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# Evaluating Suitable Rootstocks for Grafting in Organic Pepper System

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*Abstract.* **Organic farming systems are often plagued with challenges such as nutrient stress complexing with other biotic and abiotic issues. These issues can significantly affect the growth and productivity of vegetable crops, including peppers (*Capsicum* spp.). Grafting with suitable rootstocks is a promising technique to address these challenges and improve the growth and productivity of peppers in low-input systems. In the United States, grafting is not as commonly practiced in pepper as compared with other solanaceous and cucurbit crops, in part because of limited availability of rootstocks. This study examined promising rootstocks with demonstrated disease resistance and further explored their suitability for organic systems under normal and reduced fertilization. A hybrid Italian sweet pepper 'Mama Mia Giallo' was used as scion and grafted onto four different rootstocks and grown in a greenhouse in summer with natural heat stress. The plants received organic fertilizer weekly or biweekly for a period of 5 weeks. Results showed that grafting plants with the rootstocks 'CM-334', 'YC-207', 'Keystone Resistant Giant', and 'Scarface' resulted in similar or improved growth relative to nongrafted and self-grafted plants under normal and reduced fertilization. The rootstocks showed promising traits that could be explored in further stress tolerance studies. Specifically, 'YC-207'-grafted plants showed higher nitrogen utilization efficiency, shoot nitrate content, and phosphorous content as well as higher stomatal conductance, transpiration, and electron transport rate, which is indicative of stress tolerance. 'YC-207'-grafted plants also had lower malondialdehyde content, indicating less oxidative damage to the cell membrane. 'CM-334'-grafted plants showed higher antioxidant activity, nitrate reductase (NR) activity, and shoot nitrate content. This study provides evidence of the value of evaluating potential rootstocks and explores grafting as a promising technique for pepper crop to improve plant growth and performance under organic systems.**

The demand for organic foods in US market has grown exponentially in recent decades with sales increasing from $3.6 billion in 1997 to more than $60 billion in 2022 (OTA 2022). Texas has seen a remarkable surge in organic sales, with a 30% to 50% increase in 2021 compared with 2019 (USDA NASS 2021 Organic Survey). To enhance the competitiveness of the organic market, it is essential to diversify the commodity base by integrating specialty items like sweet and hot peppers. These peppers have gained popularity because of their diverse flavors, colors, nutritional benefits, and appeal to an increasing ethnically diverse consumer base. However, specialty pepper cultivars have limited tolerance to thrive in extreme climatic conditions and are susceptible to diseases and insects, resulting in low yields. It is well established that biotic and abiotic stresses cause extensive losses to agricultural production worldwide (Suzuki et al. 2014). Like conventional systems, organic production systems also face challenges such as vulnerability to nutrient stress, pests, and diseases, which can sometimes lead to lower yields (de Ponti et al. 2012). Although organic produce still commands a higher market value by 10% to 30%, lower yields by 10% to 20% relative to conventional systems have been reported (Aune 2012; Kirchmann et al. 2008). Although these yield results can vary depending on management practices and many environmental factors, the limited availability of organic tools and techniques to address biotic and abiotic stresses remains a concern. Currently, most commercial cultivars of peppers are susceptible to *Phytophthora capsici* (Barchenger et al. 2018), one of the most threatening diseases of pepper, for which management is difficult in organic systems due to limited interventions. Abiotic stresses, such as high temperatures and drought, combined with biotic stresses like soilborne pathogens, further complicate nutrient management, impacting enzymatic activity, chlorophyll content, and photosynthesis, and reducing plant resilience and further exacerbating plant vulnerability during transplant establishment (Demir et al. 2022; Suzuki et al. 2014).

The prohibition of synthetic fertilizers and chemicals in organic production systems encourages growers to explore system-based solutions, such as selecting varieties with efficient nutrient utilization and high disease resistance. Grafting is one of the methods commonly used to address soilborne pathogens in high-value vegetables such as tomatoes, cucumbers, melons, and peppers (Kyriacou et al. 2017). Grafting can improve nutrient utilization, resulting in higher shoot biomass, yield, and fruit quality (Albacete et al. 2015; Colla et al. 2010, 2011; Martínez-Andújar et al. 2016, 2017), as certain graft combinations are more effective at absorbing and transporting nutrients to shoots than nongrafted plants (Colla et al. 2011; Garcia-Bañuelos et al. 2017). Rootstocks with suitable ideotype, for example, deeper root systems for efficient nitrogen (N) acquisition and shallow lateral roots for phosphorus (P) acquisition (Kumar et al. 2019) can contribute to nutrient mobilization, uptake, and utilization. Increase in nutrient efficiency in grafted plants might be due to adaptive changes in root architecture; however, this efficiency may be correlated with other systemic and physiological signaling that influence shoot growth. A deficiency of nutrients affects photosynthetic functions at different levels, as well as chlorophyll synthesis and membrane stability (Osman 2013), which eventually causes oxidative stress in plants assessed by markers such as malondialdehyde (MDA) content. The transport of antioxidation-related metabolites, peptides, and nucleic acids from rootstock to scion enables grafted plants to tolerate oxidative stress compared with nongrafted plants (Bhatt et al. 2015; Chávez-Mendoza et al. 2013; Zhao et al. 2011). A recent study on tomatoes by Zhang et al. (2022) revealed that tomato plants grafted with a nutrient-use efficient rootstock had enhanced nitrogen absorption and utilization, leading to increased plant yield resulting from improved overall nitrogen metabolism, including increased NR activity and soluble protein content. Grafting alters nutrient concentrations in scion tissues compared with nongrafted plants (Borgognone et al. 2013), but the rootstock-mediated physiological

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effects under nutrient stress conditions have rarely been studied. Although many studies are focused on single nutrient stress, nutrient deficiency in organic systems usually occurs due to deficiency of several nutrients rather than N, P, or K alone.

Although the use of suitable rootstocks can serve as a viable approach to reduce yield losses resulting from abiotic stresses like nutrient deficiency, high temperature, water stress, and resistance against soilborne diseases, grafting is not commonly practiced in specialty peppers. This is partly attributed to the limited availability of pepper rootstocks in the United States. According to Osuna-Ávila et al. (2012), the *P. capsici*–resistant 'Criollo de Morelos CM-334' has historically been the primary rootstock used in the production of capsicum. In pepper grafting trials, the genetic resistance of 'CM-334' against *P. capsici* has been used with success (Pintado-López et al. 2017). Using landraces and wild relatives with naturally acquired stress tolerance mechanisms can enhance the resilience of domesticated crops to biotic and abiotic stress and improve nutrient uptake. However, grafting compatibility and the benefits of novel rootstocks must be assessed across commercial cultivars. Identifying suitable rootstocks for organic agricultural systems will therefore require careful consideration of the complex link among soil, nutrients, and roots.

This study explores the applicability of pepper rootstocks in organic farming systems by examining the physiological and chemical alterations in the scion caused by the rootstocks. We hypothesize that grafting pepper seedlings onto suitable rootstocks will enhance growth and adaptation through improved nutrient efficiency and stress tolerance conferred by the rootstock.

## Materials and Methods

*Plant materials and growth conditions.* In this study 'Mama Mia Giallo' (MMG), a Sweet Italian type of hybrid pepper, was selected as scion based on its flavor, productivity, and popularity. Based on preliminary studies and recommendation by breeders we also selected four rootstocks, including one landrace pepper variety 'CM-334' (CM), a Phytophthora-resistant breeding line 'YC-207' (YC), one commercial bell pepper variety 'Keystone Resistant Giant' (Keystone), and one commercial rootstock 'Scarface'. Commercial variety bell pepper 'Keystone resistant Giant' was used as rootstock based on its known resistance to many diseases, including nematodes, as well as its previous use as rootstock (Bausher et al. 2007; Kokalis-Burelle et al. 2009). Additional description of these rootstocks is provided in Table 1.

Table 1. Pepper rootstock trait descriptors.

| Rootstocks | Traits |
|---|---|
| CM-334 (CM) | A Mexican landrace, Phytophthora root rot and foliar blight - resistant accession |
| YC-207 (YC) | Breeding line resistant to Phytophthora |
| Keystone resistant Giant (K) | Commercial bell pepper resistant to mosaic virus |
| Scarface (S) | Commercial pepper rootstock |

Scarface was provided by Enza Zaden Company. Other rootstock seeds were provided by the Chile Pepper Institute, Las Cruces, NM, and Dr. Kevin Crosby.

Speedling (Ruskin, FL, USA) polystyrene 200-cell trays (Model TR200A, 2.5 × 2.5 $cm^2$ × 7.6 cm deep with 32 $cm^3$ volume per cell) with organic all-purpose container mix (Scotts Miracle-Gro, Marysville, OH, USA) were used to grow seedlings in a greenhouse with supplementary fluorescent light system (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The rootstocks were seeded 3 to 4 d earlier than the scion to match a target 1.5-mm-diameter for grafting.

Fourteen days after seedling emergence, trays were fertilized at weekly intervals using 50 ppm Organic Gem fish meal fertilizer (3% N, 3% $P_2O_5$, 0.3% $K_2O$; 50 $mg \cdot L^{-1}$ N).

At 30 d after seeding, seedlings were cut below the cotyledon, and splice grafted with the scion at a 45° angle. The graft union was held using a 1.5-mm-diameter silicone graft clip (Johnny's Selected Seeds, Fairfield, ME, USA). The grafted seedlings were acclimated in a healing chamber at 22 to 24 °C and over 95% relative humidity (RH) with no light. A dim light was introduced on the fourth day and the RH was gradually lowered up to 80% until 10 d of acclimation. Afterward, the seedlings were moved to a greenhouse for an additional 7 d of hardening. A survival rate of more than 85% was obtained across all grafting combinations, with no visual issues observed in the scion-rootstock graft connection. The seedlings were then transplanted on 7 Jun 2022 into square plastic pots (9.5 × 9.5 × 9 cm, 810 $cm^3$ vol, BWI Companies, Inc., Nash, TX, USA) filled with soil from organic certified field at the Uvalde Texas A&M AgriLife Research and Extension Center. Four holes were made in pots and mesh pads were placed in bottom of the pots before filling with soil to facilitate drainage. The soil was collected from clay soil site (hyperthermic Aridic Calciustolls of the Uvalde series). The basic soil nutrient properties were: 3.32% total organic matter, pH 8.15, electrical conductivity (EC) 0.91, an equivalent of 68.37 $kg \cdot ha^{-1}$, nitrate nitrogen, and 21.3 $kg \cdot ha^{-1}$ phosphate.

The experiment was performed using a completely randomized factorial design with two treatment factors including 1) fertilization frequency treatment ("weekly" applications of 150 $mg \cdot L^{-1}$ N (applied in volume of 200 mL, which is 0.03 g N/plant in each application, and ∼30 $kg \cdot ha^{-1}$ N) organic fish meal at 7-d intervals and "reduced" applications of 150 $mg \cdot L^{-1}$ organic fish meal at 15-d interval) and 2) grafting combinations (four unique rootstock-scion combinations, self-grafted, and nongrafted). Irrigation was hand-applied daily, providing an equal amount of water to each plant (200 to 250 mL), with frequency adjusted to once or twice a day based on weather conditions. The irrigation water used was city water with a pH of 8.15. On fertilization days, occurring at 7-d intervals for the "weekly" treatment and 15-d intervals for the "reduced" treatment, 150 $mg \cdot L^{-1}$ of fish meal was applied in place of water only. Each treatment combination had five replicates of individual plants. The experiment was conducted in a semicontrolled greenhouse and microclimatic conditions inside the greenhouse were logged hourly with a Watchdog weather station (Spectrum Technologies Inc., Aurora, IL, USA). Maximum temperature inside the greenhouse exceeded 38 to 40 °C which is considered as threshold for heat stress in peppers (Wang et al. 2021), indicating natural heat stress throughout the growth period (Fig. 1). Morphological, physiological, and biochemical measurements were taken 5 weeks after treatments were initiated.

*Growth and physiological measurements.* Growth data were measured at 30 d after

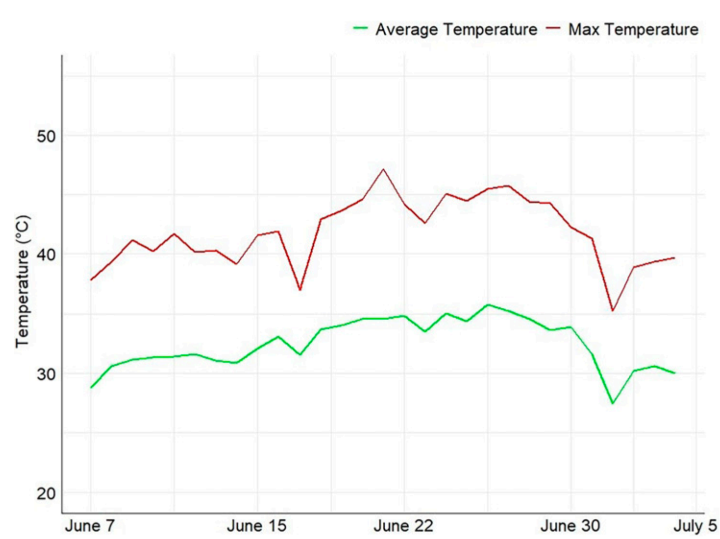


Fig. 1. Average and maximum greenhouse temperature during the experimental period.

transplanting. Shoot measurements included plant height (cm), stem diameter (mm), fresh (g; FW) and dry weight (g; DW). Leaf canopy area ($cm^2$) was measured by taking overhead photographs (Nikon AF-S DX Nikkor; D5500 DSLR; Nikon, Bangkok, Thailand) of each plant canopy and analyzing the images by measuring total area of the thresholded selection with ImageJ (version 1.53e; Laboratory for Optical and Computational Instrumentation, Madison, WI, USA).

Root morphological measurements included total root length (cm; root length), total root surface area ($cm^2$; RSA), and average root diameter (mm; RAD). Whole roots were carefully washed, cleaned, and scanned with an Epson V700 flatbed scanner (Epson, Long Beach, CA, USA) and the above root morphological variables were quantified through the WinRHIZO root image analysis software (WinRHIZO Pro V. 2002c; Regent Instruments Inc., Québec, QC, Canada). After scanning, the roots were oven-dried and weighed for root dry weight (g; RDW).

Seedling growth variables were calculated as follows:

$$\text{Seedling quality index (SQI) (Bai et al. 2014)} = \left(\frac{Stem\ diameter\ (cm)}{Seedling\ height\ (cm)} + \frac{Root\ dry\ weight\ (g)}{Shoot\ dry\ weight\ (g)}\right) \times Whole\ plant\ dry\ weight\ (g)$$

$$\text{Seedling compactness } (\text{g}\cdot\text{cm}^{-1}\text{; Compactness}) \text{ (Liu et al. 2023)} = \frac{Shoot\ dry\ weight\ (g)}{Seedling\ height\ (cm)}$$

Root-to-shoot ratio (R:S): The oven-dried root and shoot samples were weighed and the ratio was determined on a DW basis.

*Physiology.* Net photosynthesis rate ($P_n$) was measured at the end of the study using a portable photosynthesis measurement system (LI-6400, LI-COR Bioscience, Lincoln, NE, USA) between 10:00 AM and 1:00 PM on the second youngest leaf from top under the following conditions: light intensity, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LED, 10% blue 90% red); $CO_2$ concentration, 400 ppm; flow rate, 400 $\mu mol \cdot s^{-1}$.

Stomatal conductance ($g_s$), apparent leaf transpiration rate (E), and electron transport rate (ETR) were measured using the LI-600 Porometer/Fluorometer (LI-COR Inc., Lincoln, NE, USA) at end of the study. Light-adapted leaf measurements were taken between 11:00 AM and 2:00 PM on the second youngest leaf from top after ensuring the stability of the instrument under ambient conditions.

*Biochemical measurements.* Three biological replicates of leaves and roots separately with uniform appearance were taken from each treatment and were flash frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for further analysis.

Chlorophyll a (Chl a) and b (Chl b), total chlorophyll, and carotenoid contents were determined using a modified dimethyl sufoxide (DMSO) extraction method (Richardson et al. 2002). A total of 700 µL of DMSO preheated to 65 °C was added to a 2-mL Eppendorf tubes containing 10 mg of leaf tissue. The tubes were incubated for 30 min at 65 °C. The tubes were removed and 300 µL of DMSO was added to make a final volume of 1 mL. The absorbance of the solution was measured at 663, 645, and 470 nm using a MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA) and the concentration of the pigments (mg/g fresh weight) were calculated as follows:

$$\text{Chl a: } [12.7(A_{663}) - 2.69(A_{645})] \times V/1000 \times W$$

$$\text{Chl b: } [22.9(A_{645}) - 4.68(A_{663})] \times V/1000 \times W$$

$$\text{Carotenoid: } [(1000 \times A_{470} - 1.9 \times \text{Chla} - 63.14 \times \text{Chlb})/214] \times V/1000 \times W$$

where W is the weight of sample and V is the sample extract volume.

Malondialdehyde (MDA) was measured from leaves using the modified method of Lee et al. (2023a). Flash-frozen and powdered leaf samples (60 mg) were mixed with 0.5 mL 0.1% (w/v) trichloroacetic acid (TCA) and homogenized in a tube. After centrifuging the homogenized sample at 12,000 $g_n$ for 20 min at 4 °C, 1.5 mL of 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA) was added to the supernatant. The mixture was boiled at 95 °C for 25 min and quickly allowed to cool on ice. The control mixture was made by combining the supernatant and 20% TCA solution without TBA. Absorbance was measured at 440, 532, and 600 nm using a MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA).

NR activity was measured following Lopez-Delacalle et al. (2021) with some modifications: 0.1 g of fresh samples was frozen in liquid nitrogen and ground into powder; 1.2 mL volume of buffer A (50 mM phosphate buffer, pH 7.5, 2 mM EDTA, 2 mM DTT, 1% PVPP) was added to the sample, which was then centrifuged for 10 min at 4 °C. Fifteen microliters of the supernatant was transferred to a microplate; 225 µL of PBS buffer, 10 mM KNO3, and 0.2 mM NADH solution was added to it. The mixture without enzyme extract was used as blank. NR activity was calculated as NADH oxidation at 340 nm, at 0 time and after 5 min, as measured with the MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA).

The total antioxidant capacity of leaves and roots was estimated using the Ferric Reducing Antioxidant Power (FRAP) assay following Benzie and Strain (1996) with few modifications. A working reagent was made containing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6 tri-(2-pyridyl)-S-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ in a ratio of 10:1:1. Sample extract of 10 µL extracted from 100 mg of flash-frozen and crushed samples homogenized with methanol was added to the 300 µL working solution in the 96-well plate. The absorbance was measured at 593 nm after 6 min using MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA).

For nutrient analysis, oven-dried leaf and root samples were measured for total nitrogen (TKN), $NO_3^-$, $NH_4^+$, and phosphorous content using the EasyChem Plus analyzer (Chinchilla Scientific, Oak Brook, IL, USA). Total nutrient content was obtained as the product of nutrient concentration and plant DW (mg N) (Lawlor 2002). Nitrogen utilization efficiency (NUtE) of plants were calculated as follows:

NUtE = Total plant dry weight/Total N accumulation (mg DW/mg N) based on the formula of Elliott and Läuchli (1985) and Siddiqi and Glass (1981).

*Statistical analysis.* Data presented corresponds to the mean value ± standard error. Descriptive and summary statistics, analysis of variance (ANOVA), principal component analysis (PCA), and heatmap for various measurements were calculated using R software (R version, 2020). Data were analyzed by two-way ANOVA to assess main effects (Grating and Fertilization treatment) and their interaction (Grafting × Fertilization treatment). To better visualize the interaction effect, the effect of grafting within each fertilization treatment was analyzed. Group means were separated by the Tukey's honestly significant difference test at a significance level of $P \leq 0.05$. The data were subjected to PCA and heatmap illustrations to visualize general clustering and trends among fertilization, grafting, and measured parameters.

## Results

*Shoot and root growth.* All shoot growth parameters measured in our study decreased significantly in response to reduced fertilization. The shoot dry weight (SDW) of Scarface-grafted plants was significantly higher than nongrafted plants under weekly fertilization, whereas under reduced fertilization, Keystone-grafted plants had higher SDW than nongrafted plants. The SDW of self- and Scarface-grafted plants decreased under reduced fertilization (Fig. 2A). The leaf canopy area under each fertilization was lowest for nongrafted plants (Fig. 2B). The YC- and Keystone-grafted plants had ~55% more leaf canopy area than nongrafted plants in both fertilizations, whereas Scarface-grafted had the highest leaf canopy area in reduced fertilization with ~72% more area than non- and self-grafted plants. (Figs. 2B and 3). The stem diameter of CM-grafted plants was significantly higher than nongrafted plants under reduced fertilization (Fig. 2C). Root DW was significantly lower in Keystone-grafted plants compared with YC-, Keystone-, and Scarface-grafted plants under weekly fertilization, whereas under reduced fertilization, RDW of Scarface-grafted plants decreased significantly (Figs. 2D and 4). Under the reduced fertilization, root length was highest in YC- and CM-grafted plants compared with nongrafted plants (Fig. 2E). Root average diameter was highest in nongrafted plants, under reduced fertilization compared with other grafted plants (Fig. 2F).

The plant compactness of self-, CM-, and Scarface-grafted plants was significantly higher than nongrafted plants under weekly fertilization, whereas under the reduced fertilization, only CM-grafted plants exhibited improved compactness (Table 2). The seedling quality index (SQI)

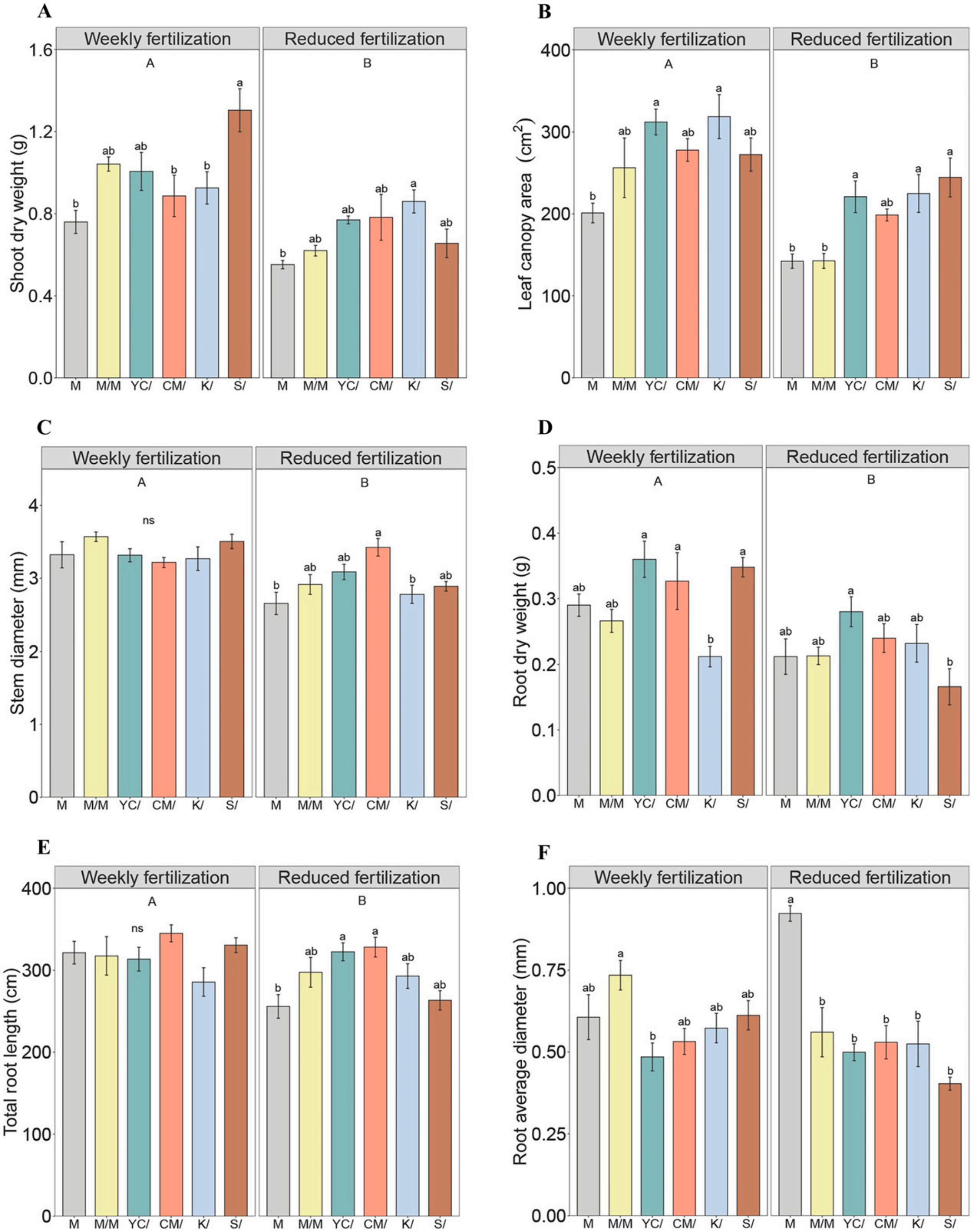


Fig. 2. Effects of grafting on the shoot dry weight (**A**), leaf canopy area (**B**), stem diameter (**C**), root dry weight (**D**), root length (**E**), and root average diameter (**F**) of pepper under different fertilization treatments. Different small letters indicate significant differences among grafts within a fertilization treatment, and capital letters indicate significant difference between fertilization treatment at $P < 0.05$ according to the Tukey test. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; and S/ = scion grafted with 'Scarface' rootstock.

of Keystone-grafted plants under weekly fertilization was significantly lower than YC-, CM-, and Scarface-grafted plants, whereas under reduced fertilization, there was no significant difference. The root:shoot ratio was numerically higher in nongrafted plants under both fertilizations (Table 2).

The Biplot PCA projection of growth parameters revealed that PC1 explained 47% variability and had higher loadings from shoot growth parameters, whereas PC2 explained 15.8% variability with higher loadings from root growth parameters. Weekly fertilization treatments showed positive scores on PC1, indicating better performance in shoot growth

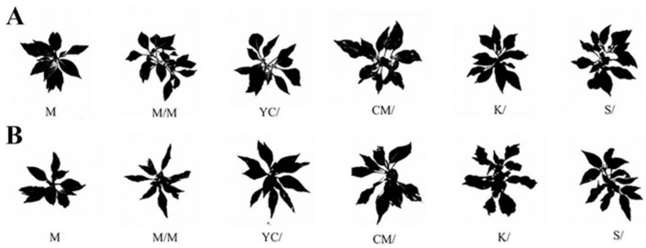


Fig. 3. Images of leaf canopy area of graft combinations under Weekly (**A**) and Reduced (**B**) fertilization. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted 'Mama Mia Giallo'; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; and S/ = scion grafted with 'Scarface' rootstock.

parameters such as leaf area, compactness, stem diameter, SDW, and total dry weight (TDW), compared with reduced fertilization treatment. Under reduced fertilization, non-, self-, Keystone-, and Scarface-grafted plants were strongly negative on PC1 indicating their shoot parameters were significantly affected by the reduced fertilization, whereas CM- and YC-grafted plants were only slightly negative on PC1 indicating that the shoot growth was less affected by reduced fertilization compared with other grafting combinations. CM- and YC-grafted plants had positive PC2 scores under both fertilizations indicating that their root characteristics were not highly compromised by reduced fertilization. Grafting with Scarface showed significant improvement in shoot growth under weekly fertilization whereas a significant reduction under reduced fertilization. The negative PC2 score of Scarface-grafted plants also shows that root system of this rootstock was not tolerant to reduced fertilization (Fig. 5).

*Leaf physiology.* The net photosynthesis rate ($P_n$) remained higher in YC-grafted plants compared with non- and self-grafted plants under both fertilization treatments. Scarface-grafted plants had similar $P_n$ values as YC-grafted under weekly fertilization but the $P_n$ values under reduced fertilization were significantly less than YC-grafted plants. (Fig. 6A). The stomatal conductance rate ($g_s$) was significantly affected by the fertilization treatment as it decreased under reduced fertilization and was higher in YC-grafted plants compared with self- and nongrafted plants under weekly fertilization (Fig. 6B). Transpiration rate (E) was significantly lower under reduced fertilization. Mean E values of YC-grafted plants were higher than non-, self-, and CM-grafted plants under weekly fertilization, whereas Scarface- and Keystone-grafted had higher rates than nongrafted plants only. Scarface- and YC-grafted plants had a significantly higher mean E values than non- and CM-grafted plants under reduced fertilization and (Fig. 6C). The ETR was unaffected by fertilization alone, and YC-grafted plants had significantly higher ETR than non-, self-, Keystone- and Scarface-grafted plants under both fertilization treatments (Fig. 6D).

*Leaf pigment, malondialdehyde, and antioxidant activity.* Total chlorophyll content was statistically similar in nongrafted as well as all grafted plants under weekly fertilization, whereas under reduced fertilization, the nongrafted plants had significantly less total chlorophyll content than Scarface-, YC-, and self-grafted plants (Fig. 7A).

Oxidative stress, assessed by the malondialdehyde (MDA) content, was higher under reduced fertilization. MDA content was not significantly different among the grafting combinations under weekly fertilization, whereas the YC- and CM-grafted plants had significantly less MDA content than non- and self-grafted plants under reduced fertilization (Fig. 7B). The antioxidant activity in shoots, assessed by FRAP content was also higher under reduced fertilization. Under weekly fertilization, Shoot FRAP content in Scarface-grafted plants was significantly lower than CM- and YC-grafted plants (Fig. 7C). Under reduced fertilization, CM-grafted plants had significantly higher shoot FRAP content. Root FRAP activity was significantly higher in YC- and CM-grafted plants under weekly fertilization compared with non-, Keystone- and Scarface-grafted plants. The root FRAP activity among grafting combinations was not significantly different in reduced fertilization except for Scarface-grafted plants, which had the lowest mean values (Fig. 7D).

*Nutrient properties.* Shoot nitrate content was significantly higher in YC- and CM-grafted plants under weekly fertilization, whereas there was no significant difference under reduced fertilization (Fig. 8A). CM-grafted plants had significantly higher phosphorous content compared with non-, self-, Scarface-, and Keystone-grafted plants under weekly fertilization, whereas under reduced application, the phosphorous content was significantly reduced in all plants (Fig. 8B).

YC-grafted plants had significantly higher NUtE than self-grafted plants under weekly fertilization, whereas significantly higher than all graft combinations except nongrafted plants under reduced fertilization (Fig. 8C).

There was a significant effect of grafting within fertilization treatment on leaf NR but there was no significant effect of fertilization treatment alone. The YC- and CM-grafted plants had higher leaf NR activity than nongrafted plants under both fertilizations (Fig. 8D).

A heatmap was generated to elucidate the relationships between variables under different fertilization treatments for each graft combination and to cluster the variables based on their responses. The heatmap identified three distinct cluster groups with complete linkage method reflecting the performance of the graft combinations (Fig. 9). The first cluster from the top (first to fifth row) represents the least stressed group, which includes weekly fertilized YC-, CM-, Scarface-, and self-grafted plants, as well as YC-grafted plants under reduced fertilization. These plants showed higher values for shoot growth, nutrient content, and physiological parameters, indicating less stress. YC-grafted plants with reduced fertilization also fell into this cluster, which demonstrated enhanced performance due to moderate shoot growth and physiological parameters, increased nutrient utilization efficiency, higher nitrate content and NR activity, and elevated root antioxidant activity, coupled with low oxidative stress (MDA content). The second cluster from top, composed solely of nongrafted plants (sixth row), represents the most stressed group. These plants exhibited the lowest values in all shoot

Table 2. Mean seedling indices of graft combination under different fertilization treatments.

| | Plant compactness ($g \cdot cm^{-1}$) | | Seedling index | | Root:Shoot | |
|---|---|---|---|---|---|---|
| Fertilization | Weekly | Reduced | Weekly | Reduced | Weekly | Reduced |
| Grafts | | | | | | |
| M | 0.0372 d | 0.0328 b | 0.5737 ab | 0.4212 a | 0.386 a | 0.387 a |
| M/M | 0.0534 ab | 0.0360 b | 0.5756 ab | 0.4289 a | 0.257 c | 0.343 ab |
| YC/ | 0.0476 bcd | 0.0399 ab | 0.7062 a | 0.5525 a | 0.362 ab | 0.363 ab |
| CM/ | 0.0486 bc | 0.0516 a | 0.6617 a | 0.5501 a | 0.367 a | 0.321 ab |
| K/ | 0.0424 cd | 0.0472 ab | 0.4342 b | 0.4619 a | 0.232 c | 0.267 ab |
| S/ | 0.0606 a | 0.0414 ab | 0.7127 a | 0.3583 a | 0.271 bc | 0.248 b |

Data represents mean values. Values within each fertilization treatment followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's honestly significant difference test. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; and S/ = scion grafted with 'Scarface' rootstock.

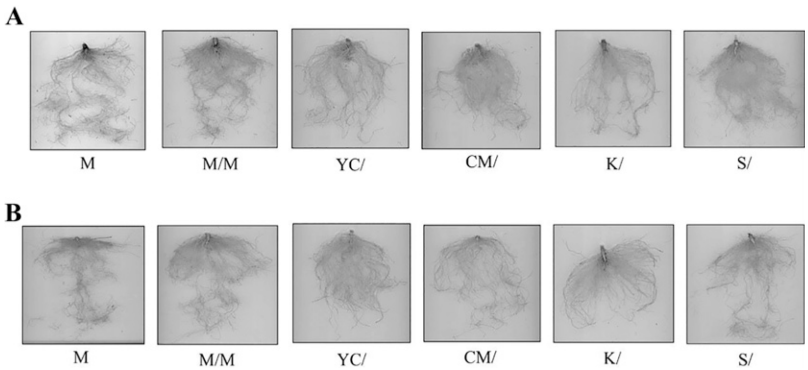


Fig. 4. Images of root system of graft combinations under Weekly (**A**) and Reduced (**B**) fertilization. M = nongrafted scion ‘Mama Mia Giallo’; M/M = self-grafted scion; YC/ = scion grafted with ‘YC-207’ rootstock; CM/ = scion grafted with ‘CM-334’ rootstock; K/ = scion grafted with ‘Keystone resistant Giant’ rootstock; and S/ = scion grafted with ‘Scarface’ rootstock.

and physiological parameters, lowest chlorophyll content, moderate biochemical activity, and very high oxidative stress, denoted by MDA values. The third cluster from the top (7th to 12th) represents moderately stressed plants, which consists of weekly fertilized keystone- and nongrafted plants as well as all the grafted plants under reduced fertilization except YC-grafted plants. This cluster is represented by moderate or low values in most shoot parameters and physiology. Some graft combinations in this cluster perform well in a few parameters but lack heavily in others, for example, Keystone-grafted plants under weekly fertilization show better performance in shoot growth and leaf pigments content but performed poorly in biochemical and physiological traits.

## Discussion

This research was conducted with the hypothesis that grafting with potential rootstocks that included a disease-resistant landrace, a breeding line resistant to *Phytophthora*, and commercially available vigorous and resistant pepper varieties could provide new opportunities for organic pepper production in challenging abiotic stress conditions. Nitrogen (N) and phosphorous (P) are two of the most important macronutrients taken up from soil by plants, thus their deficiency reduces the biomass accumulation and growth of crops. Rootstocks can significantly alter the concentration of nutrients in leaves of grafted plants, affecting their overall growth and development. In our study, when fertilizer application was reduced, various growth parameters decreased significantly. For example, the SDW of Scarface-grafted plants was highest under weekly fertilization, but significantly decreased under reduced fertilization (Fig. 2A), indicating that the benefit of commercial rootstock Scarface diminished when fertilization was reduced. Similar results were observed in Lee et al. (2023b), where the rootstock-grafted tomato seedlings had higher shoot FW and DW than nongrafted plants under no stress condition, but the benefits were reduced when heat stress was applied. In a study by Kalozoumis et al. (2021), it was observed that grafting with tomato cultivar M82, which is known to be vigorous under water stress (Rigano et al. 2016), did not protect plants from combined nutrient and water stress even though it increased shoot growth under nonstress conditions. This indicates that although rootstocks can enhance plant growth under optimal conditions, their effectiveness may be compromised under stressful conditions. During nutrient stress, plants can often experience younger leaf curling and a reduction in canopy area, which has been observed in crops including strawberry (Jiang et al. 2023) and pepper (Massimi and Radocz 2021). The curling could be caused by various nutrient deficiencies, such as phosphorus, potassium, and some other micronutrients. In this study, under reduced fertilization, we observed non- and self-grafted plants had the lowest leaf canopy area, whereas YC-, Scarface-, and Keystone-grafted plants had significantly higher leaf area than non- and self-grafted plants. This suggests that these rootstocks can partially mitigate the adverse effects of nutrient stress by maintaining a larger leaf area, which is crucial for photosynthesis and overall plant health. Roots play a crucial role in adapting to changes in nutrient availabilities and signaling plants to alter their root system (Gruber et al. 2013). Changes in nitrate and phosphate availability have been found to have contrasting effects on root system architecture (López-Bucio et al. 2003). In our experiment, where plants were exposed to the combined stress of many nutrients, no clear root architecture pattern was observed. Although the root:shoot ratio of nongrafted plants did not change significantly between fertilization treatments, the root length decreased more than 20% in reduced fertilization and the root average diameter increased compared with weekly fertilization (Fig. 2E and F). The higher root length with less root average diameter (thus finer roots) of the rootstocks has been associated with a higher yield in tomato (Bayõndõr and Kandemir 2023). Therefore, these two traits can be identified as important phenotypic characters when selecting pepper rootstocks. Overall, this experiment’s results revealed considerable variations in

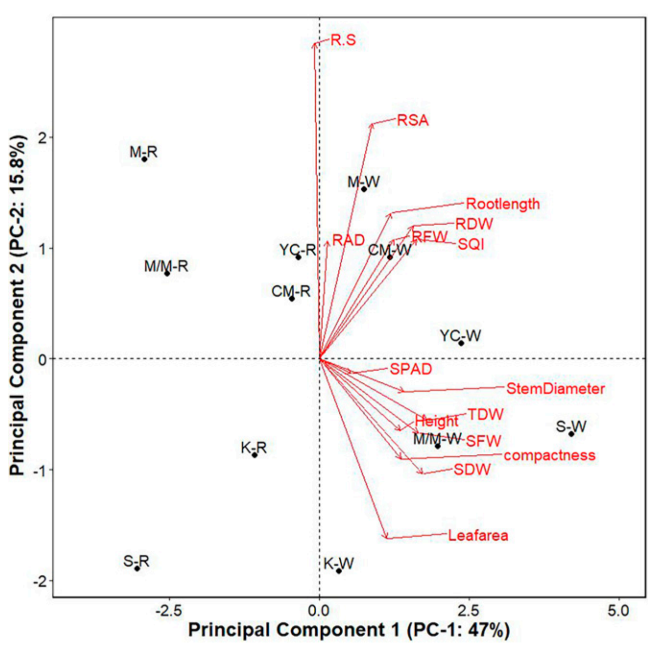


Fig. 5. Principal component analysis (PCA) demonstrates the clustering of shoot and root parameters in response to grafting and fertilization. Biplot for the first two components for growth parameters (scores) and treatments (loadings) as vectors for grafts and fertilization treatment. M = nongrafted scion ‘Mama Mia Giallo’; M/M = self-grafted scion; YC/ = scion grafted with ‘YC-207’ rootstock; CM/ = scion grafted with ‘CM-334’ rootstock; K/ = scion grafted with ‘Keystone resistant Giant’ rootstock; S/ = scion grafted with ‘Scarface’ rootstock; W = Weekly fertilization, R = Reduced fertilization; R.S = root to shoot ratio; RSA = root surface area; RAD = root average diameter; RFW = root fresh weight; RDW = root dry weight; SPAD = chlorophyll index value in SPAD-meter; SQI = seedling quality index; SDW = shoot dry weight; SFW = shoot fresh weight; TDW = total dry weight.

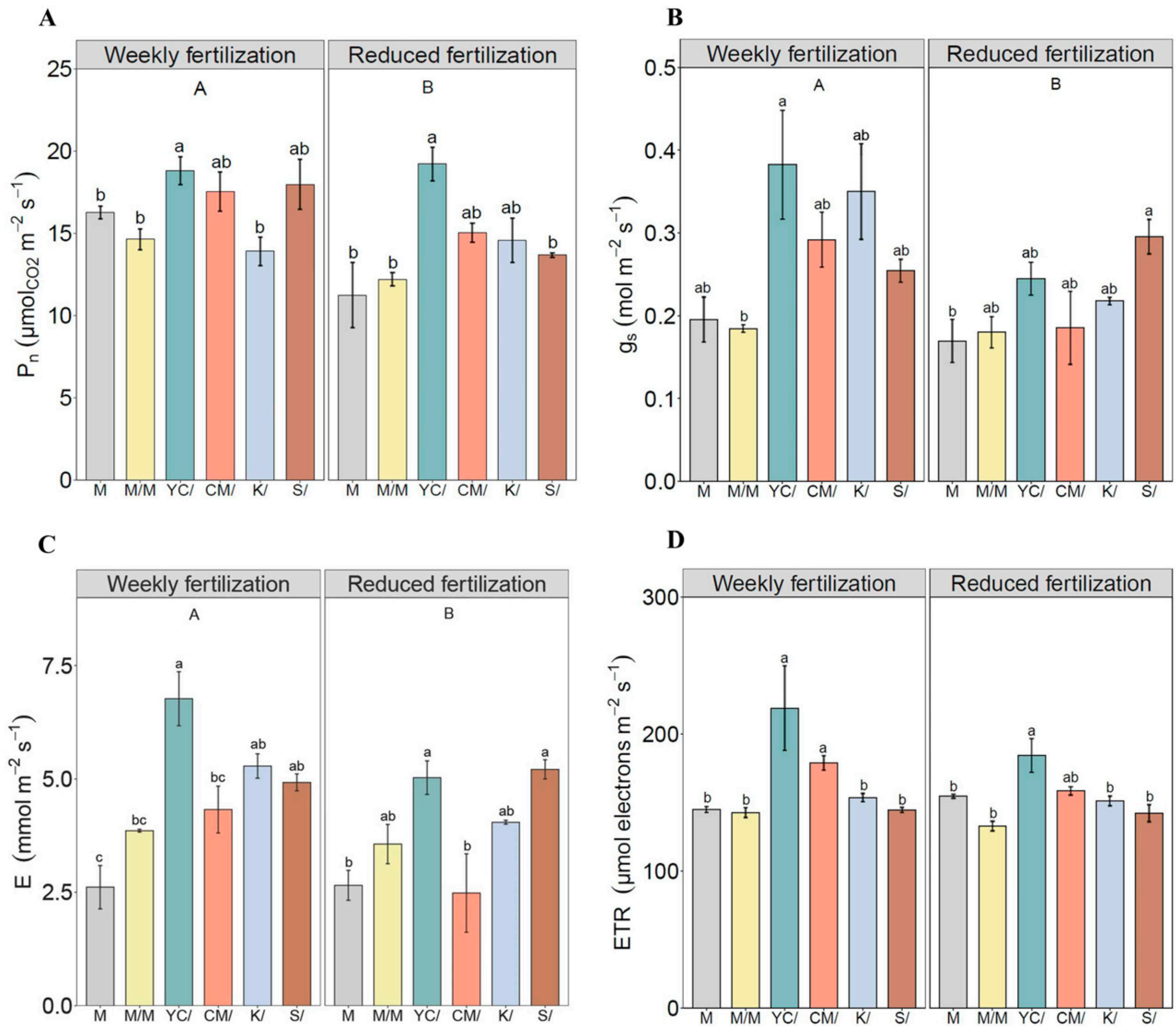


Fig. 6. Effect of grafting on net photosynthesis rate, $P_n$ (**A**); stomatal conductance, $g_s$ (**B**); transpiration rate, E (**C**); and electron transport rate, ETR (**D**) under different fertilization treatments. Bars and arrows represent means with standard error. Different small letters indicate significant differences among grafts within a fertilization treatment, and capital letters indicate significant difference between fertilization treatment at $P < 0.05$ according to the Tukey test. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; and S/ = scion grafted with 'Scarface' rootstock.

root morphological traits among grafted, nongrafted and self-grafted plants (Fig. 4). The PCA (Fig. 5) shows that growth parameters (including plant compactness, leaf area, SDW, root dry weight, TDW) were highly correlated with root morphological characteristics, especially root length, whereas slightly less correlated to root average diameter and root-to-shoot ratio, indicating that root length is a more critical factor in determining the overall growth performance of plants under nutrient stress. This agrees with Comas et al. (2013), who indicated that there is limited scientific evidence to make inferences on root morphology based on root:shoot ratio only and should be focused on other traits such as root length density.

Plants undergo several physiological alterations when exposed to stressful conditions, including a reduction in transpiration rate, stomatal conductance, net photosynthetic rate, photochemical quenching, and related processes. Temperature and nutrient stress can disrupt the balance of light energy absorption and utilization in plant photosynthesis, leading to changes in photosynthetic activity (Chaitanya et al. 2003). Although elevated $CO_2$ initially enhances carboxylation rates, photosynthesis declines over the long-term exposure. This acclimation of photosynthesis to elevated $CO_2$ is more pronounced in nutrient-limited plants compared with well-fertilized ones. This decline is partly due to the accumulation of nonstructural carbohydrates, which can suppress photosynthetic gene expression, including Rubisco. Nitrogen limitation, essential for Rubisco production, also contributes to this reduction, leading to decreased photosynthesis (Stitt and Krapp 1999). With lower stomatal conductance and reduced photosynthesis, the demand for NADPH decreases in the Calvin cycle, eventually blocking electron transport and causing overexcitation of chlorophyll molecules, which can cause accumulation of reactive oxygen species (ROS) and oxidative stress to plants (Sachdev et al. 2023). Thus, in C3 plants facing nutrient stress, selecting genotypes with appropriate $g_s$ to maintain high $CO_2$ concentration, and investing more nitrogen in bioenergetics for a high ETR is recommended (Mu and Chen 2021). In this study, we observed that YC-grafted plants had higher $P_n$ and ETR in both weekly and reduced (Fig. 6A and D) compared with non- and self-grafted plants, which suggests that suitable rootstock could positively impact the photosynthesis process even in stress environments. Nutrient stress has a close relationship with stomatal conductance. Nitrate is the primary N source for plants, playing a significant role in stomatal movement by influencing signaling molecules, such as abscisic acid, that regulate stomatal aperture (Chen et al. 2020). Nitrate helps in maintaining $g_s$ by regulating turgor and hormones in guard cells development, in conjugation with other ions and solutes, which are crucial for stomatal opening and closing (Broadley et al. 2000). Increased $g_s$ is known to

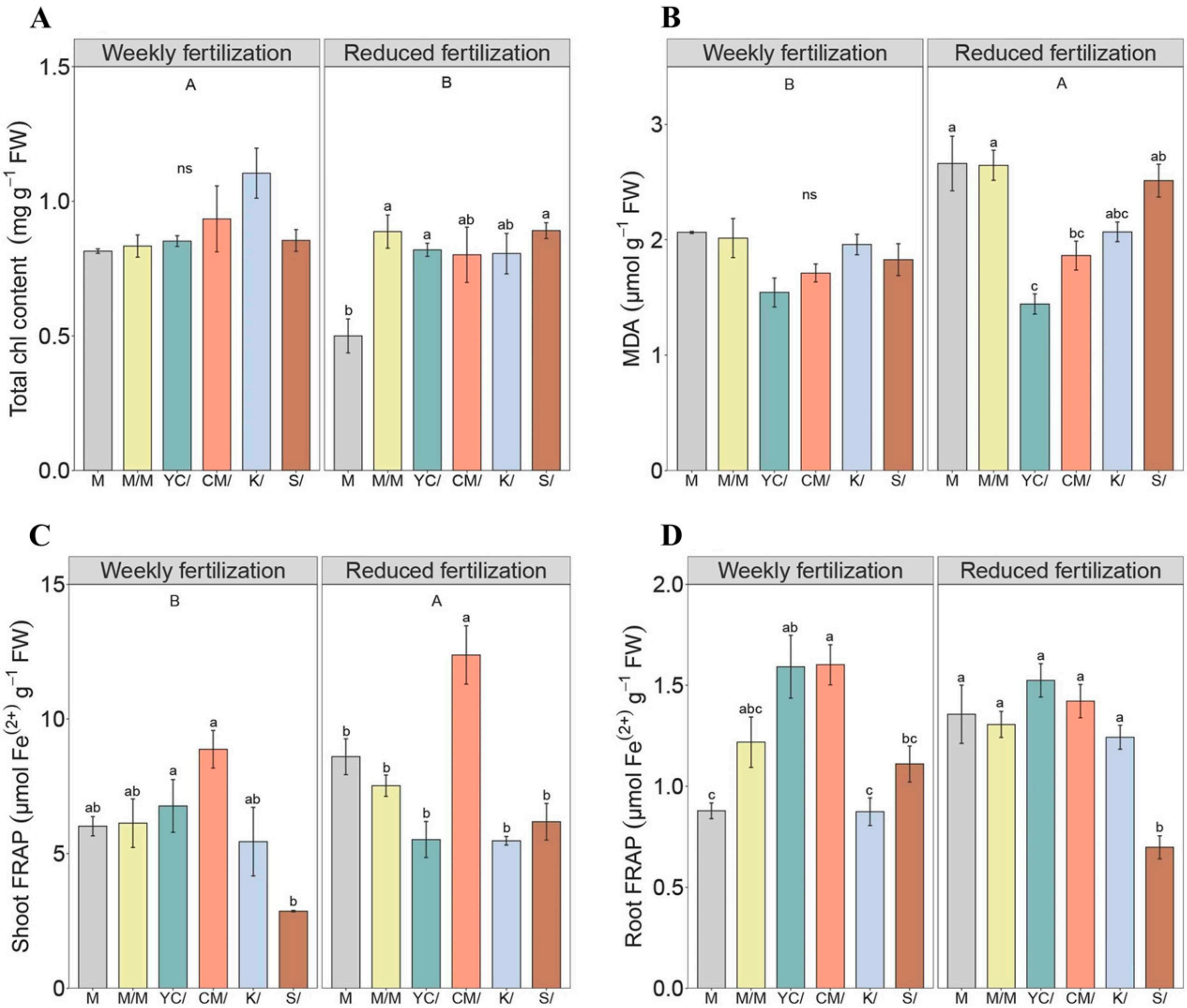


Fig. 7. Effect of grafting on total chlorophyll (**A**), malondialdehyde (MDA) content (**B**), shoot Ferric Reducing Antioxidant Power (FRAP) antioxidant activity (**C**), root FRAP antioxidant activity (**D**) under different fertilization treatments. Bars and arrows represent means with standard error. Different small letters indicate significant differences among grafts within a fertilization treatment, and capital letters indicate significant difference between fertilization treatment at $P < 0.05$ according to the Tukey test. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; S/ = scion grafted with 'Scarface' rootstock; SFW = shoot fresh weight.

have tradeoff of lower intrinsic water use efficiency ($P_n/g_s$), but because water availability is not a limiting factor in our study, maintaining optimum $g_s$ is beneficial for photosynthesis as well as transpirational cooling of plants to prevent heat stress. It is advantageous for the plant to trade an increased rate of water loss to obtain more photosynthetic products, which are essential for growth and reproduction. Significantly higher transpiration rates in a heat-tolerant cultivar were observed in a study in hot peppers, which suggested a mechanism for heat tolerance via photosynthetic rate possibly due to increased stomatal conductivity and transpiration rate (Rajametov et al. 2021). In our study, non- and self-grafted plants had less $g_s$ and E values, compared with rootstocks-grafted plants in both fertilization treatments, with the exception of CM-grafted plants in reduced fertilization. The long-distance nitrate transfer is through the root-to-shoot xylem transport driven by transpiration, as stated by Wang et al. (2012). This is consistent with the research of Van der Vliet et al. (2007), who confirmed that transpiration did influence inorganic mineral ions accumulation and translocation in plants. The higher nitrate content (Fig. 8A) and the possible heat tolerance capacity could have played a role in maintaining a higher $g_s$ and E of YC-grafted plants regardless of fertilization treatment. However, in spite of higher nitrate content, the E values in CM-grafted plants were not significantly higher than non- or self-grafted plants (Fig. 6C), which is not entirely justifiable, but unlike YC, CM might have a different tolerance mechanism. Grafted plants have also been found to exhibit tolerance to stress, by exhibiting low stomatal conductance and reduced transpiration rate, as a feedback mechanism in which reductions in stomatal conductance help prevent further decreases in water potential by reducing transpiration, which was also observed in grafted tomatoes as reduced transpiration was linked as an abiotic stress tolerance trait (Dash et al. 2023).

The effectiveness of nitrate uptake systems and modulation of nitrate signaling are crucial components of nitrogen use efficiency in plants, as nitrate is the main form of N accessible to and absorbed by plants in soil. The utilization of certain rootstocks has been found to stimulate NR activity and N metabolism in melon and tobacco plants (Pulgar et al. 2000; Ruiz et al. 2006). The functional characteristics of the rootstocks such as water and nutrient uptake efficiency could result in increased absorption, upward transport, and accumulation of $NO_3^-$ in the scion, thereby stimulating NR and $NO_3^-$ assimilation (Martínez-Ballesta et al. 2010). NR activity is linked to many aspects of carbon and nitrogen metabolism. $NO_3^-$ plays a significant role in regulating the movement of photosynthate between starch and nitrogen assimilation components. Colla et al. (2010) reported higher NR activity in grafted melon plants under low-nitrogen conditions, suggesting that grafted plants have the potential for greater nitrogen uptake efficiency and utilization for shoot biomass production. In our study, we could see that YC-grafted and CM-grafted plants had higher NR activity (Fig. 8D) with corresponding

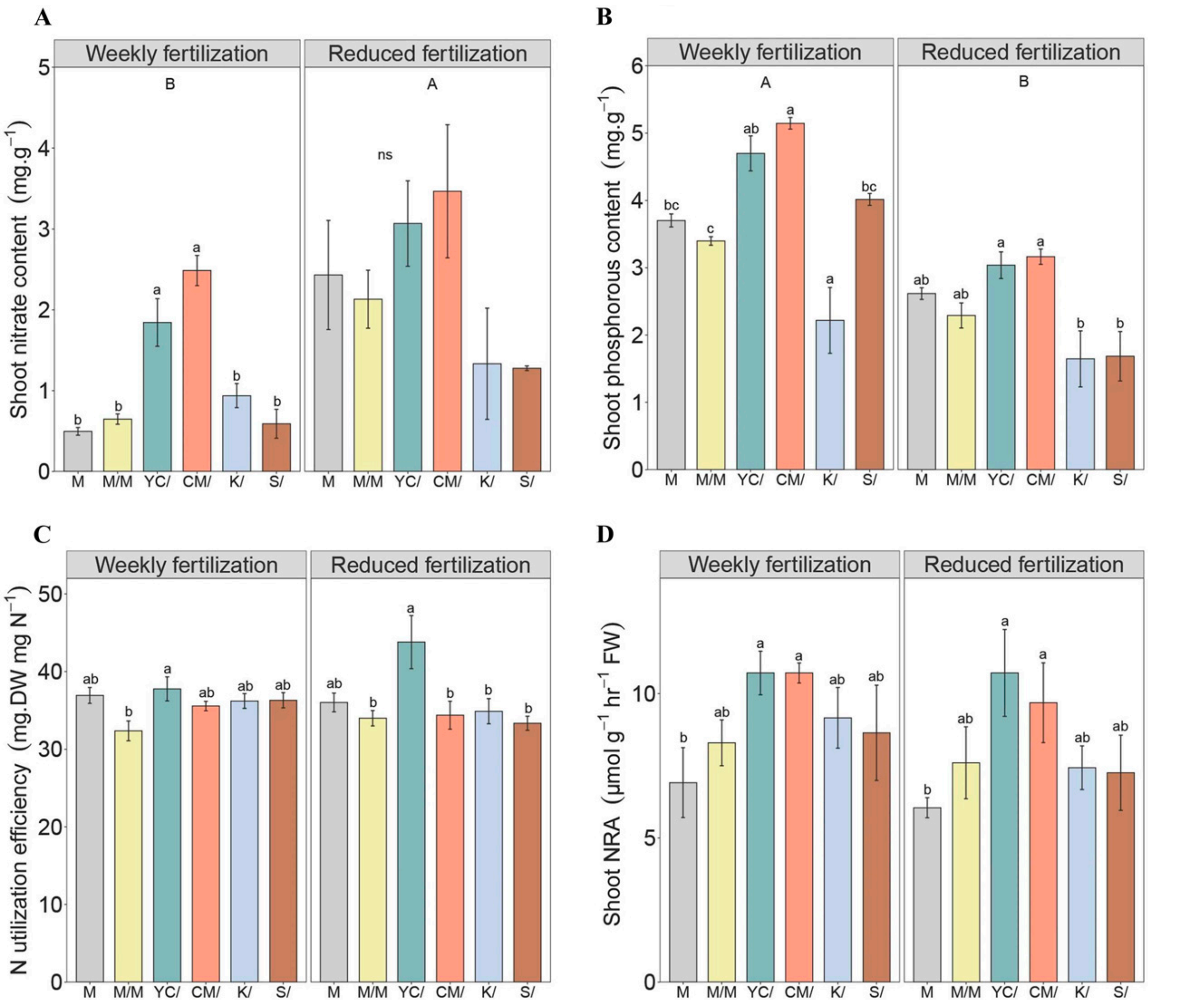


Fig. 8. Shoot nitrate content (**A**), and shoot phosphorous content (**B**), nitrogen (N) utilization efficiency (**C**), and shoot nitrate reductase activity (NRA) (**E**) of graft combinations under different fertilization treatments. Bars and arrows represent means with standard error. Different small letters indicate significant differences among grafts within a fertilization treatment, and capital letters indicate significant difference between fertilization treatment at $P < 0.05$ according to the Tukey test. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; and S/ = scion grafted with 'Scarface' rootstock.

higher nitrate accumulation in leaves compared with nongrafted plants (Fig. 8A). N use efficiency (NUE) is a complex trait that describes the efficiency of N uptake and utilization by crops. Improving nitrate capture has been considered the most accessible way to increase N efficiency. A previous study in *Brassica* spp. has reported that when nitrate was more distributed to shoot, NUE increased (Han et al. 2016). In our study, YC-grafted plants also had higher NUtE under reduced fertilization (Fig. 8C). Similarly, the increased uptake of phosphorous by YC-grafted plants (Fig. 8B) could also explain the tolerance of this rootstock, as phosphorous is an integral component of sugar-phosphates intermediates of respiration and photosynthesis. It has been reported that grafted plants increase the absorption of phosphorus in solanaceous plants (Kawaguchi et al. 2008) such as tomato (Fernández-Garcí et al. 2004) and eggplant (Leonardi and Giuffrida 2006). Another study by Ruiz et al. (1996) in grafted melon plants found rootstock positively influenced foliar levels of total phosphorus, resulting in increased shoot vigor. This effect was accompanied by higher concentrations of carbohydrates such as glucose, sucrose, fructose, and starch in shoots. That study concluded that efficient phosphorus uptake by the roots led to a decrease in carbohydrate concentration in the roots, with these components being transported to the shoot, and ultimately enhancing the aboveground plant vigor. An extensive root system is important for the uptake of P due to its low mobility in the soil. In our study, the low P accumulation in Keystone-grafted plants could be explained by their smaller root system. Total root length has been found to decrease with P deficiency in many crops (Lopez et al. 2023). We observed a numerical decrease in root length for nongrafted plants when fertilizer was reduced. Because the phosphorus content of the soil used in this study was already lower than the recommended levels, reduction in fertilizer might have influenced root and shoot growth of plants as response to phosphorus deficiency.

Adverse stress conditions trigger the substantial production of ROS, leading to damage in plants (Sharma et al. 2003). Excessive accumulation of ROS results in cellular damage due to lipid peroxidation, which breaks down various complex compounds such as MDA and disrupts the normal physiological processes of plants (Hasanuzzaman et al. 2020). Our study revealed that YC-grafted plants under reduced fertilization had significantly less MDA, supporting that ideal rootstocks will have less lipid peroxidation under nutrient stress. The nonenzymatic antioxidant capacity measured by the FRAP method was significantly and numerically highest in CM-grafted plants under reduced and weekly fertilization, respectively. The CM-grafted plants also had similar root FRAP activity in both fertilization treatments, an indication of the capacity of CM rootstock to naturally

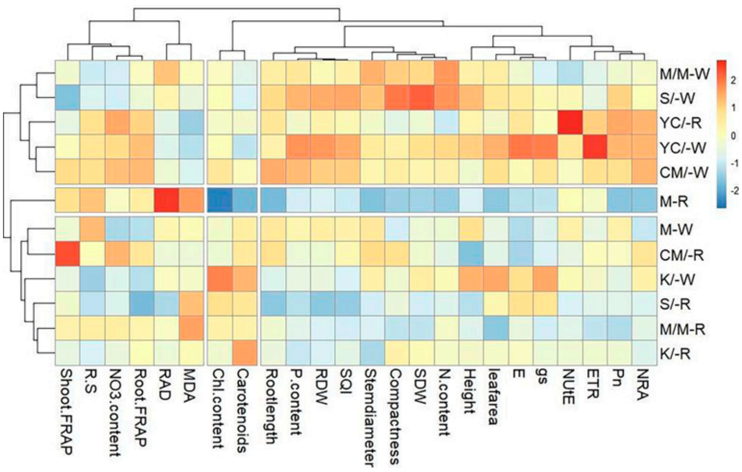


Fig. 9. Heatmap and clustering of varieties based on the scaled values of the measured variables. Each row represents a graft combination in each fertilization treatment and each column indicates a measured parameter. The variables that are clustered together have a positive correlation. Euclidean distance and complete linkage method was used for clustering of rows and Pearson correlation and complete linkage was used for clustering of columns. Cells with red and blue color have high and low relative expression, respectively. M = nongrafted scion 'Mama Mia Giallo'; M/M = self-grafted scion; YC/ = scion grafted with 'YC-207' rootstock; CM/ = scion grafted with 'CM-334' rootstock; K/ = scion grafted with 'Keystone resistant Giant' rootstock; S/ = scion grafted with 'Scarface' rootstock; W = weekly fertilization; R = reduced fertilization; NRA = nitrate reductase activity; Pn = photosynthesis rate; ETR = electron transport rate; NutE = nitrogen utilization efficiency; $g_s$ = stomatal conductance; E = transpiration rate; SDW = shoot dry weight; SQI = seedling quality index; RDW = root dry weight; Chl = total chlorophyll; MDA = malondialdehyde; RAD = average root diameter; FRAP = Ferric Reducing Antioxidant Power.

produce high levels of antioxidants that are transferred efficiently to the scion (shoot) during stress. Whereas YC-grafted plants having lower MDA as well as shoot FRAP could suggest that they might not have experienced high stress as shown by the high expression of physiological tolerant traits such as $P_n$, $g_s$, E, ETR, and nitrate content as well as NUtE. In comparison, although CM-grafted plants had also lower MDA content, they had very high antioxidant activity, suggesting that CM-grafted plants counteracted the stress by producing more antioxidants effectively. All rootstocks had similar antioxidant activity in both fertilization treatments, except for Scarface, which showed lower values than other graft combinations. Whether Scarface involves the production of antioxidant enzymes remains unknown in this study and therefore we have limited information to explore the total antioxidant mechanism as also reported by another study in tomato rootstocks by Lee et al. (2023a) that some grafted plants focused on the accumulation of nonenzymatic antioxidants rather than the enzymatic antioxidant capacity.

## Conclusion

This study showed that using suitable pepper rootstocks could be an option in pepper production, especially for resource limited small-scale organic growers. The four rootstocks we used were all compatible with the scion and no significant reduction in shoot parameters was observed due to grafting. Especially under reduced fertilization, YC-207 was shown to be the most effective rootstock, maintaining shoot growth, tolerant physiological traits, and with an increase in NUtE, suggesting this rootstock could be valuable in organic nutrient system. Because there is a lack of commercially available pepper rootstocks in the US market, our research findings are encouraging to further explore a wide range of rootstocks including disease-resistant landraces, their wild relatives, and new breeding lines to validate their effectiveness in promoting growth and yield in field conditions.

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