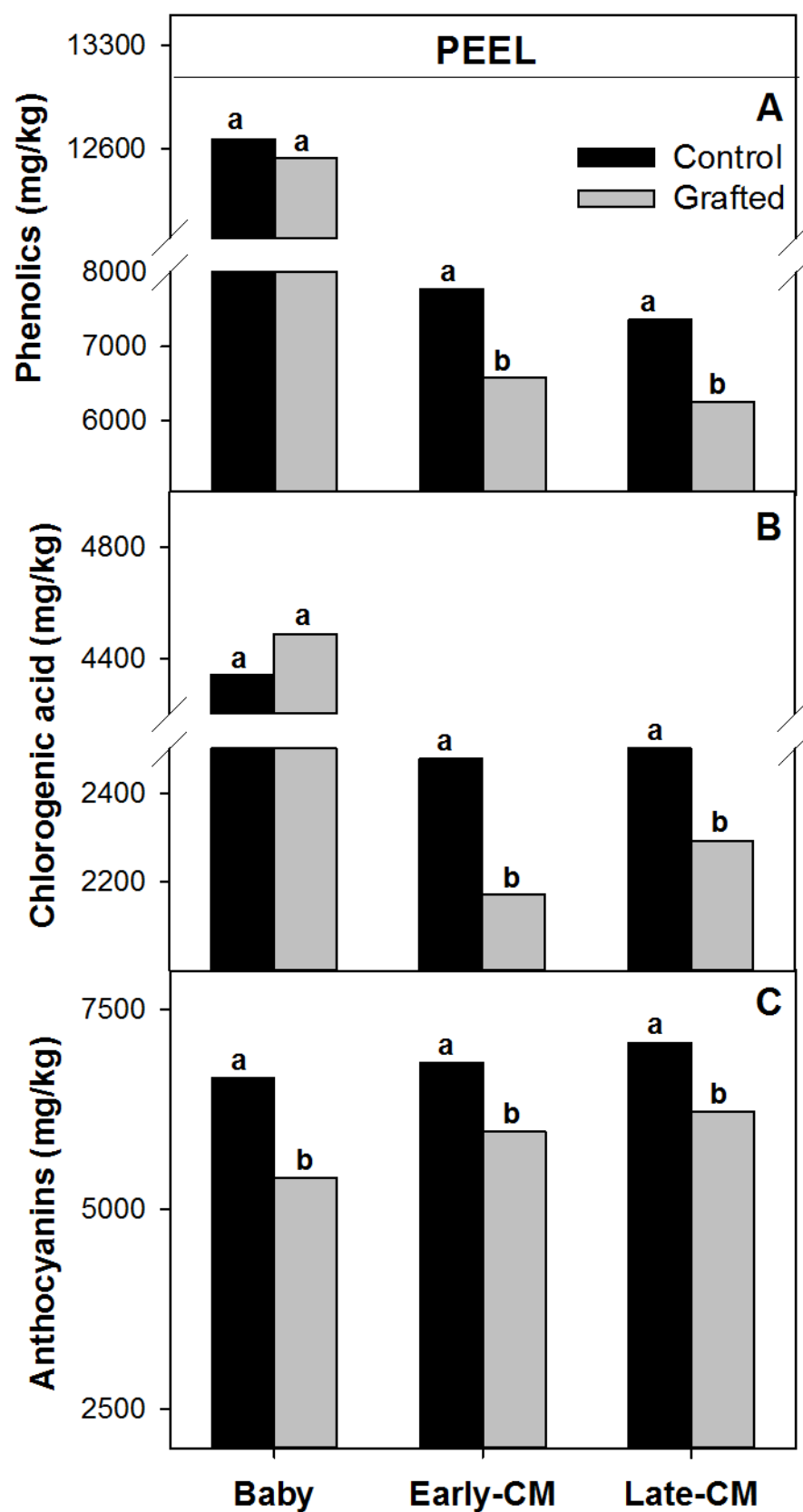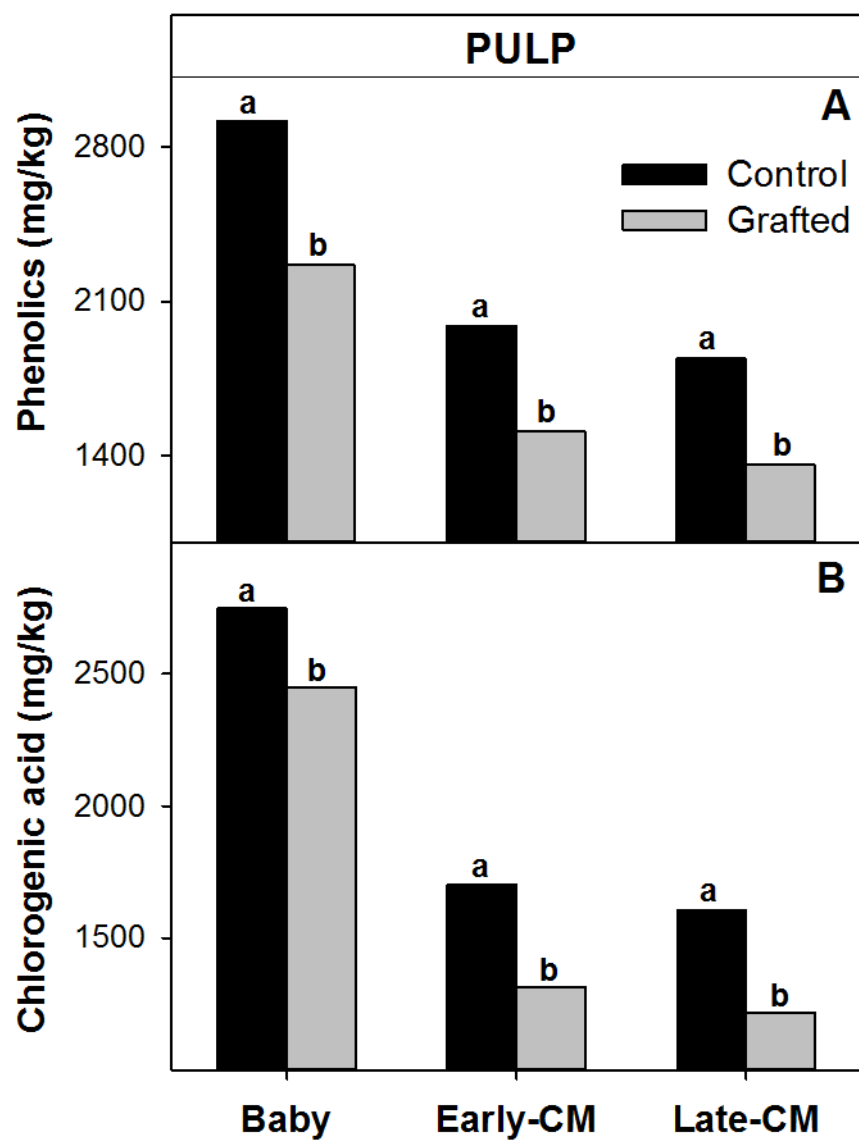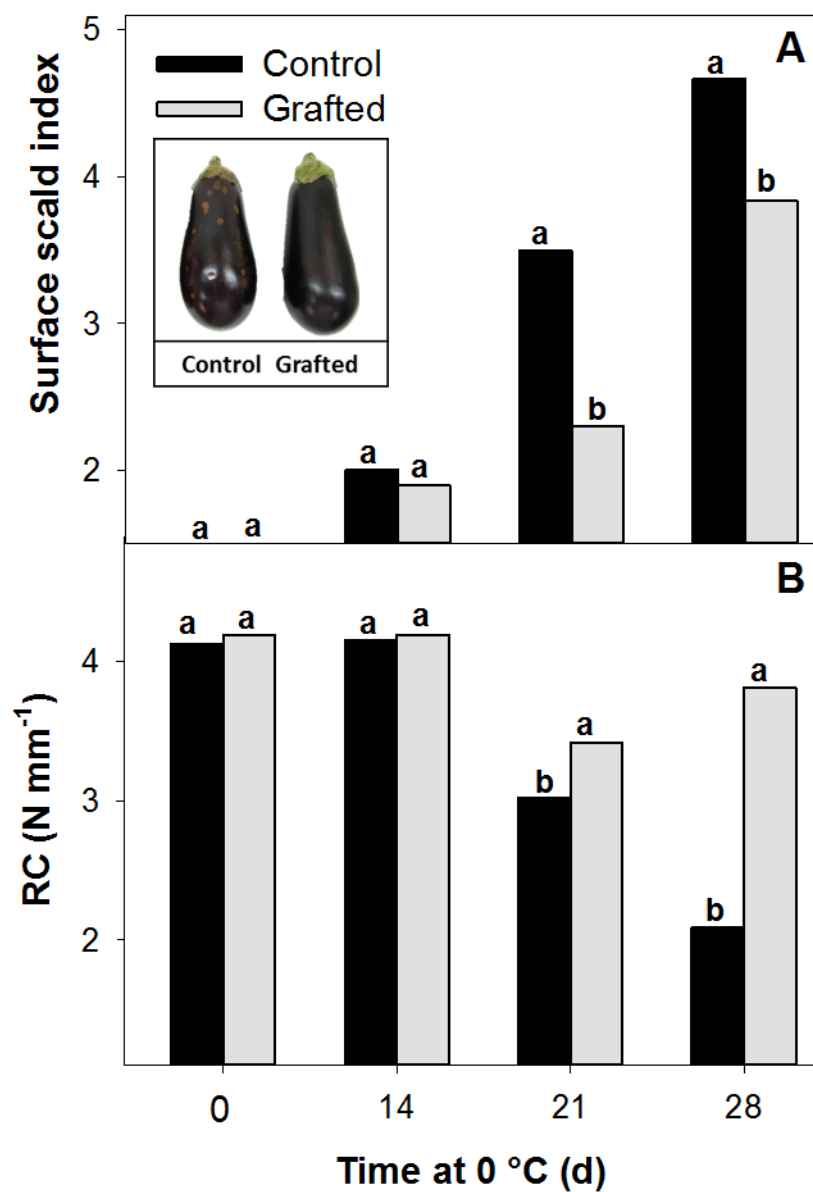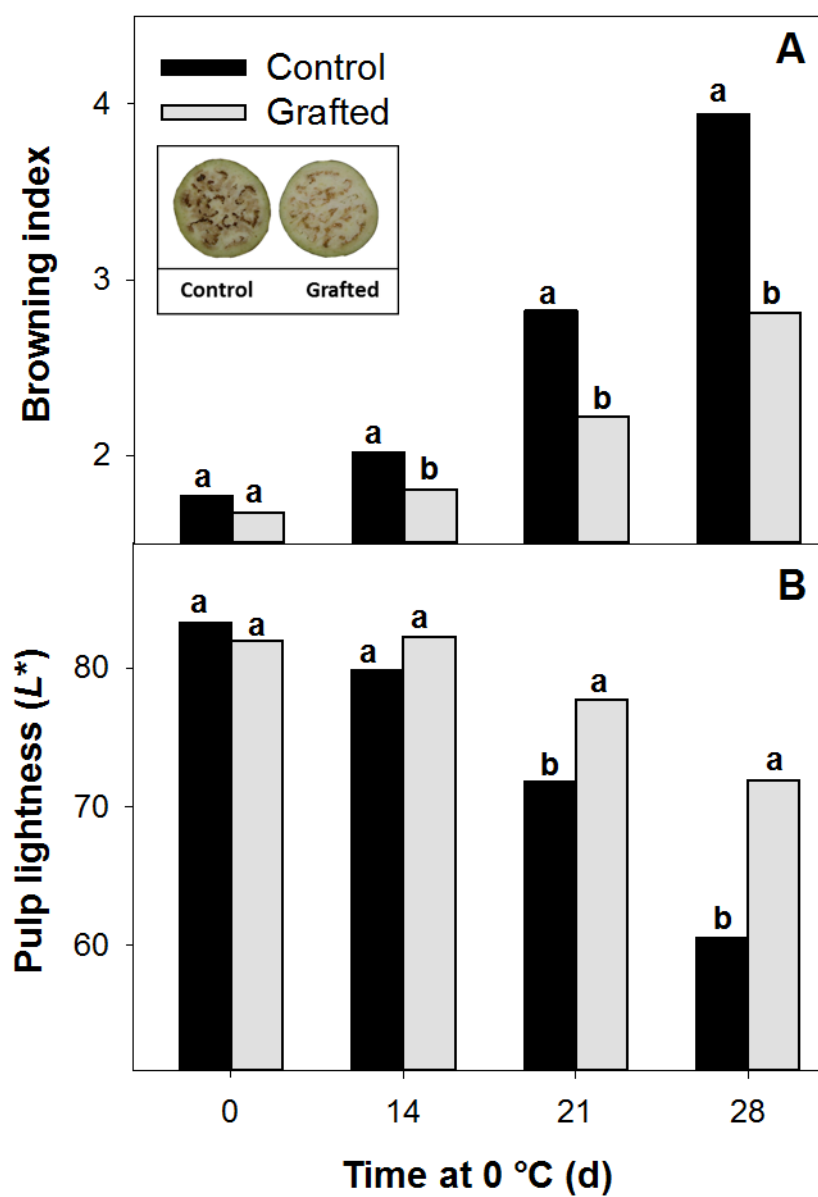
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