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Grafting and Inoculation of Grafted Plants With *Bacillus amyloliquefaciens* Enhances Biotic and Abiotic Stress Tolerance in Organic Tomato

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Abstract

Biotic and abiotic stress factors pose significant challenges to organic tomato production in Alabama and the southeastern United States. High temperature, drought, soil, and foliar-borne diseases have rendered it exceedingly difficult for organic tomato growers in Alabama to produce profitable crops. Our objective was to evaluate plant response to imposed biotic (*Verticillium dahliae*) and abiotic (drought) stress in the popular tomato variety 'Roma' through grafting unto the resistant rootstock 'Maxifort', and the incorporation of the plant growth-promoting rhizobacterium (PGPR) *B. amyloliquefaciens*, to mitigate challenges posed by these stress factors. The research was conducted in a greenhouse in north Alabama where both soilborne pathogens (*V. dahliae*) and drought stress treatments were applied. The experimental design was a split plot with 4 main plots (Grafting, PGPR, Grafting + PGPR, and Control) and 3 subplots (drought, pathogen, and control) treatments with 4 replications. *B. amyloliquefaciens* was applied in the rhizosphere of grafted and non-grafted plants at 1×10^8 CFU/ml/plant. Pathogen-treated plants were also inoculated with *V. dahliae* at 1×10^5 propagules/ml. The study revealed that grafting, and grafting + *B. amyloliquefaciens* caused a significant increase in stem girth, plant biomass, early flowering, and fruiting of tomatoes compared to the non-grafted and control treatments. Integrating grafting and PGPR could be beneficial in enhancing plant resilience and performance under biotic and abiotic stress conditions.

Keywords: abiotic and biotic, grafting, plant growth promoting rhizobacteria, rootstock, stress tolerance

1. Introduction

Tomato is the third most important vegetable in the world after potatoes. There is an assortment of substances found in tomatoes (*Solanum lycopersicum*) that may be good for human health (Atkinson et al., 2011). It is a major component of diet and nutrition on a global scale. Vegetable production in Alabama and the southeastern US is subject to environmental challenges of drought, high temperatures, and humidity during the summer. It is expected that as climate change advances, biotic and abiotic stressors will proliferate and intensify more, increasing the likelihood that crops will be subjected to both kinds of stress (Kissoudis et al., 2015). Abiotic stresses like drought, salinity, and heavy metal contamination have also been shown to pose a threat to tomato production, in addition to a host of biotic obstacles like diseases spreading through the soil and foliage. Tomato crops with restricted access to water and/or nutrients have found improvement with both grafting and PGPR inoculation (Kalozoumis et al., 2020). In recent years, these conditions have been exacerbated by climate change effects thus necessitating the implementation of novel cultural practices to mitigate these effects on crop growth and production. Vegetable grafting started in the 1920s with the use of resistant rootstocks to manage soilborne diseases (Davis et al., 2008). According to Naik et al. (2021), an essential technique for reducing the dangers of intensive vegetable production systems such as soilborne diseases is grafting. Grafting works best as a redox homeostasis (maintaining the balance of oxidized and reduced biomolecules in the plant system) in integrated pest management method to control biotic stress when it is used in conjunction with sustainable

agricultural system practices and with a growing understanding of the biology, diversity, and population dynamics of the pathogen or other pests (Louws et al., 2010). Soare et al. (2018) indicated that grafting had a positive effect on growth and production traits. Grafting using specific rootstocks can improve growth and yields in tomato production in addition to managing soilborne diseases (Djidonou et al., 2013). Grafting tomatoes augments marketable fruit yields and generates large gross returns that cover the expected cost of using grafted seedlings. Plant growth-promoting rhizobacteria (PGPR) are a diverse group of bacteria located in the rhizosphere, on root surfaces, and associated with roots (Moustaine et al., 2017). Plant growth regulators are produced at the root interface after inoculation with plant growth-promoting rhizobacteria (PGPR). The regulators promote root development and improve the uptake of water and nutrients from the soil (Sharafzadeh, 2012). According to Cordero et al. (2018), in salinity-affected soils, inoculation with specific PGPR is a practical, affordable, and ecologically friendly substitute for chemical fertilization. PGPR defense appears to be mediated via increase in proline production, enhanced activities of antioxidant enzymes, stimulation in the activities of protease and polyphenol oxidases, increased contents of phenolics, protein and chlorophyll. PGPRs decreased oxidative stress caused by cadmium (Cd) in *L. esculentum* seedlings by modifying the expression of antioxidative defenses, as shown by both quantitative and qualitative antioxidant measurements (Khanna et al., 2018). The formulation of biopesticides involving PGPR comprises an environmentally friendly and sustainable approach to overcome insect infestation (Muqarab & Bano, 2017). According to Gashash et al. (2022), the combined application of *B. subtilis* and *B. amyloliquefaciens* positively influenced tomato production, leading to an increase in fruit yield. It was observed that grafting using particular rootstocks and applying PGPR boost plant resilience and tolerance to both abiotic and biotic stresses and promote plant growth and development in musk melon (Zhao et al., 2017). The objective of this study was to integrate grafting with the application of plant growth-promoting rhizobacteria to alleviate biotic and abiotic stress in greenhouse tomato production.

2. Materials and Methods

2.1 Plant Material

Tomato seeds of the resistant hybrid variety ‘Maxifort’ used as the rootstock were obtained from Johnny’s Selected Seeds (Winslow, ME), and the susceptible heirloom variety ‘Roma’ used as scion shoot was purchased from C.T. Garvin Seed and Feed (Huntsville, AL) and germinated in Promix BX (Premier Horticulture Inc., Mattaponi, VA) soilless media in a greenhouse in north Alabama.

2.2 Grafting

A susceptible heirloom tomato variety, ‘Roma’ was used as the scion and was grafted onto a resistant ‘Maxifort’ hybrid variety used as rootstock. The plants were grafted using the Top grafting method (Johnny’s Selected Seeds, 2021) and placed in a healing chamber at 21-26 °C and 85-95% RH and total darkness for 5 days followed by acclimatization (gradual exposure to increasing light intensity) in the open greenhouse until new growth (leaves) was evident.

2.3 Transplanting of Grafted Seedlings

Three-part field soil and one-part soilless media (Promix BX) were mixed, autoclaved at 121 °C and 1.1 kg/cm^2 for 40 min, cooled and potted in 1-gallon pots, followed by seedling transplant. The field soil, a Decatur silty clay loam (Clayey, Kaolinitic, Thermic Rhodic Paleudults), was collected from an organically managed field plot for vegetables, on fallow at Alabama A&M University’s Winfred Thomas Agricultural Research Station (WTARS). The tomato plants were supported by 1 m tall bamboo stakes and were fertilized with composted poultry manure (Back to Nature^{TM}) providing 45 kg N/ha.

2.4 Plant Inoculations

The microorganisms used in the study were obtained from the American Type Culture Collection (ATCC, Manassas, VA). They included the plant growth-promoting rhizobacterium (PGPR) *Bacillus amyloliquefaciens* strain BAA-390 and the soilborne fungal pathogen *Verticillium dahliae*. *B. amyloliquefaciens* was grown on nutrient agar and nutrient broth at 30 °C for 48 hours, followed by centrifugation at 7000 rpm and resuspension of the pellet in 0.01% saline. *V. dahliae* was grown on potato dextrose agar for 14 days at 26-28 °C under white light.

After graft union was fully established, *B. amyloliquefaciens* strain BAA-390 was inoculated at the rhizosphere of grafted and non-grafted plants at 1×10^8 CFU/ml/plant, and 7 days later *V. dahliae* were also applied in the rhizosphere of the designated plants at 1×10^5 propagules/ml. The propagules consisted of microsclerotia and mycelia fragments from 14-day-old cultures.

2.5 Experimental Design, Data Collection, and Analyses

The experimental design was a split-plot design with 4 main plots (Grafted, Grafted + *B. amyloliquefaciens*, *B. amyloliquefaciens*, and Control) and three subplots (drought, pathogen, and control) treatments. Plants in the drought sub-treatment were watered with 500 ml/pot (1 gallon) with tap water every two days throughout the duration of the experiment. The measurement of total solids content of fruits (TSC) was done by first weighing the fruits, drying the sample (70 °C for 4 days) to remove all water, and then weighing the remaining dried material. The percentage of TSC was calculated by comparing the weight of the dried solids to the original weight of the fresh sample (USDA, 1970):

$$\text{Total Solids Content (\%)} = (\text{Wet Weight/Dry Weight}) \times 100 \quad (1)$$

Experimental data collected including stem girth, early flowering (number of flowers/plant), number of fruits/plants, total solids content and fruit weight, measurements were subjected to the analysis of variance (ANOVA) procedure of the general linear model (GLM) in SAS statistical package (SAS 9.4). Treatment means were separated using the Tukey Kramer multiple comparison test at $P \leq 0.05$.

3. Results

Greenhouse research focused on evaluating the effects of four main treatments on plants: Grafted, PGPR (Plant Growth-Promoting Rhizobacteria), Grafted + PGPR, and a Control group, under three sub-treatments or stressors: Drought, Pathogen, and Control. The combined Grafted + PGPR treatment showed a highly significant improvement in plant performance (stem girth, number of early flowers/plant and marketable fruit weight) ($P < 0.05$) compared to plants treated with only PGPR and the untreated Control group. This suggests that the synergy between grafting and PGPR may offer enhanced resilience or benefits to plants under stressful conditions.

3.1 Stem Girth

We observed that grafted tomato plants had bigger and sturdier stems. The stem girth was measured at a height of 4cm to 5cm from the soil surface. The Graft and PGPR + Graft treatments significantly ($P \leq 0.05$) increased stem girth compared to the Control and PGPR alone (Figure 1). The application of grafting techniques appears to enhance the stem diameter of vegetable plants.

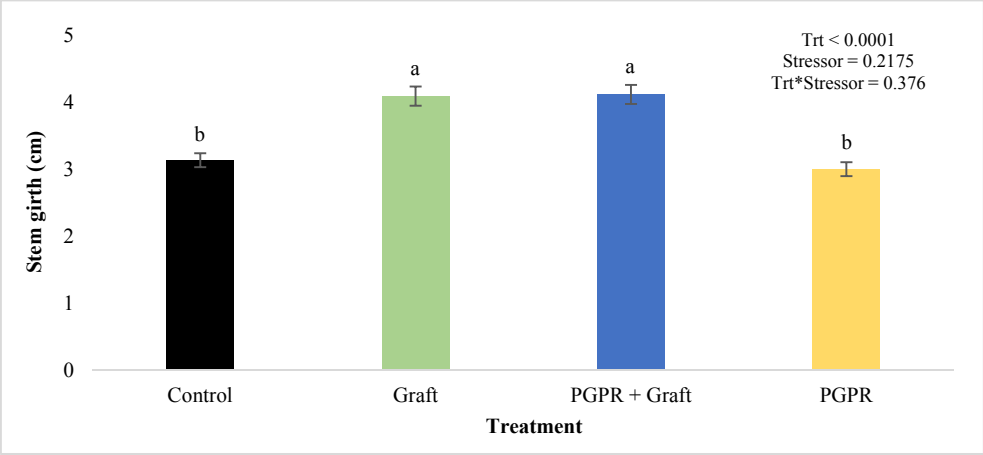

Figure 1. Stem girth of all the tomato plants in the greenhouse

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.2 Plant Height

Plant height was measured from the base of the stem to the tip to determine the impact of the stress treatments. Plants under drought stress were significantly shorter than the control and pathogen stress treatments (Figure 2).

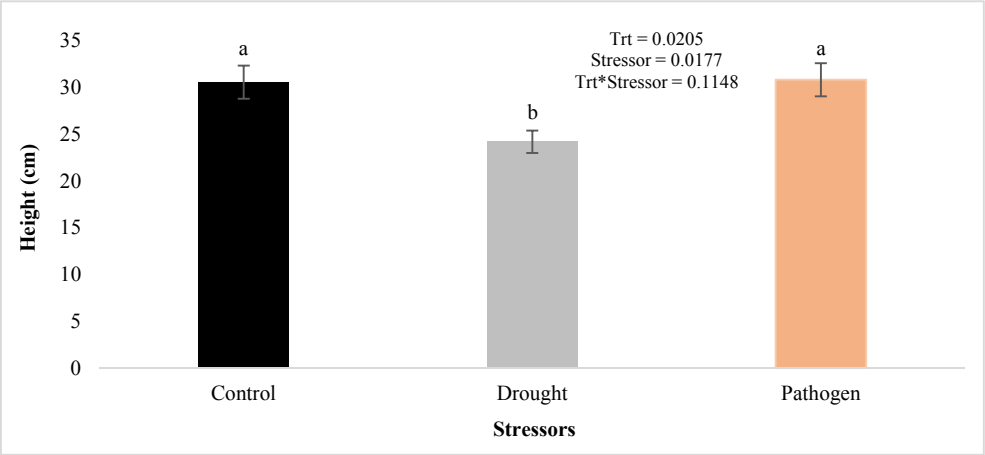

Figure 2. Tomato plant height is influenced by the subplot stress treatments in the greenhouse

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.3 Flowering

The Graft + PGPR treatment significantly ($P \leq 0.05$) increased the number of flowers/plants compared to the Control and PGPR treatments (Figure 3). The flowering in tomatoes is directly correlated with fruit production, meaning that more blooms will lead to more fruits being produced. In this study, we demonstrated that grafting and PGPR work together to improve tomato plants' early flowering leading to fruit production.

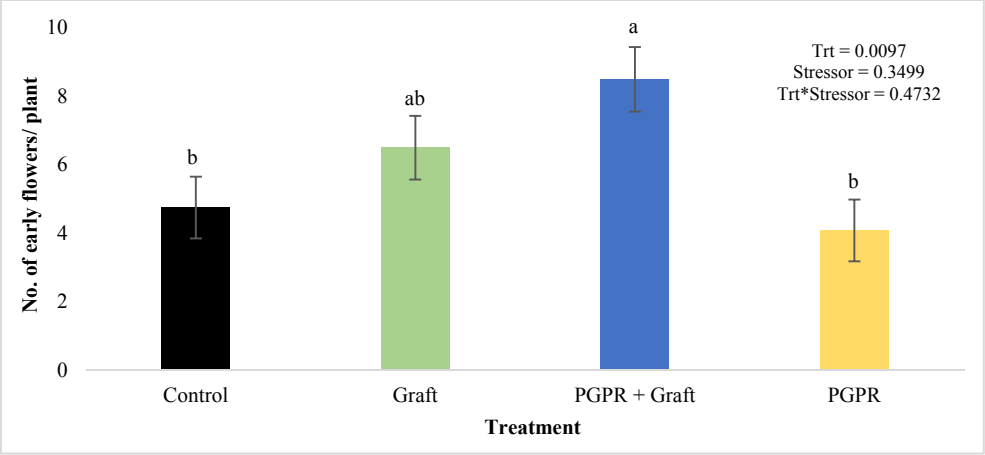

Figure 3. Early flowering of tomato plants

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.4 Fruiting

There were no significant differences between the treatments for fruit production per plant (Figure 4). Fruit production did not follow the same trend in the various treatments as the early flowering because fruits were assessed only at harvest. The PGPR + Graft treatment increased the number of fruits produced per plant more than other treatments.

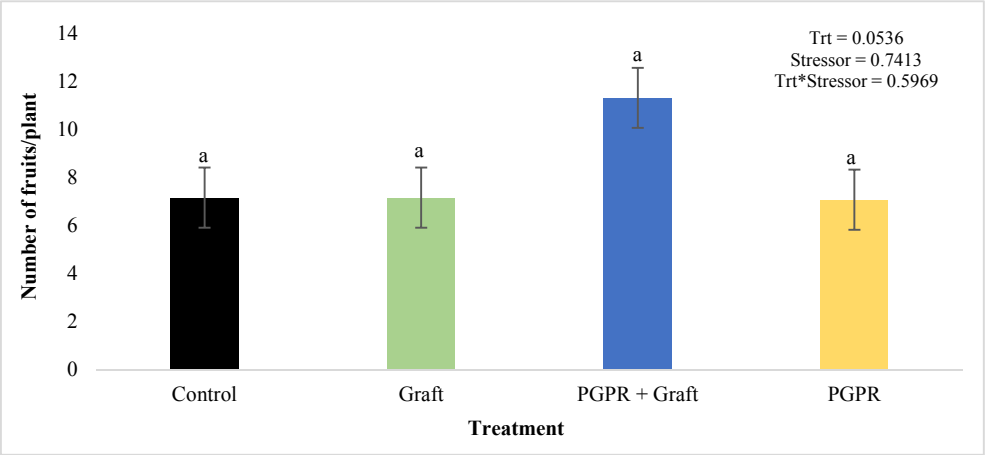

Figure 4. Tomato fruit count per plant

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.5 Fruit Weight

The average fruit weight per plant was assessed in the study, with a particular emphasis on comparisons between drought stress, control, and pathogen conditions. The average fruit weight significantly ($P \leq 0.05$) decreased under drought stress (Figure 5). The assignment of the letter 'b' to represent the Drought condition and 'a' for both the Control and Pathogen conditions further indicated this disparity in outcomes. This difference highlights how negatively drought stress affects fruit weight and how sensitively plants respond physiologically to water scarcity compared to other stressors like exposure to pathogens in this research.

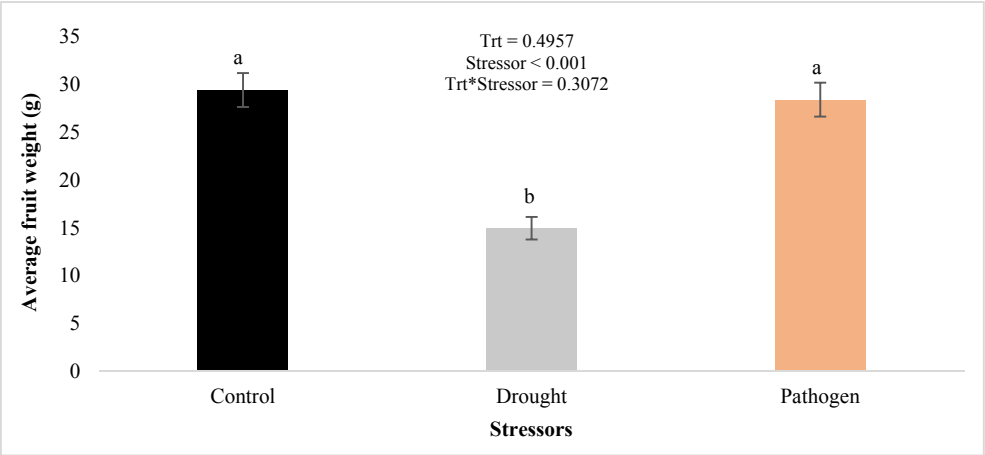

Figure 5. Tomato fruit weight in response to imposed stress factors

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly ($P \leq 0.05$) different according to Tukey's test.

3.6 Plant Biomass

The imposition of drought stress treatment on tomato plants led to a significant reduction in plant biomass (Figure 6). However, inoculation of plants with *V. dahliae* had no significant effect on plant biomass compared to the control.

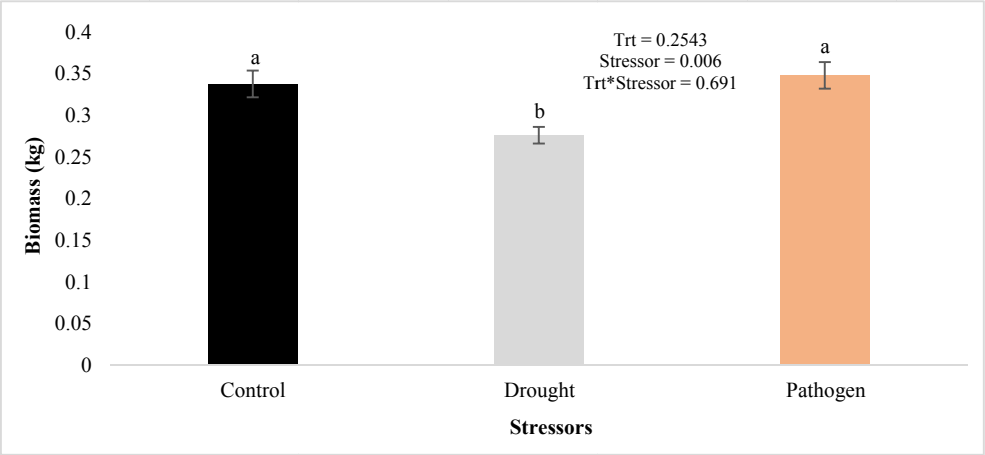

Figure 6. Biomass of tomato plants grown in the greenhouse

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.7 Marketable Fruits

Tomato fruits were categorized based on size to evaluate their marketability. Sizes ranged significantly, with the smallest and largest fruits weighing approximately 7.4 grams (drought treatment) and 55.7 grams respectively in the greenhouse. According to the established criteria, larger fruits were designated as marketable and suitable for retail. Conversely, smaller fruit sizes are designated for processing into various tomato-based by-products, optimizing the utilization of the entire harvest.

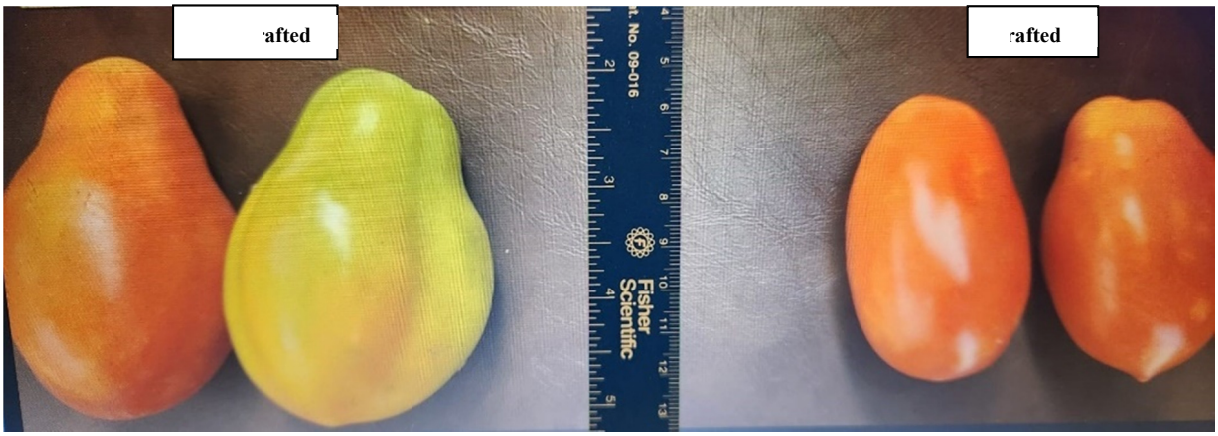

Figure 7. Fruit size range of tomato fruits harvested from grafted and non-grafted plants

Fruits obtained from grafted plants treated with *B. amyloliquefaciens* (Grafted + PGPR treatment) were the heaviest, and thus potentially the most marketable based on weight, compared to the fruits from other treatments.

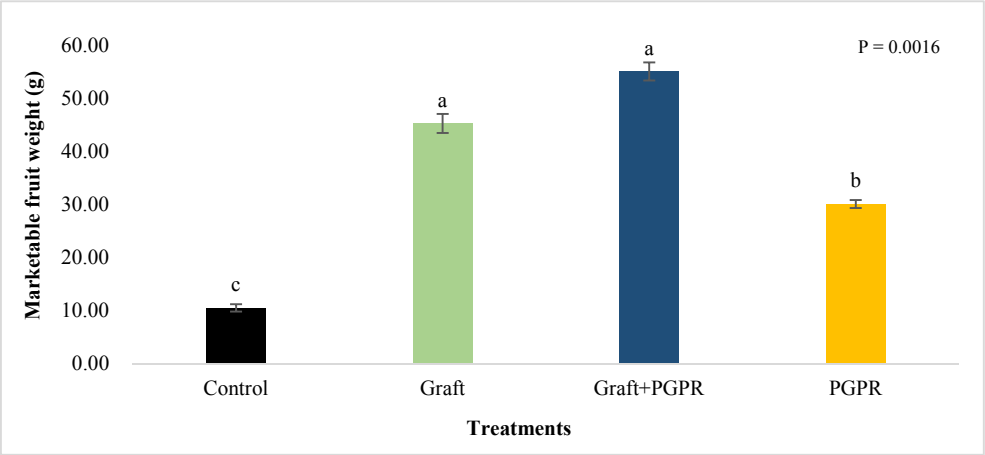

Figure 8. Marketability of fruits from tomato plants grown in the greenhouse

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey's test.

3.8 Total Solids Content

Total solids content in tomato fruits was determined post-harvest and was stratified by the distinct treatment variables utilized throughout the study. This is the percentage of the total solid content of tomato fruits across different treatments (Control, Grafted, PGPR, Grafted + PGPR) under normal, pathogen, and drought conditions.

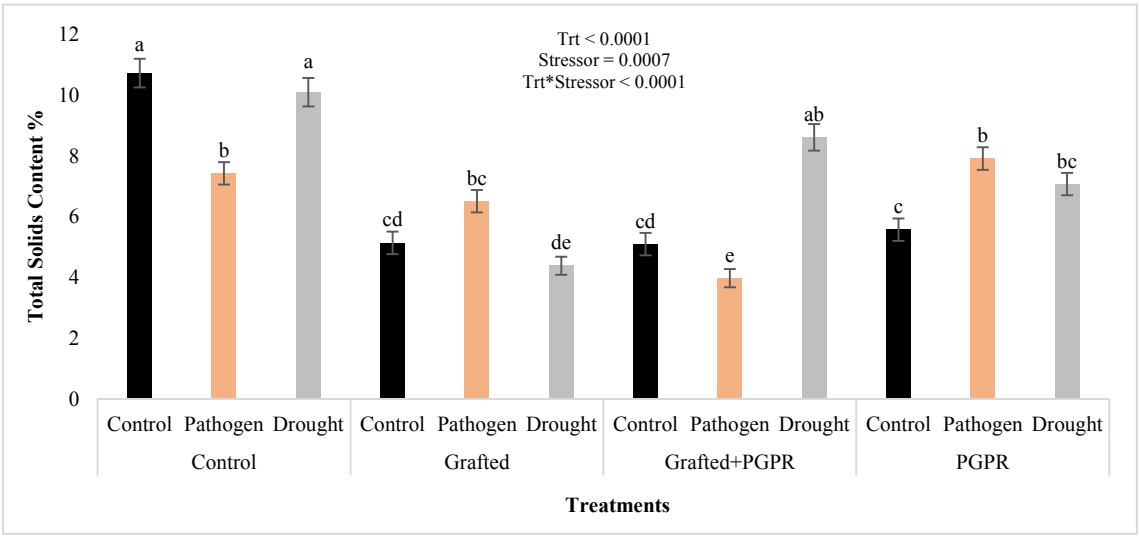

Figure 9. Total Solids Content of tomato fruits grown in the greenhouse

Note. Values (bars) are presented as the mean±SE. Means with the same letter are not significantly ($P \leq 0.05$) different according to Tukey's test.

3.9 Catalase Activity

An increase in catalase activity in treatments including Graft, Graft + PGPR and PGPR under pathogen and drought stresses was observed (Figure 10). This study demonstrates how grafting in conjunction with plant growth-promoting rhizobacteria (PGPR) and PGPR alone can strengthen plants' defensive mechanisms against drought and pathogenic stress. These increases in enzyme activity point to a strong physiological response which may enhance plant health and resilience under challenging environments.

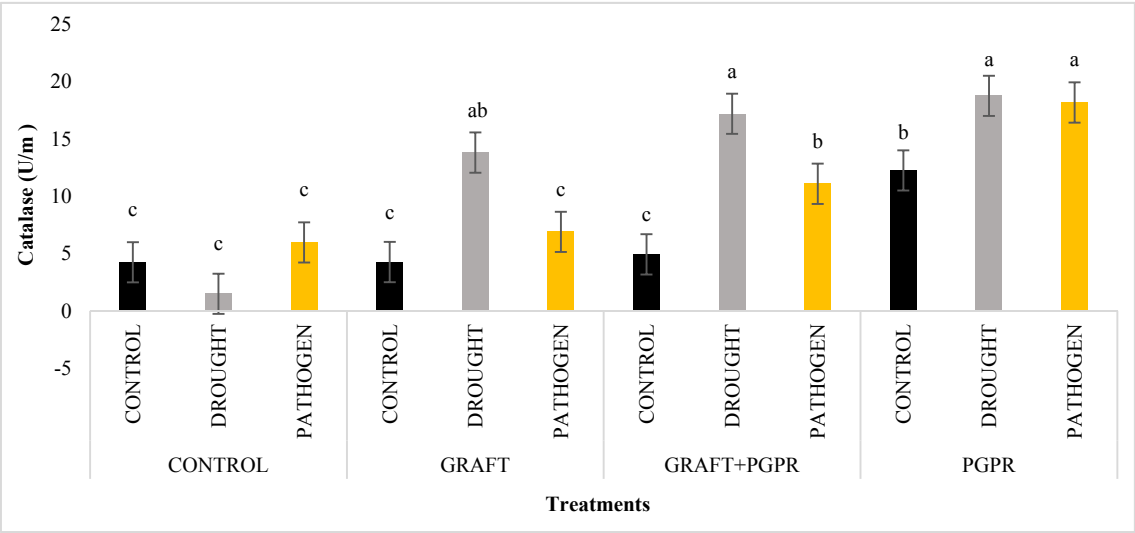

Figure 10. Effect of main (Graft, Graft + PGPR, PGPR, and Control) and sub (Control, Drought, Disease) treatments on the expression of catalase activity (U/ml)

Note. Values are means±SE. Means (bars) with the same letter are not significantly (P ≤ 5%) different according to Tukey's test.

4. Discussion

This study utilized grafting techniques alongside plant inoculation with *B. amyloliquefaciens* to effectively mitigate the effects of biotic (*V. dahliae*) and abiotic (drought) stresses. It was noted that grafted tomatoes exhibited significantly larger stem girths than non-grafted (Figure 1) as previously reported (Al-Harbi et al., 2017), highlighting the potential benefits of grafting in enhancing plant robustness. In this study, the synergistic effect of grafting and the inoculation of *B. amyloliquefaciens*, was the substantial enhancement in tomato crop performance. This was evidenced by significant increases in parameters such as stem girth, flowering intensity, fruit count, and fruit weight compared to control plants. However, the tomatoes that were subjected to drought stress treatment produced low fruit weight, indicating that fruit weight was significantly affected by drought (Figure 4). Similar findings were previously reported (Begum et al., 2019; Seleiman et al., 2021). Tomato plants exposed to drought conditions exhibited a statistically significant reduction in height compared to the control plants, similar to the findings reported by Imana et al. (2010).

Grafting and Graft + PGPR treatments significantly increased the number of initial flowers on the tomato plants (Figure 3). Similar observations were previously made by Khah et al. (2006). In general, grafted plants seem to produce more flowers than non-grafted ones, and these flowers naturally translate into fruits. Tomato plants subjected to PGPR treatment in conjunction with drought conditions exhibited a reduction in biomass (Figure 4) when contrasted with those that received the control and pathogen treatments. Kalozoumis et al. (2021) highlighted that in environments facing both water and nutrient shortages, the application of PGPR-T3 markedly diminished aboveground vegetative growth, underscoring the significant impact of specific PGPR strains under stress conditions. Additionally, it was observed that tomato plants inoculated with *Verticillium dahliae* in the greenhouse displayed no apparent symptoms of *V. dahliae* infection, as noted by Rahou et al. (2022).

The fruits with the highest marketability (Figure 8) were those from the Grafted and Grafted + PGPR treatments, as these treatments enhanced fruit weight. According to Mavlyanova et al. (2020), employing grafting techniques across various varieties based on the rootstock significantly enhanced production by 16–20% and improved fruit marketability by 97–100%. Furthermore, the tomato plants subjected to Grafting and PGPR + Grafting treatments exhibited resilience, showing no disease infection. Rivard and Louws (2008) reviewed that grafting served as a potent strategy for organic farmers in the southeast United States to mitigate the risk of crop failure due to soilborne diseases and represents an essential element of an integrated pest management system.

The employment of grafting techniques and Plant Growth-Promoting Rhizobacteria markedly enhanced catalase activity under drought conditions. *B. subtilis*, a specific strain of PGPR, activated antioxidant processes in grafted plants (Padro et al., 2022). This indicates that inoculation with this growth-enhancing bacterium may offer a biotechnological strategy to enhance the efficacy of tomato grafting as suggested by Padró et al. (2022).

The varied effects of the sub-treatments—drought, pathogen exposure, and control, can be used to explain the different levels of disparity observed across treatments and sub-treatments in the greenhouse. Fruits from plants that were exposed to drought and the control treatment had a higher Total Solids Content than fruits from grafted plants alone and in conjunction with *B*. *amyloliquefaciens*. These results corroborated those of Quadir et al. (2006) who found that management practice and environmental factors had a major impact on TSC in fruits produced in the greenhouse.

The Catalase enzyme analysis was used to determine the physiological effects of grafting and *B*. *amyloliquefaciens* on plants. Such enzymatic analysis may assist in identifying oxidative stress responses in plant tissues which in turn offers valuable information about the metabolic well-being of the plants that are exposed to different treatments and sub-treatments. The ability of the enzyme to break down hydrogen peroxide, a frequent consequence of plant metabolism that can harm cells if not properly regulated, was specifically assessed using the catalase activity. This approach may shed light into the biochemical pathways affected by grafting and PGPR, as well as the assessment of the effectiveness of these treatments in strengthening the plants' defense mechanisms against oxidative stress. Catalase activity (Figure 10) in the PGPR, Grafted + PGPR, and Grafted was significantly greater than in the control, and this cuts across all three sub-treatments within the main treatments. Similar findings were previously reported by Srivalli et al. (2003).

An essential biochemical assay for determining a plant's ability to withstand oxidative stress and to detoxify hazardous compounds is the Glutathione S-Transferase (GST) test which was carried out on tomato leaves. Due to their ability to catalyze the conjugation of the antioxidant glutathione to a variety of endogenous and exogenous hazardous chemicals and heavy metals which increases their water solubility and facilitates their excretion, GST enzymes are essential components of the detoxification process (Csiszár et al., 2014). Detectable levels of Glutathione S-Transferase (GST) activity were found in the plant tissue, however, GST assay conducted on tomato leaves did not show statistically significant values suggesting that GST was not upregulated in the stress treatments.

5. Conclusion

Grafting and further inoculation of grafted plants with *B*. *amyloliquefaciens*, was beneficial in enhancing fruit size, weight, and quality of tomatoes and providing protection against soil-borne pathogens like *Verticillium dahliae*, and drought. For both conventional and organic tomato growers, grafting tomatoes onto disease-resistant rootstocks is essential since it increases vigor and stress tolerance. This study reveals the benefits of grafting in, growing tomatoes even under biotic and abiotic stress conditions and promoting sustainable vegetable production in southeastern US. Grafting and application of *B*. *amyloliquefaciens* will be advantageous if incorporated in organic tomato production system or as an integrated disease management tool in sustainable tomato production systems. Future work will entail evaluating more PGPR strains to identify ecologically adapted and effective strains that can be harnessed for greenhouse tomato production.

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Authors Contributions

Dr. Nyochembeng was responsible for the study design and revising. Dr. Ogunkunle was responsible for biochemical analyses. Ms. Adeniyi was responsible for conducting the experiments, data collection, analyses and drafting the manuscript. Dr. Hopkinson revised it. All authors read and approved the final manuscript and contributed equally to the study.

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Data Sharing Statement

No additional data are available.