

# Enhancing Hydroponic Organic Tomato Resilience through Grafting and Bioprotection Strategies

**Prosanta K. Dash**

*Mechanical Engineering Program, Texas A&M University at Qatar, P.O. Box 23874, Doha, Qatar; and Agrotechnology Discipline, Life Science School, Khula University, Khulna 9208, Bangladesh*

**Bing Guo**

*Mechanical Engineering Program, Texas A&M University at Qatar, P.O. Box 23874, Doha, Qatar*

**Daniel I. Leskovar**

*Texas A&M AgriLife Research, 1619 Garner Field Road, Texas A&M University, Uvalde Texas 78801, USA*

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**Abstract.** The infestation of major greenhouse pests such as whiteflies, leafminers, and thrips and hot and humid conditions pose significant challenges in controlled environment horticulture, particularly for organic tomato production. Low tomato productivity is attributed to pests and inadequate stress tolerance of existing cultivars, which hinder the ability to optimize fruit set and yield. Although the individual effects of bioprotection and grafting strategies of conventional production systems have been extensively studied, their combined effects in controlled environments have been less explored. This study aimed to assess the efficacy of grafting ('Maxifort' × 'Valdeon RZ') and bioprotection strategies (yellow sticky traps, Spinosad, and *Bacillus thuringiensis*) on the mitigation of greenhouse pest infestation and enhancement of the yield of organically grown hydroponic tomatoes in adverse environmental conditions in Qatar. The experimental design used a strip plot with grafted 'Valdeon RZ' and nongrafted 'Valdeon RZ' as the main plot treatments; Yellow sticky traps, Spinosad, and *Bacillus thuringiensis* were randomly assigned to the subplots. Tomato cultivar Valdeon RZ grafted on Maxifort exhibited superior seedling quality, as evidenced by increased stem diameters and improved root attributes. Grafted 'Valdeon RZ' plants treated with Spinosad demonstrated an enhanced net assimilation rate (27%) and stomatal conductance (17%), as well as reduced transpiration loss (22%) and electrolyte leakage (18%); however, the intercellular CO<sub>2</sub> concentration was maintained. Flowering of grafted 'Valdeon RZ' plants treated with Spinosad occurred 4 days earlier than that of untreated and nongrafted counterparts. Compared with the control plants, grafted plants treated with Spinosad exhibited superior fruit sets (22%) and pollen viability (18%), as well as fewer incidences of flower drops (28%). Grafted 'Valdeon RZ' plants treated with 'Spinosad' outperformed the control in terms of marketable fruit yields, with a significantly higher yield (26%). Additionally, fruits collected from grafted plants demonstrated superior postharvest quality, including firmness, soluble solids content, acidity, and color dynamics. Among the bioprotection strategies, Spinosad exhibited superior pest control efficiency, followed by *Bacillus thuringiensis* and yellow sticky traps. Spinosad-treated plants showed a 40% reduction in leafminer, 28% reduction in whitefly, and 22% reduction in thrips compared with untreated control plants. Our findings can lead to practical strategies that minimize greenhouse pest infestations while improving tomato yield in an organic hydroponic system within a protected environment.

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P.K.D. is the corresponding author. E-mail: prosanta.dash@qatar.tamu.edu.

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Challenges associated with environmental pollution and the degradation of food quality resulting from the excessive use of agricultural malpractices require alternative approaches. Organic crop production faces inherent pest and disease issues, particularly in hot and humid climates. Because of the increasing costs of chemical control and pesticide resistance and increased consumer awareness of pesticide residues, the demand for organic methods is growing worldwide. However, pest and disease pressures, which are exacerbated by climate change, limit the ability of growers to achieve better yields and ensure

food security (Phophi and Mafongoya 2017). Despite the common use of chemicals to manage insect pests and diseases in vegetable production, the overuse of pesticides poses health problems and environmental impacts when not handled properly (Jallow et al. 2017). Two economically important insect pests of tomatoes are whiteflies and thrips, and some species act as vectors for plant viruses. Additionally, the sweet-potato whitefly (*Bemisia tabaci*) and western flower thrip (*Frankliniella occidentalis*) are significant pests of tomatoes and have been recognized as supervectors in the emergence and global spread of destructive plant viruses (Gilbertson et al. 2015). Whiteflies and thrips exhibit high reproductive rates, and studies have demonstrated their propensity to develop resistance to insecticides, thereby presenting tomato producers with limited options for effective control (Naveen et al. 2017). Additionally, the tomato leafminer (*Tuta absoluta*) has become a global pest that can cause severe damage in greenhouse cultivation, particularly in pesticide-independent production systems (Nonomura and Toyoda 2020). The tomato leafminer exhibits high reproductive rates and can develop resistance to insecticides, thus limiting effective control options for tomato producers; furthermore, it is a well-known pest that is harmful to tomatoes and causes significant yield losses (Saeidi and Raeesi 2021). Damage caused by larvae of *Tuta absoluta* can result in 100% yield loss because larvae feed on tomato leaves and produce extensive galleries, burrow in stems, and consume apical buds as well as developing and mature tomato fruits (Desneux et al. 2010).

Considering both environmental and economic perspectives, bioprotection strategies in the greenhouse environment are viable alternatives to pesticide use (Yang et al. 2014). Various biological control strategies, including the use of Spinosad, *Bacillus thuringiensis*, and yellow sticky traps, as well as grafting, could provide more efficient and sustainable pest management, thus leading to improved tomato yields. Grafting enhances plant resistance, nutrient uptake efficiency, yield potential, and fruit quality (Dash et al. 2023); furthermore, it serves as a climate-resilient technology adaptable to unfavorable conditions such as drought, heat, diseases, and pest infestations (Colla et al. 2014). Implementing a bioprotection system reduces chemical applications, thus leading to improved bumble bee pollination and, consequently, higher tomato yields (Glenister 2020). Although biological control measures have resulted in success against western flower thrips in greenhouse pepper, controlling them in tomatoes remains challenging (Stanly et al. 2004). Spinosad is effective against *T. absoluta* larvae in tomato seedlings over an extended period (Erasmus et al. 2023), thus making it a promising option, particularly in organic farming (Biondi et al. 2013). Spinosad is a mixture of spinosyns A and D and is a product of the fermentation of a naturally occurring actinomycete, *Saccharopolyspora spinosa* (Smith et al. 2024). *Bacillus thuringiensis* has shown effectiveness against whiteflies on different host plants, resulting in more than 92% whitefly

nymph mortality (Salazar-Magallon et al. 2015), and the use of native strains in Colombia resulted in significant mortality against whitefly *B. tabaci* on tomatoes (Cabra and Fernandez 2019). However, compared with pheromone traps, yellow sticky traps are highly effective for controlling whiteflies and thrips in cotton crops (Murtaza et al. 2019). Additionally, rectangular yellow sticky traps have more successfully controlled whiteflies and aphids in tomatoes under protected environments than other shapes and colors tested (Nair et al. 2021).

Greenhouses provide an excellent opportunity to cultivate high-quality products in large quantities on a small surface area (van Lenteren 2000). In response to climate change, food security concerns, population growth, and geopolitical factors, the Qatari government has invested significantly in developing new agricultural strategies to achieve at least a modest level of self-sufficiency in food production (Qatar Food Security Department 2020). More than 90% of greenhouse crop production in Qatar occurs under protected environments, thus making it essential to explore whether bioprotection and grafting strategies can serve as potential alternatives to pesticides for insect control and enhance tomato yields in the challenging hot and humid conditions of Qatar. This study aimed to assess the efficacy of grafting ('Maxifort' × 'Valdeon RZ') and bioprotection strategies (yellow sticky traps, Spinosad, and *Bacillus thuringiensis*) for mitigating greenhouse pest infestation and improving the yield of organically grown hydroponic tomatoes in controlled environmental conditions in Qatar.

## Materials and Methods

### Plant materials and growth conditions.

Hybrid tomato cultivar Valdeon RZ (Fitogricola, Avenida Benicasim, Castellon, Spain) was grafted onto an interspecific hybrid rootstock 'Maxifort' (*Solanum lycopersicum* L. × *Solanum habrochaites* S. Knapp and D.M. Spooner). The organic tomato seeds were sown in polystyrene 50-cell trays (dimensions: 4.8 × 3.8 × 5.8 cm; 80-cm<sup>3</sup> cell volume; XQ50; Wilson Garden Co. Ltd., Zhengzhou, China) filled with growing media comprising 90% cocopeat and 10% compost (LivePlant Biotech; Hortalan Group, LivePlant Biotech, Almeria, Spain). Trays were irrigated and incubated at 24 °C and 80% relative humidity for 72 h in an insulated cold room. 'Maxifort F1' (Johnny's Selected Seeds, Fairfield, ME, USA) tomato seeds were sown 5 d earlier than 'Valdeon RZ' to match stem diameters between the rootstock and scion. The seedlings were grown in a propagation unit until the stem diameter reached the target size (2.5 mm) for grafting (30 d). The trays were fertilized at 3-d intervals using organic nitrogen (N), phosphorous (P), and potassium (K) fertilizer (N20-P10-K30; 200 mg·L<sup>-1</sup> of N) and trace elements (iron, zinc, bromine, molybdenum, copper, manganese; 10 mg·L<sup>-1</sup>) (Yara; Hortalan Group, Madrid, Spain) beginning 22 d after seedling emergence. The seedlings were cut below the cotyledon, and the splice method of grafting was followed. The graft union was

## Grafting timeline

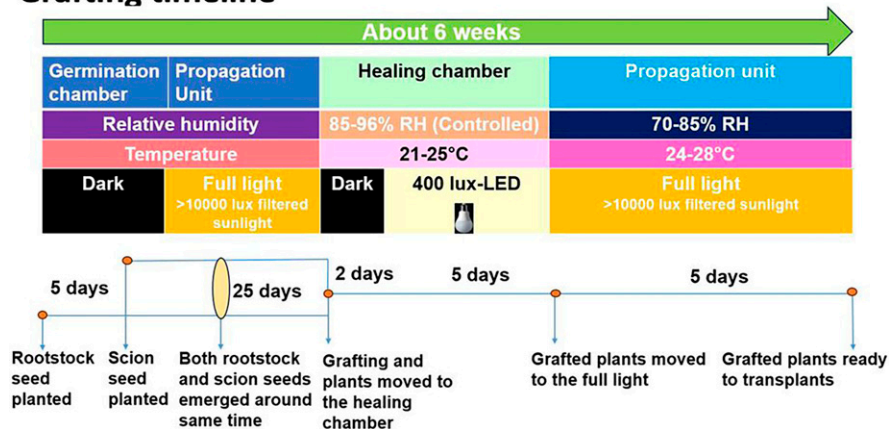


Fig. 1. Tomato seeding, grafting, healing, and transplant rearing management scenario in a nursery at AGRICO Organic Farm in Al-Khore, Qatar.

attached tightly using a 2.5-mm-diameter silicone graft clip (Johnny's Selected Seeds). The grafted seedlings were immediately transferred to a healing chamber for acclimatization at 22 to 24 °C and 85% to 96% relative humidity. The dark condition was maintained for 2 d, a dim light-emitting diode light was added outside of the chamber on day 3, and the relative humidity was decreased gradually until 7 d of acclimation. Afterward, the grafted seedlings were transferred to the main propagation unit for an additional 5 d of hardening (Fig. 1); then, they were transferred into the grow bag (1.0 × 0.2 × 0.1 m) filled with a cocopeat (90%) and compost (10%) mix (Polydime; Kirulapone, Colombo, Sri Lanka) located at AGRICO Organic Farm in Al-Khore, Qatar (lat. 25°41'N, long. 51°30'E). The grow bags were positioned on a metal bench with a 1.2-m

center-to-center distance and 0.2-m blank space between them; a drip tube hose was inserted for each plant to provide irrigation and nutrients.

The experiment was performed using a strip plot design with grafted 'Valdeon RZ' and nongrafted 'Valdeon RZ' as the main plot treatments; yellow sticky traps, Spinosad, and *Bacillus thuringiensis* were randomly assigned to the subplots with four replications. One yellow sticky trap (20 cm × 25 cm; Isphani Agro Ltd., Matijheel Commercial Area, Dhaka, Bangladesh) was hung in each grow bag near the canopy area and replaced every month. Spinosad (Tracer\*45 SC) was applied at 0.4 mL·L<sup>-1</sup> in water with an interval of 7 d during the vegetative, flowering, and fruiting stages as a foliar spray (Auto Crop Care Ltd., Banani, Dhaka, Bangladesh). Similarly, *Bacillus thuringiensis* var. kurstaki 5% at 1.5 g·L<sup>-1</sup> in water was

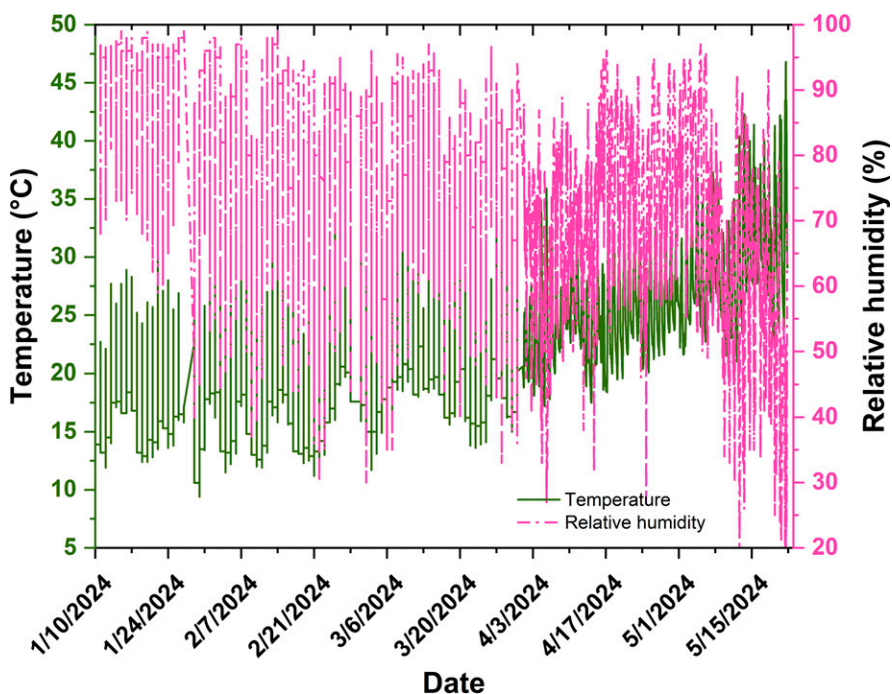


Fig. 2. Real-time air temperature and relative humidity inside the greenhouse at AGRICO Organic Farm in Al-Khore, Qatar.

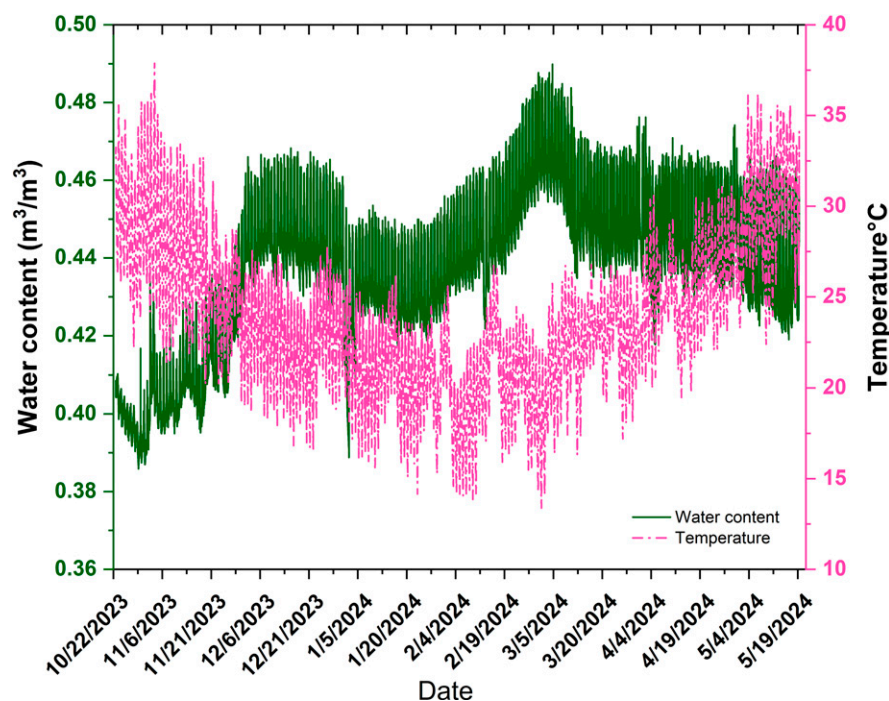


Fig. 3. Real-time growing media temperature and water content at a depth of 2 cm at AGRICO Organic Farm in Al-Khore, Qatar.

applied using a knapsack sprayer with 7-d intervals during the vegetative, flowering, and fruiting stages (Russel IMP, Flintshire, UK). Each experimental unit consisted of 12 plants. The greenhouse air temperature (°C) and relative humidity (%) were monitored continuously during the experiments (model WS80BN; Ambient weather, Chandler, AZ, USA) (Fig. 2). Growing media temperatures and water contents ( $\text{m}^3 \cdot \text{m}^{-3}$ )

were recorded using data loggers at a depth of 2 cm (HOBO® MX, MX2307; Onset, Bourne, MA, USA) (Fig. 3). The electrical conductivity of growing media was monitored at a depth of 2 cm using a digital electrical conductivity meter (EC-1385; ServoVendi SL, Malaga, Spain) (Fig. 4). The plants were irrigated daily between 0800 and 1600 HR with a drip irrigation system (flow rate, 0.3 L/emitter/h) and

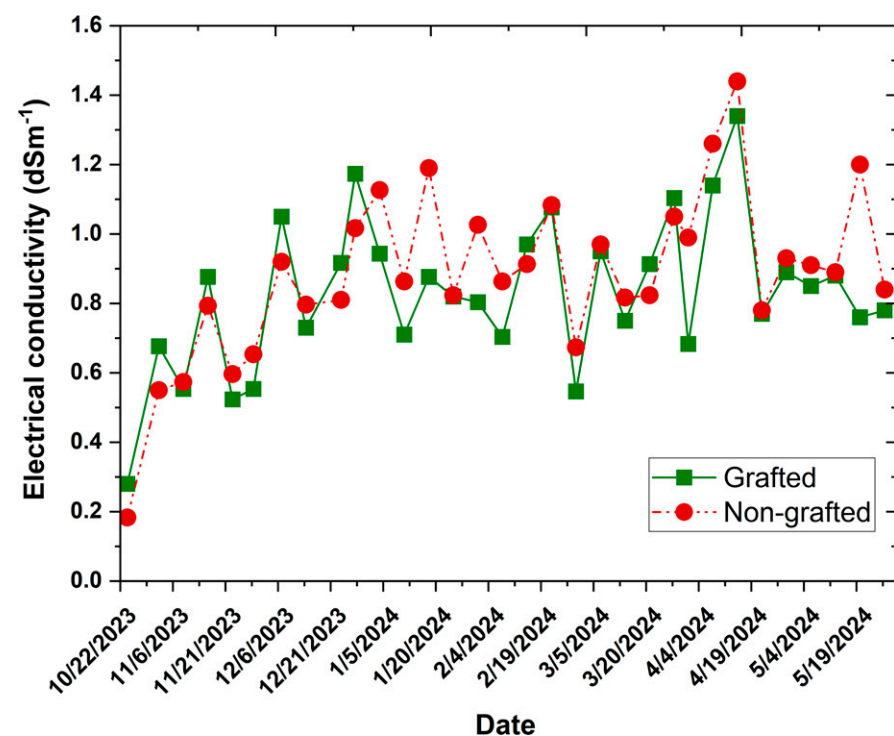


Fig. 4. Electrical conductivity of growing media over time at AGRICO Organic Farm in Al-Khore, Qatar.

fertilized weekly with N20–P10–K30 ( $200 \text{ mg} \cdot \text{L}^{-1} \text{ N}$ ); this continued until 25 May 2024.

**Seedlings growth and root attributes assessment.** The evaluated growth and root attributes of grafted and nongrafted seedlings were as follows: stem diameter; plant height; leaf number; leaf area; soil plant analysis development (SPAD) value; gas exchange; the presence of fine, medium, and large roots; and total roots. Leaf area was calculated using ImageJ software (version 1.53e; Madison, WI, USA) (Dash et al. 2023). The SPAD values were recorded using a portable chlorophyll meter (SPAD-502 plus; Konica Minolta, Tokyo, Japan). Gas exchange variables, including the transpiration rate, assimilation rate, intercellular  $\text{CO}_2$ , and stomatal conductance to water vapor, were measured using a portable photosynthesis system (LI-6800; LI-COR Inc., Lincoln, NE, USA). Root characteristics were analyzed (WinRHIZO™ 2021; Regent Instrument Inc., Sainte Foy, Quebec City, Canada) before the transplanting phase.

**Plant growth and physiological measurements.** Commencing 30 d after planting, the canopy area ( $\text{cm}^2$ ) was calculated weekly by capturing images from the top of the plant using a Nikon AF-S DX camera (D5500 DSLR; Bangkok, Thailand); image analysis was performed using ImageJ software (version 1.53e). The SPAD value was recorded using a portable chlorophyll meter (SPAD-502 Plus; Konica Minolta) on the third fully expanded leaf from the top. Gas exchange data, including the assimilation rate, transpiration rate, intercellular  $\text{CO}_2$ , and stomatal conductance to water vapor, were measured on the same leaf using a portable photosynthesis system (LI-6800; LI-COR Inc.) between 1000 and 1300 HR, with a flow rate of  $500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , reference  $\text{CO}_2$  concentration of  $400 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , fan speed of 10,000 rpm, fluorometer set point of  $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , and aperture size of  $6 \text{ cm}^2$ . Electrolyte leakage was computed using the following formula (Mukherjee et al. 2023).

$$\text{EL}(\%) = \frac{\text{EC1}}{\text{EC2}} \times 100 \quad [1]$$

where EL is electrolyte leakage, EC1 is the initial electrical conductivity reading after the leaf sample was immersed in deionized water for 20 h, and EC2 is the final electrical conductivity after boiling the solution at  $60^\circ\text{C}$ .

**Phenological dynamics.** Flowering (days to first flowering and 50% flowering) was evaluated by keeping a record of the number of days from planting to the first flower opening and 50% flower opening from each experimental unit. To assess the flower drop and fruit set performance against abiotic stresses, five mature flower clusters (containing 5–12 flowers) were tagged in each plot and the existing information was collected regularly. Pollen viability was determined according to the method outlined by Dash et al. (2023) using an iodine potassium iodide staining test. Pollen grains stained dark (dark red or brown) were considered viable, and further examinations were conducted using a

microscope (DM 2700M; Leica Microsystems Inc., Deerfield, IL, USA).

**Pest infestations evaluation.** The prevalences of three greenhouse pests, leafminer (*Tuta absoluta*), whitefly (*Bemisia tabaci*), and thrips (*Thrips tabaci*), were determined through mathematical calculations. Images of the top five leaves of eight plants per experimental unit were captured, and quantification of insects was accomplished using ImageJ software. The larval instars of whiteflies were compared using the images provided by Barbedo (2014). Similarly, the larval instars of leafminers were identified based on the images provided by El-Shafie (2020). Additionally, the larval instars of thrips were examined using the images provided by Joseph et al. (2019). Subsequently, the incidence of each pest was computed by methodically documenting the number of infected leaves and the total number of leaves. The formula used for this calculation was as follows:

$$IC(\%) = \frac{n}{N} \times 100 \quad [2]$$

where IC represents the pest incidence, n denotes the number of infected leaves, and N represents the cumulative number of leaves (both healthy and infected leaves).

**Yield and postharvest quality evaluation.** Fully ripe marketable fruits were harvested every other day for a total of 54 harvests, commencing on 9 Jan 2024 and concluding on 22 May 2024. The total yield was calculated based on these harvests. Various postharvest quality attributes, including fruit weight, firmness, color dynamics, acidity, and soluble solids content, of the stored fruits were assessed. The harvested tomato fruits were promptly stored in the laboratory (Mechanical Engineering Program, Texas A&M University in Qatar). These fruits were stored under ambient conditions of 23 °C and 75% relative humidity to evaluate their postharvest quality. Fruit firmness was measured using a digital force gauge (Chatillon force measurement, DFS3; Ametek, Largo, FL, USA) equipped with a 2-mm probe. The force applied was calculated in N/cm<sup>2</sup>. Color traits of the stored fruits, such as lightness (L\*),

redness/greenness (a\*), yellowness/blueness (b\*), chroma (C\*), and hue angle, were recorded using a portable Chroma Meter (CR 410; Konica Minolta). The percentage of soluble solids and acidity in tomato juice following a dilution ratio of 1:50 were determined using a pocket Brix-Acidity meter (PAL-BXIACID3; Atago Co. Ltd., Shiba-Koen, Minato-ku, Tokyo, Japan).

**Statistical analysis.** A two-factor strip plot design was used for this experiment. The collected data were analyzed using Origin 2023 (version: 9.6.5; OriginLab Corporation, Northampton, MA, USA). The data were subjected to a two-way analysis of variance to determine the statistical differences among the treatments, and pairwise mean comparisons were estimated using Tukey's honestly significant difference test at  $P < 0.05$ . Additionally, a principal component analysis (PCA) was conducted to investigate the relationships between variables and treatments, as well as to visually display trends and patterns in the data. A heatmap was created using the scaled values of each parameter, and a cluster analysis was performed using the correlation distance.

## Results

**Seedling attributes.** Tomato seedlings 'Valdeon RZ' grafted on 'Maxifort' grown using the standard containerized system demonstrated significantly enhanced vegetative growth, improved physiological characteristics (including SPAD value, transpiration, assimilation, stomatal conductance, and intercellular CO<sub>2</sub> concentration), and superior root development of fine, medium, large, and total roots in comparison with those of nongrafted plants (Table 1).

**Plant growth and physiological characteristics.** Canopy growth of grafted 'Valdeon RZ' plants treated with Spinosad was significantly (26%) higher than that of nongrafted plants. Over time, grafted plants consistently outperformed nongrafted plants in terms of canopy growth. Additionally, grafted 'Valdeon RZ' plants exhibited higher SPAD (chlorophyll index) values than those of nongrafted plants. There were notable interactions between

grafting and bioprotection strategies; specifically, grafted 'Valdeon RZ' plants treated with Spinosad showed a 12% greater increase in SPAD values compared with those of nongrafted and untreated plants (data not shown).

The assimilation rate of grafted plants was significantly higher than that of nongrafted plants (Fig. 5A). Grafted 'Valdeon RZ' plants treated with Spinosad exhibited a 27% increase in the leaf assimilation rate compared with that of untreated and nongrafted plants. Furthermore, the leaf transpiration was significantly lower (22%) in grafted plants treated with Spinosad than in control plants (Fig. 5B). Similarly, grafted plants showed a 17% increase in stomatal conductance (Fig. 5C) and an 18% reduction in electrolyte leakage (Fig. 5D). Grafted plants treated with Spinosad also maintained a significantly higher intercellular CO<sub>2</sub> concentration than that of nongrafted plants (data not shown).

**Phenological dynamics.** There were significant interactions between grafting and bioprotection strategies for phenological attributes. The impact of grafting on the days required to reach the first flower stage differed from that of nongrafted plants. Flowering occurred 4 d earlier in grafted 'Valdeon RZ' plants treated with Spinosad compared with that of nongrafted plants. A similar trend of the time to reach 50% flowering was observed, with nongrafted plants experiencing a delay of more than 4 d compared with grafted plants, regardless of the bioprotection strategies used (Fig. 6A). Additionally, compared with nongrafted plants, grafted plants treated with Spinosad exhibited fewer flower drops (28%). Grafted 'Valdeon RZ' plants exhibited a higher (22%) fruit set than nongrafted transplants (Fig. 6B). Furthermore, grafted 'Valdeon RZ' plants treated with Spinosad had higher pollen viability (18%) than nongrafted plants.

**Insect incidence pattern.** Compared with untreated and nongrafted plants, significant interactions between grafting and bioprotection strategies were observed for greenhouse pests, including leafminers, whitefly, and thrips, with distinct effects, as evidenced by the interaction of grafting with Spinosad (Fig. 7). These

Table 1. Effects of grafting on seedlings growth, physiology and root attributes of tomato.

Grafting status	Height (cm)	Stem diam (cm)	Leaf no.	Leaf area (cm <sup>2</sup> )	SPAD value	Transpiration rate (mol·m <sup>-2</sup> ·s <sup>-1</sup> )	Assimilation rate (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )	Stomatal conductance (mol·m <sup>-2</sup> ·s <sup>-1</sup> )	Intercellular CO <sub>2</sub> (μmol·mol <sup>-1</sup> )
'Maxifort' × 'Valdeon RZ'	14.0 b	0.35 a	4.0	10.5	27.8 a	0.011 a	4.4 a	0.77 a	374.4 a
Nongrafted	17.0 a	0.26 b	4.0	10.2	26.3 b	0.007 b	3.3 b	0.63 b	361.3 b
Significance	**	**	NS	NS	**	*	**	*	*
Root length (cm)									
	Root diam (mm)	Fine (0–0.5 mm)	Medium (0.5–1.5 mm)	Large (1.5–2.5 mm)	Total				
'Maxifort' × 'Valdeon RZ'	0.77 a	213.4 a	32.1 a	8.6 a	254.1 a				
Nongrafted	0.63 b	175.6 b	18.5 b	3.2 b	197.3 b				
Significance	*	**	**	**	**				

All-pairwise comparisons were performed using Tukey's honestly significant difference test at  $P < 0.05$ . Columns with dissimilar letters are statistically different, whereas columns with no letters are statistically nonsignificant.

\* $P < 0.05$ ; \*\* $P < 0.01$ .

NS = nonsignificant; SPAD = soil plant analysis development.

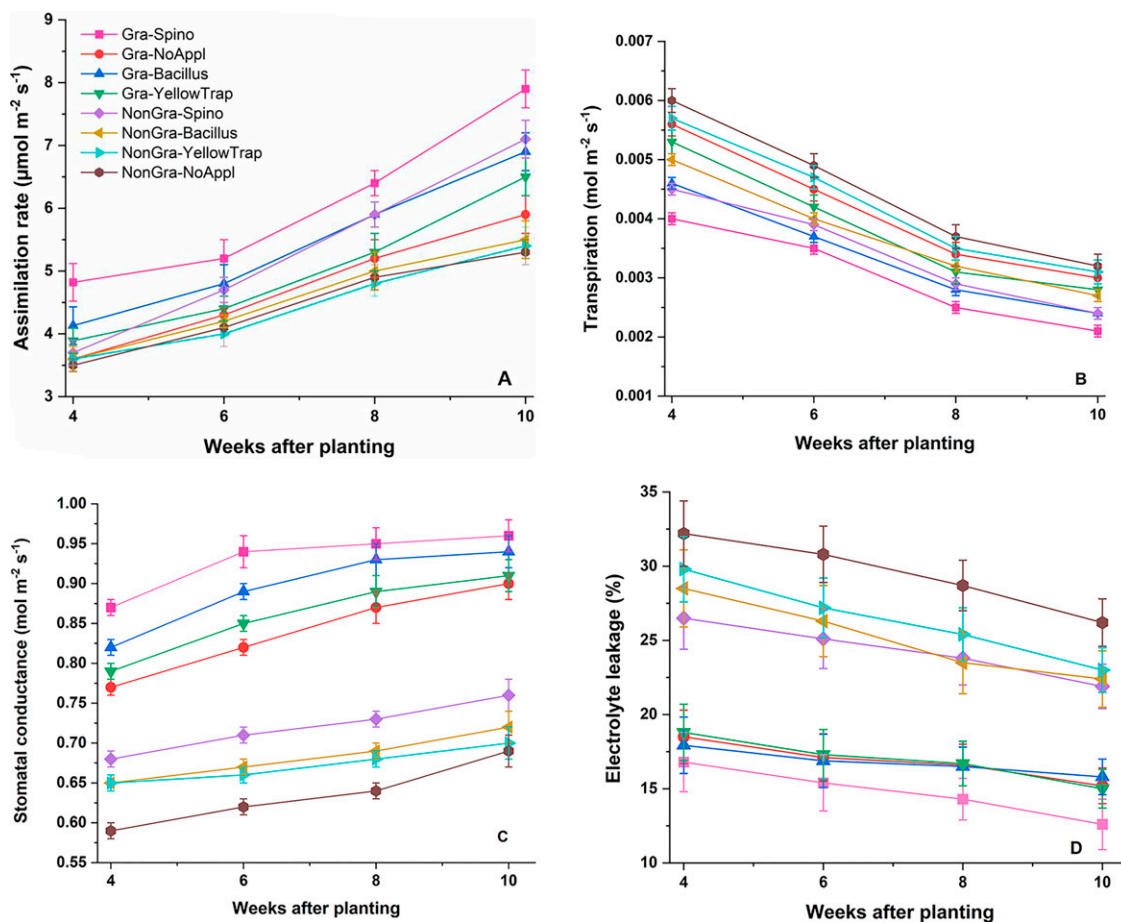


Fig. 5. Effects of grafting and bioprotection strategies on the leaf assimilation rate (A), transpiration rate (B), stomatal conductance (C), and electrolyte leakage (D) of tomato. The vertical bar represents the SE. Gra-Spino = grafted + Spinosad; Gra-NoAppl = grafted + no application; Gra-Bacillus = grafted + *Bacillus thuringiensis*; Gra-YellowTrap = grafted + yellow sticky trap; NonGra-Spino = nongrafted + Spinosad; NonGra-Bacillus = nongrafted + *Bacillus thuringiensis*; NonGra-YellowTrap = nongrafted + yellow sticky trap; NoGra-NoAppl = nongrafted + no application. Location: AGRICO Organic Farm in Al-Khore, Qatar.

findings indicated that grafted plants treated with Spinosad exhibited a substantial reduction in the incidence of leafminers by 40%, a 28% reduction in whiteflies, and a 22% reduction in

thrips compared with untreated control plants, thus demonstrating the efficacy of this bioprotection strategy over time. However, grafted plants treated with *Bacillus thuringiensis*

exhibited a substantial reduction in the incidences of leafminers, whiteflies, and thrips by 34%, 13%, and 12%, respectively, compared with untreated control plants. Similarly, grafted

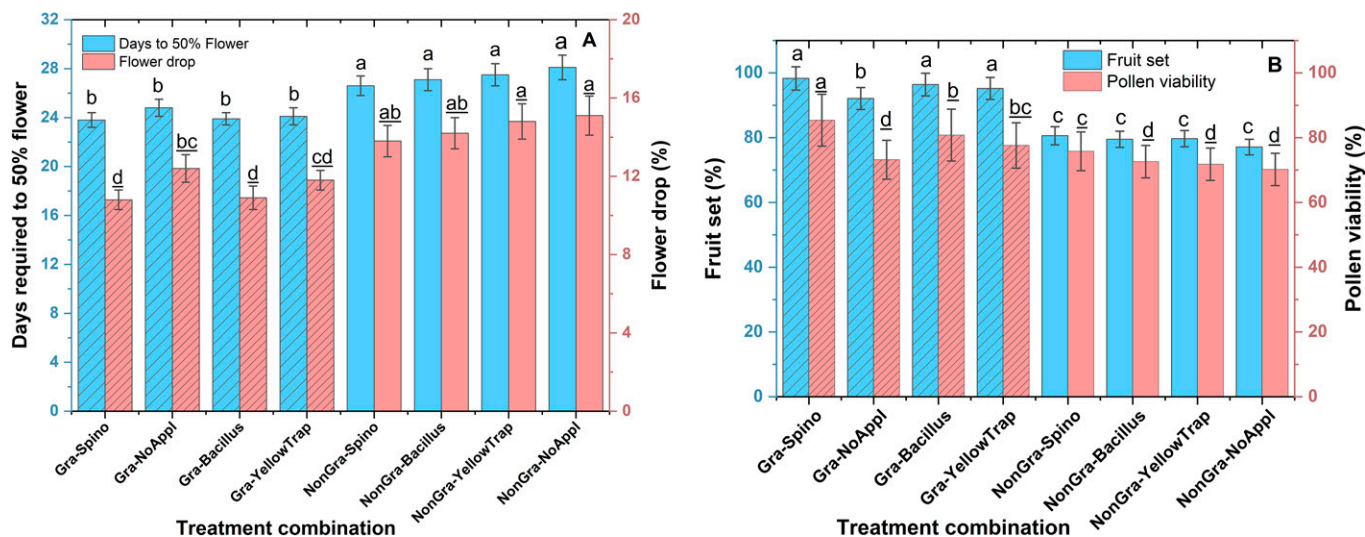


Fig. 6. Effects of grafting and bioprotection strategies on days required to 50% flowering and flower drop (A) and on fruit set and pollen viability (B) of tomato. Vertical bars represent the SE. Bar graphs with dissimilar letters are statistically different, whereas bars with the same letter are statistically similar according to Tukey's honestly significant difference test at  $P < 0.05$ . Location: AGRICO Organic Farm in Al-Khore, Qatar.

plants protected by yellow sticky traps showed significant reductions in the incidence of leafminers, whiteflies, and thrips by 25%, 9%, and 6%, respectively, compared with control plants.

**Yield and postharvest quality.** The marketable fruit yield was significantly influenced by the interaction between grafting combinations and bioprotection strategies ( $P < 0.05$ ). Grafted 'Valdeon RZ' plants treated with Spinosad had a 26% higher marketable fruit yield than that of nongrafted and untreated plants (Fig. 8). Similarly, grafted 'Valdeon RZ' plants treated with *Bacillus thuringiensis* had a 22% higher marketable fruit yield compared with that of nongrafted and untreated plants. Grafted 'Valdeon RZ' plants protected with yellow sticky traps also had a 17% higher marketable fruit yield than that of control plants. Overall, grafted plants exhibited higher fruit production efficiency than that of nongrafted plants.

Grafted 'Valdeon RZ' plants maintained postharvest quality, with fruits having a storage life 3 d longer than that of nongrafted plants (Table 2). At the end of the storage period, grafted 'Valdeon RZ' fruits had an 11% higher weight than that of nongrafted fruits. Additionally, grafted 'Valdeon RZ' fruits maintained significantly higher firmness levels (24%) than those of fruits from nongrafted plants. There were no significant differences in Brix, acid levels, and color dynamics among the treatments.

**Principal component analysis, heat map, and cluster analysis assessment.** A PCA of 26 growth, physiology, phenology, yield, and postharvest quality attributes was conducted to evaluate the overall variability and identify the key variables that contribute to experimental variations. Of all the PCAs performed, the first two principal components, PC1 and PC2, accounted for 80.5% and 9.5% of the total explained variability, respectively (Fig. 9). The biplot is an effective method used to visualize the results of the PCA that depicts the principal component scores and loading vectors in a single graph. The biplot illustrated that variables such as fruit yield, fruit set, pollen viability, intercellular CO<sub>2</sub> concentration, SPAD value, assimilation rate, stomatal conductance, hue angle, C\*, acidity, firmness, Brix, fruit weight, shelf life, a\*, L\*, and b\* were positively correlated. In contrast, variables such as flower drop, days to the first flower, electrolyte leakage, days to 50% flowering, transpiration, leafminer incidence, whitefly incidence, and thrips incidence did not appear to be strongly linked or negatively correlated. The analysis indicated that all the studied variables had varying effects on understanding the experimental variance, either positively or negatively. Additionally, tomato plant physiological and phenological traits had more pronounced influences on modulating plant growth and development, enhancing fruit yield while maintaining postharvest quality. However, PC2 did not provide as much clarification of the experimental variations. However, a heatmap (Fig. 10) was generated to illustrate the relationships between variables among different treatment groups (grafting × bioprotection

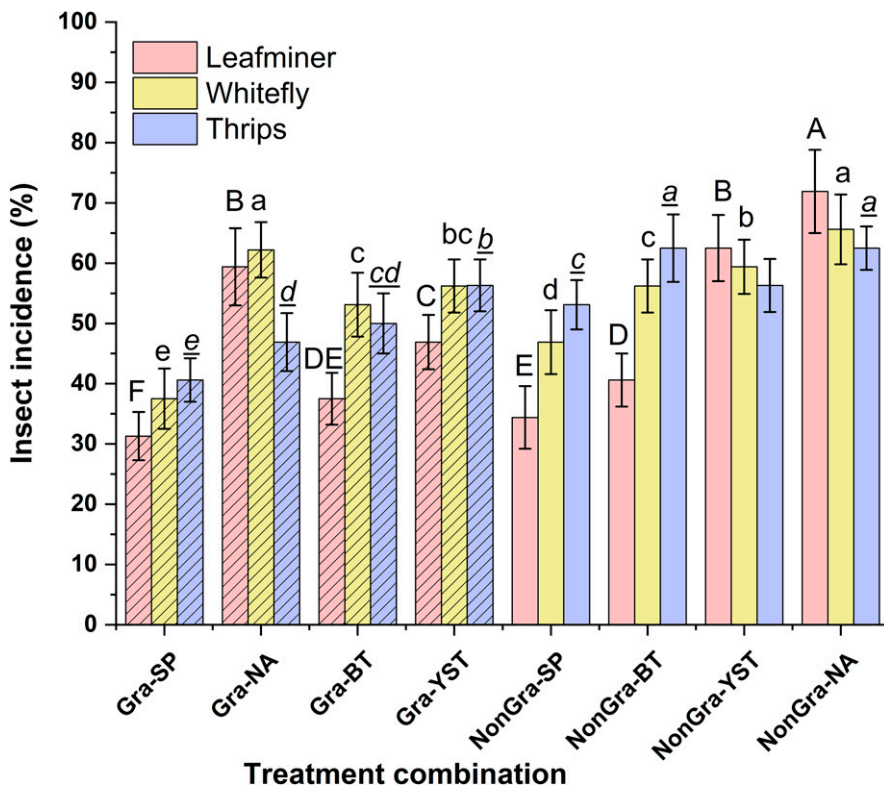


Fig. 7. Effects of grafting and bioprotection strategies on insect incidence of tomato. Vertical bars represent the SE. Bar graphs with dissimilar letters are statistically different, whereas bars with the same letter are statistically similar according to Tukey's honestly significant difference test at  $P < 0.05$ . Location: AGRICO Organic Farm in Al-Khore, Qatar.

strategies) and cluster variables based on their responses. The clusters were clearly distinguished based on variables such as flower drop, thrips incidence, days to the first flower, days to 50% flowering, electrolyte leakage, whitefly incidence, thrips incidence, and transpiration. Additionally, another broad cluster grouped all growth, development, and postharvest

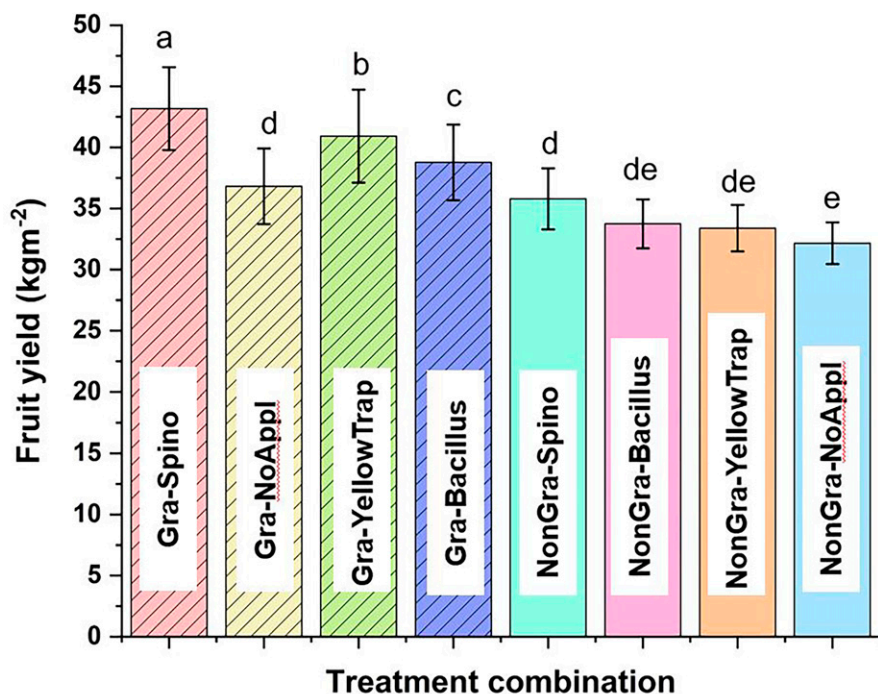


Fig. 8. Effects of grafting and bioprotection strategies on fruit yield of tomato. Vertical bar represents the SE. Bar graphs with dissimilar letters are statistically different, whereas bars with the same letter are statistically similar according to Tukey's honestly significant difference test at  $P < 0.05$ . Location: AGRICO Organic Farm in Al-Khore, Qatar.



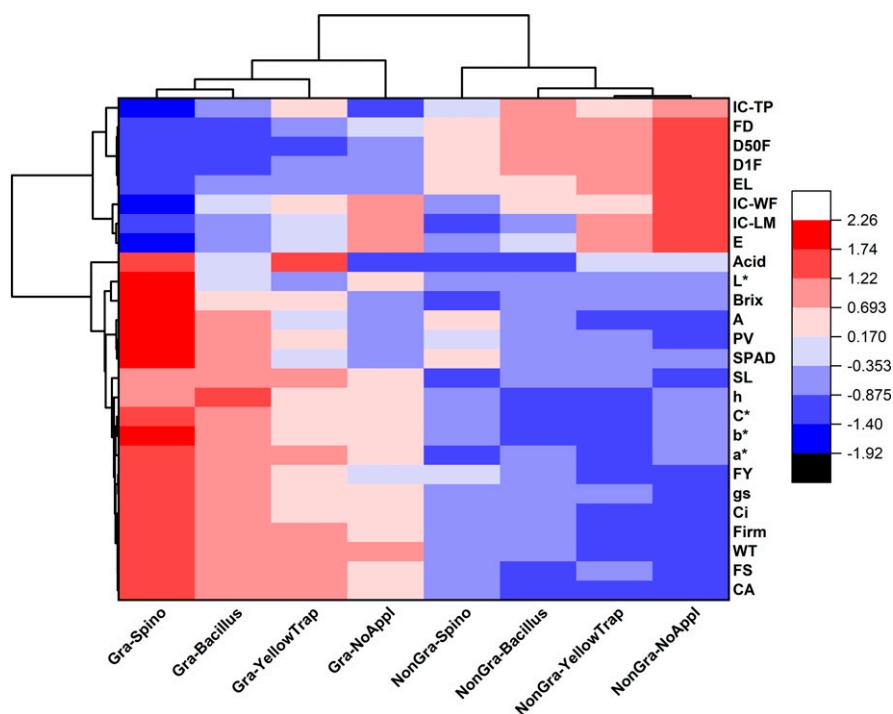


Fig. 10. Heatmap and clustering of grafting and bioprotection strategies based on the scaled values of the measured variables attained under greenhouse environments. Each column represents a combination of grafting and bioprotection strategies, and each row indicates a measured variable. Treatment combinations are clustered based on their measured variables and variable groups are clustered based on their correlation. The variables that are clustered together have a high positive correlation. Red and blue cells have high and low relative appearances, respectively.

independent genetic mechanisms because no specific quantitative trait loci were identified for these effects in grapevines. Dash et al. (2023) also noted that grafted plants reduced transpiration loss under abiotic stress conditions. Mauro et al. (2020) found that the tomato grafting combination ('Dreamer' × 'Maxifort') exhibited dominant growth and reduced leaf electrolyte leakage compared with those of nongrafted or self-grafted plants, particularly under root hypoxia conditions.

In our study, grafted tomato plants treated with Spinosad had accelerated flowering time because grafted plants mitigated the negative effects of transplant shock established earlier because of robust root systems and absorbed more nutrients from the growing media than the nongrafted plants. Additionally, Spinosad reduced pest pressure, thus helping to maintain plant vigor. Penella et al. (2017) reported that grafted plants accumulated nutrients faster than the nongrafted plants. Meyer et al. (2017) found that a combination of grafting and 50% leaf removal resulted in a higher flower count compared with that of nongrafted tomatoes, suggesting that this combination influences flowering timing. Mahbou et al. (2022) noted that grafted tomato plants flowered 4 d earlier than nongrafted plants. Similarly, Dash et al. (2023) reported that grafted plants ('Maxifort F1' × 'Velocity F1') established faster by mitigating abiotic stresses, thereby accelerating physiological and phenological activities and resulting in flowering 3 d earlier.

Abiotic stresses reduce the pollen viability of tomatoes and other vegetable crops, and they are a critical indicator of the ability of a plant to undergo flowering events such as pollination, fertilization, seed, and fruit development (Halo et al. 2023). Extreme heat disrupts several physiological and biochemical processes in plants, leading to flower drop, poor flower set, and, consequently, lower fruit yield (Osei-Bonsu et al. 2022). Alsamir et al. (2021) noted that extremely high temperatures not only reduce flower and fruit set but also affect fruit development and maturity. Strong root systems in grafted plants may enhance hormone levels, resulting in better physiological responses in tomatoes (Som and Madhava 2013). The improved flower and fruit setting, reduced flower drop, and higher pollen viability in grafted plants suggested that they performed well under abiotic stress, thus supporting proper growth and development.

In this study, the use of Spinosad, *Bacillus thuringiensis*, and yellow sticky traps significantly reduced leafminer, whitefly, and thrips infestations in tomatoes, respectively. Erasmus et al. (2023) highlighted the effectiveness of Spinosad against *T. absoluta* larvae in tomato seedlings, with foliar applications causing 96% mortality of second instar larvae through rapid dissolution of spinosyn A and spinosyn D via photolysis. Cabra and Fernandez (2019) reported that in Colombia, *Bacillus thuringiensis* effectively induced significant mortality of whitefly *B. tabaci* on tomatoes. Salazar-Magallon et al. (2015) found that *Bacillus*

*thuringiensis* (40 mg·mL<sup>-1</sup>) caused up to 90% mortality of the second and third instar *B. tabaci* under controlled greenhouse conditions. Al-Shayji and Shaheen (2008) also demonstrated the effectiveness of *Bacillus thuringiensis* against whitefly nymphs at a concentration of 500 µg·mL<sup>-1</sup>, achieving mortality rates up to 68.2%. The toxicity of *Bacillus thuringiensis* against whiteflies is linked to the number of *Cry* proteins expressed, as noted by Cabra and Fernandez (2019). Nair et al. (2021) found that yellow rectangular sticky traps were the most effective for managing aphids and whiteflies on tomatoes in protected conditions. Murtaza et al. (2019) similarly found that yellow sticky traps were highly effective for monitoring and controlling thrips and whiteflies in cotton crops. Overall, sticky traps were effective against sucking-type insects such as whiteflies and thrips.

The finding that grafted tomato plants yield more fruit than nongrafted plants aligned with those of previous studies. Kalozoumis et al. (2021) reported that grafted tomato plants ('M82' × 'Belladonna F1') produced 11% more fruit than nongrafted ones. Similarly, Dash et al. (2023) found that grafted tomatoes ('Maxifort F1' × 'Velocity F1') had 26% higher marketable fruit yields compared with those of nongrafted plants. Grafted tomatoes demonstrated vigorous growth, suppressed soil-borne diseases, and improve yields in both greenhouse and field conditions (Kubota et al. 2008). Selecting appropriate rootstock and scion combinations is crucial for maximizing growth, yield, and fruit quality (Aloni et al. 2010). For instance, using 'Maxifort F1' rootstock increased marketable fruit yield by more than 50% compared with that of nongrafted plants (Djidonou et al. 2017). Similarly, grafted seedlings ('MS-150' × '0301111') boosted yield by 45% over that of nongrafted seedlings (Zhang et al. 2021), consistent with the observations of Kunwar et al. (2017). In organic systems, grafting improved fruit weight by 12%, fruit number by 22%, and marketable yield by 43% compared with those of nongrafted plants (Moreno et al. 2019). Latifah et al. (2023) also noted that grafted tomatoes 'Cervo' and 'Timoty' produced 30% more marketable fruits than their nongrafted counterparts.

In our study, the interaction effects of grafting and bioprotection strategies had a significant impact on the postharvest quality of tomatoes, particularly shelf life, fruit weight, and firmness. Abu Glion et al. (2019) found that the tomato grafting combination 'Lorka' × 'Register' resulted in better improvements in fruit shelf life and other quality parameters, likely because of the appropriate selection of scion and rootstock, thus enhancing the hormonal balance of the plants and optimizing source-sink relationships, as noted by Aloni et al. (2010). Latifah et al. (2023) reported that fruits from grafted 'Cervo' and 'Timoty' tomato plants had 10% longer shelf life and 20% greater firmness compared with those of nongrafted plants, supporting our findings. The combination of grafting ('Maxifort' × 'Optima F1') and 50% shading increased marketable yields and maintained storage quality by enhancing the firmness, micronutrients (Fe, Zn),

macronutrients (Ca), higher malic acid content, and by reducing the sugar content (Milenkovic et al. 2020). After 21 d of storage, tomatoes from grafted plants were firmer than those from nongrafted plants (Dash et al. 2023), corroborating our results. However, although Krumbein and Schwarz (2013) found that the titratable acid content increased by 9% in tomatoes grafted with 'Maxifort' × 'Classy' and maintained under 50% shade, our study did not observe significant changes in acid content caused by grafting. The increase in titratable acid content attributable to grafting suggested that the grafting combination of rootstock and scion played a crucial role in determining fruit quality. Additionally, biocontrol agents such as *Bacillus* sp., known for strong biofilm-forming abilities, can protect tomatoes from pathogens and extend shelf life when applied to the fruit surface (Cheng et al. 2023). However, the results indicated that grafting and bioprotection strategies did not significantly impact the postharvest quality attributes of stored tomatoes. The average values recorded for the stored tomato fruits were as follows: soluble solids content, 3.6%; acidity, 1.1%; L\*, 39.2; a\*, 22.1; b\*, 24.3; C\*, 33.1; and hue angle, 48.2.

The PCA, heatmap, and cluster analyses highlighted the key variables that affect tomato growth, physiology, phenology, yield, postharvest quality, and insect incidence. The heatmap and dendrogram support the PCA findings by clustering variables based on similarity indices. Variables such as flower drop, transpiration rate, electrolyte leakage, time to first flowering, time to 50% flowering, and insect incidence showed patterns in contrast to those associated with fruit set, pollen viability, stomatal conductance, assimilation rate, SPAD value, canopy area, fruit yield, and postharvest quality. This study of tomato grafting and bioprotection strategies revealed their complex impacts on growth, physiology, phenology, yield, postharvest quality, and insect incidence in organic hydroponic tomato production, as illustrated in the PCA and heatmap analyses.

Previous reports highlighted organic hydroponic vegetable production as a key solution for food security and safety in growing populations, especially in arid climates. Qatar and similar countries face challenges in meeting domestic vegetable demand because of adverse climatic conditions. Optimal hydroponic management using grafted tomato plants such as the 'Maxifort' × 'Valdeon RZ' combination and bioprotection strategies with 'Spinosad' at 0.4 mL·L<sup>-1</sup> can significantly reduce greenhouse pest pressures. These hydroponic strategies offer a promising pathway to enhance the agricultural sector, particularly for organic farmers who aim to increase long-term farm income, and serve as a model for other organic hydroponic farms.

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