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Article

# Grafting Eggplant Onto Underutilized *Solanum* Species and Biostimulatory Action of *Azospirillum brasilense* Modulate Growth, Yield, NUE and Nutritional and Functional Traits

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**Abstract:** The grafting of vegetable crops is considered a valuable mean for ensuring the yield and quality under different cultivation conditions. Simultaneously, there are increasing research efforts in exploiting underutilised plants as potential rootstocks for vegetables to increase the sustainability of horticultural systems. In accordance with the European Green Deal, the application of biostimulants is a fashionable and ecological agronomic practice to enhance the production and quality of vegetables. Thus, the current research appraised the synergistic effect of grafting eggplant onto various allied potential rootstocks (*Solanum torvum*, *S. aethiopicum* and *S. macrocarpon*) and of applying a plant growth-promoting bacteria (*Azospirillum brasilense* DSM 2298) on eggplant growth, production, fruit quality traits (nutritional and functional features) and nitrogen use efficiency (NUE). The findings showed that 'Gloria' $F_1$ plants grafted onto *S. torvum* or *S. aethiopicum* had a significant increase in plant height 50 DAT by 11.6% and 9%, respectively, compared with not grafted plants. Simultaneously, plants inoculated with *A. brasilense* DSM 2298 acquired a significant upsurge of plant height 50 DAT by 6% compared with the control. Our results revealed that *S. torvum* and *S. aethiopicum*-grafted plants improved their marketable yield by 31.4% and 20%, respectively, compared with not grafted ones. Furthermore, *A. brasilense* DSM 2298 significantly boosted the yield compared with the control plants. Plant type had no effect on fruit dry matter and firmness, whereas plants grafted onto *S. macrocarpon* showed a significant increase in the soluble solids content (SSC) and fruit K concentration compared with not grafted plants. Plants grafted onto *S. torvum* rootstock and inoculated with *A. brasilense* DSM 2298 had a significant increase in fruit protein concentrations compared with the combination not grafted × control. Moreover, *S. torvum*-grafted plants and those inoculated with the microbial biostimulant revealed the highest NUE values. The results evidenced that *S. torvum* and *S. macrocarpon*-grafted plants, inoculated with *A. brasilense* DSM 2298, had the highest ascorbic acid (average 7.33 and 7.32 mg 100 $g^{-1}$ fw, respectively). Interestingly, *S. torvum* rootstock increased the chlorogenic acid concentration and reduced the glycoalkaloids concentration compared with not grafted plants. Our data also showed that *A. brasilense* DSM 2298 significantly increased SSC by 4.5%, NUE by 5.5%, chlorogenic acid concentration by 2.0% and the total anthocyanins by 0.2% compared to the control. Thus, our study underlined that *S. aethiopicum* rootstocks inoculated with *A. brasilense* DSM 2298 could represent a valid substitute to the common *S. torvum* rootstock.

**Keywords:** *Solanum melongena*; *S. torvum*; *S. aethiopicum*; *S. macrocarpon*; microbial biostimulant; PGPB

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## 1. Introduction

Three eggplants species attracted human attention and were domesticated [1]. Among them, the most significant is the common eggplant (*Solanum melongena*), whose first cultivation started in Southeast Asia [2], whereas *S. aethiopicum* (scarlet eggplant) and

*S. macrocarpon* (gboma eggplant) are the other species, which were domesticated in Africa [3]. Scarlet and gboma eggplants are less notorious than common eggplant and, as reported by Schippers [4], are frequently grown in sub-Saharan Africa and sporadically in other world locations, such as Southeast Asia. Noteworthy, Sunseri et al. [5] reported that scarlet eggplant is also cultivated in the Caribbean, Brazil and in a few areas of Southern Italy. Taher et al. [6] declared that, in line with the AVGRIS and Genesys databases, there are 6632 accessions of cultivated eggplants preserved in gene banks (5665 *S. melongena*, 798 *S. aethiopicum* and 169 *S. macrocarpon*). As concerning wild eggplant relatives, among the 1304 accessions deposited in germplasm banks, *S. incanum* is the most representative species (167 accessions), followed by *S. torvum* (132 accessions).

Within the Mediterranean Basin, Sicily represents one of the most important centres of out-of-season cultivation, and it is considered an important area of eggplant ecotypes conservation [7]. Currently, due to the absence of resilient genotypes to biotic and/or abiotic distress, grafting is recognised as a valid toolbox for ensuring production and fruit quality under favourable or unfavourable grown conditions [8–13]. Currently, the vegetable plug plant sector is highly specialised. However, the higher labour and time required for propagation determined an increase in the grafted vegetable plug plant cost compared with the not grafted ones. Although grafted plantlets have a higher initial cost, they permit an increased yield and reduced cost of the control measures for soilborne diseases and pests. *Solanum torvum* is the most widespread rootstock for *S. melongena*, as it permits to overcome different soilborne diseases [14,15]. Nevertheless, other authors [16–18] have proposed wild eggplant relatives or interspecific hybrids as potential rootstocks for eggplant.

In agreement with the European Green Deal strategies, biostimulants, comprising non-microbial and microbial ones, are considered sustainable means to support the yield and quality of vegetables [19–26]. Among the microbial biostimulants, plant growth-promoting bacteria (PGPB) contain a cluster of microorganisms with an aptitude for colonising the root apparatus, the rhizosphere and the internal part of the plant tissues [27,28]. As reported by Cassan et al. [29], PGPB can provoke plant growth by inducing a number of mechanisms, such as nitrogen fixation and nitrate reductase activity. Furthermore, significant actions on hormones biosynthesis, the solubilisation of phosphate and organic plant defence were reported [30–34]. Currently, an eclectic collection of PGPB was documented, including the *Azospirillum* genus [35–37], which is a free-living genus generally established worldwide and employed in horticulture [24]. The inoculation of PGPB increases the yield of vegetables due to its capacity in improving NUE, stimulating mineral absorption and use proficiency [24]. Even though copious studies have been conducted to study the combinatorial effects of grafting and of other agronomical practices [38–40], no specified study has been performed to investigate the synergistic influences between wild and allied eggplant relatives' rootstocks and PGPB inoculation in enhancing the eggplant yield and quality.

Starting from the aforementioned consideration, the aim of the current study was to evaluate the interactive action of eggplant wild/allied relatives' rootstocks and *A. brasiliense* PGPB application on plant growth, yield and yield-related traits, fruit quality and NUE of 'Gloria' $F_1$ eggplant cultivated in a protected environment. The findings of the current research should deliver precious data on new potential eggplant rootstocks and on their responses to PGPB inoculation.

## 2. Materials and Methods

### 2.1. Experimental Location and Type of Plants

The trial was conducted for two consecutive years (2020/2021 and 2021/2022 during the fall–spring periods) at the experimental farm of the University of Palermo (SAAF), near Marsala (Trapani Province). The research was performed in a polyethylene-coated greenhouse. Five plant types (T) represented by the *solanaceous* species *S. melongena* 'Gloria' $F_1$ not grafted, self-grafted or grafted onto *S. torvum*, *S. aethiopicum* gr. *gilo* or *S. macrocarpon* rootstocks, were studied. The self-grafted plant type was comprised, because there are proofs that self-grafted plants need to be included in grafting experiments to better understand the real effects of grafting [16,17,41–43]. On 12 October 2020 (first year) and on 11 October 2021 (second year), eggplants were transplanted with a plant density of 2 plants $m^{-2}$ (1 m × 0.5 m). A data logger was installed in the greenhouse to record the maximum and minimum temperatures (Figure S1). The soil hosting the experiment was a medium-textured soil characterised by pH 7.0, 1.6% total N and 2.9% organic matter. Throughout the growing cycles, all eggplants were managed according to Tesi [44].

### 2.2. *Azospirillum brasilense* Culture Preparation and Inoculation

The *A. brasilense* DSM 2298 microorganism strain employed for this research is conserved in the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures). As reported by Keswani et al. [45], *A. brasilense* belongs to the biosafety level-1 (BSL-1, low individual and low community risk), including organisms that are non-pathogenic and declared safe for laboratory personnel and the environment.

*A. brasilense* was cultivated on an agar plate. The colony was inoculated in 100-mL Erlenmeyer flasks at a temperature of 28 °C with 10 mL of nutrient broth (NB), containing 5.0 g $L^{-1}$ of peptone and 3.0 g $L^{-1}$ of meat extract spinning at 150 rpm in a centrifuge for 24 h. After the incubation period, the bacterial culture was centrifuged (4000 rpm for 10 min); then, the supernatant was eliminated, and the pellet was mixed with NaCl (0.8%). The final optical density was about $10^9$ CFU/mL. Two millilitres of this suspension were inoculated with 120 mL of NB. The growth was established optically at 600 nm (Beckman DU730 spectrophotometer). The plants were inoculated with *A. brasilense* in two phases: during the transplanting phase (2 min of roots soaking) and 15 days after transplanting (100 mL of solution per plant). The control plants received only water.

### 2.3. Plant Development and Productive Traits

The plant growth and productive parameters were assessed for all plants. Plant heights 50 days after transplant (DAT) and the number of leaves 50 DAT were recorded. Fruits were harvested only at the commercial maturity stage, as reported by Sabatino et al. [16], and six harvests in total were accomplished. After harvest, the production was divided into marketable and unmarketable. Hence, the total production (kg $plant^{-1}$), marketable yield (kg $plant^{-1}$) and number of marketable fruits (n. $plant^{-1}$) were recorded. Furthermore, the mean mass of marketable fruits (g) was determined.

### 2.4. Fruit Nutritional Traits, Element Concentration, Functional Compounds and Nitrogen Use Efficiency

All analyses on the fruit quality and functional features were accomplished on fruits from the 2nd–4th harvests; in particular, 6 fruits, randomly chosen from each replicate, were used. Fruits firmness (Newton, N) was assessed using a penetrometer (Trsnc, Milan, Italy).

The soluble solids content (SSC), expressed as °Brix, was measured in 120 g of fruit pulp. The pulp was blended and filtered; then, the analysis was realised using an optical refractometer.

The fruit dry matter percentage was achieved by drying 200 g of fruit at 102 °C.

The Kjeldahl method was employed to measure the fruit nitrogen concentration; then, the value was transformed into the protein content (N content × 6.25). The values were reported as g 100 $g^{-1}$ dry weight (dw). The phosphorous concentration in fruits was assessed by the methos described by Fogg and Wilkinson [46], whereas the potassium concentration was measured via atomic absorption spectroscopy (SavantAA, 200 ERRECI, Milan, Italy). For Ca and Mg measurements, atomic absorption spectroscopy was employed as described by Morand and Gullo [47]. The mineral determinations were reported as mg 100 $g^{-1}$ dw.

To determine the fruit ascorbic acid concentrations, a reflectometer (Merck RQflex® 10) and ascorbic acid test strips were used (Merck, Darmstadt, Germany). Distilled $H_2O$ was utilised to dissolve the fruit pulp sample. Water was added and mixed until the solution was filtered. Then, the test strips were immersed in the prepared samples and placed into the reflectometer. The values were presented as mg 100 $g^{-1}$ fresh weight (fw).

Fruit chlorogenic acid content (mg 100 $g^{-1}$ dw) was measured following the procedure of Stommel and Whitaker [48]. Briefly, a binary mobile phase gradient of methanol (Sigma-Aldrich, St. Louis, MO, USA) in 0.01% aqueous phosphoric acid (Titolchimica spa, Rovigo, Italy) was used. The quantification of chlorogenic acid (CA), conducted after a RP-HPLC separation, was based on absorbance at 325 nm relative to the sesamol internal standard and an external standard of authentic CA (Sigma-Aldrich, St. Louis, MO, USA).

The fruit glycoalkaloids concentration (mg 100 $g^{-1}$ dw) was assessed as reported by Sabatino et al. [16] using liquid chromatography (HPLC) (Agilent, 6546 Quadrupole Time of Flight LC/MS, Santa Clara, CA, USA). Partially purified solasonine and solamargine (Sigma-Aldrich, St. Louis, MO, USA) were used as the external standard.

The total anthocyanins determination was performed via HPLC, as reported by Mennella et al. [49]. Purified delphinidin-3-rutinoside (D3R, Polyphenols Laboratories AS, Sandnes, Norway) was used as the external standard.

The nitrogen use efficiency (NUE) was calculated on all plants using the following formula: NUE = yield (t)/nitrogen application rate (kg).

### *2.5. Experimental Layout and Statistics*

To evaluate the effect of the year, a preliminary three-way ANOVA (type of plant (T) × biostimulant (B) × year (Y)) was accomplished. The treatments were identified by a factorial combination of five plant types (not grafted, self-grafted or grafted onto *S. torvum*, *S. aethiopicum* or *S. macrocarpon*) and two biostimulant treatments (control or inoculation with *A. brasilense* DSM 2298). The study was organised in a completely randomised block design with 3 replicates (30 plants per treatments), obtaining a total of 300 plants. All recorded data were analysed by a two-way ANOVA (type of plant × biostimulant). A separation of means was accomplished via Tukey's HSD test at $p \leq 0.05$.

## 3. Results

### *3.1. Plant Growth and Yield*

The research was repeated over two consecutive years applying the same experimental set-up and achieving comparable data (Table S1). Consequently, the results from the 2020–2021 trial are presented.

The ANOVA for plant heights 50 DAT did not show a significant effect of the interaction between T and B (Figure 1A), whereas, for the N of leaves 50 DAT, a significant interaction was found (Figure 1B).

The 'Gloria' $F_1$ plant grafted onto *S. torvum* and *S. aethiopicum* rootstocks showed the highest plant height 50 DAT, followed by self-grafted plants. The lowest height values were measured in plants grafted onto *S. macrocarpon*. On the other hand, the biostimulant enhanced the plant height compared to the control. Plants grafted onto *S. torvum*, inoculated with *A. brasilense* DSM 2298 or not, gave the highest number of leaves 50 DAT, followed by those self-grafted and treated with the biostimulant. The lowest values were in the control plants grafted onto *S. macrocarpon*.

The ANOVA for eggplant production traits (total yield, marketable yield, number of marketable fruits and mean mass of marketable fruits) revealed no significant effect of the interaction of T × B (Table 1).

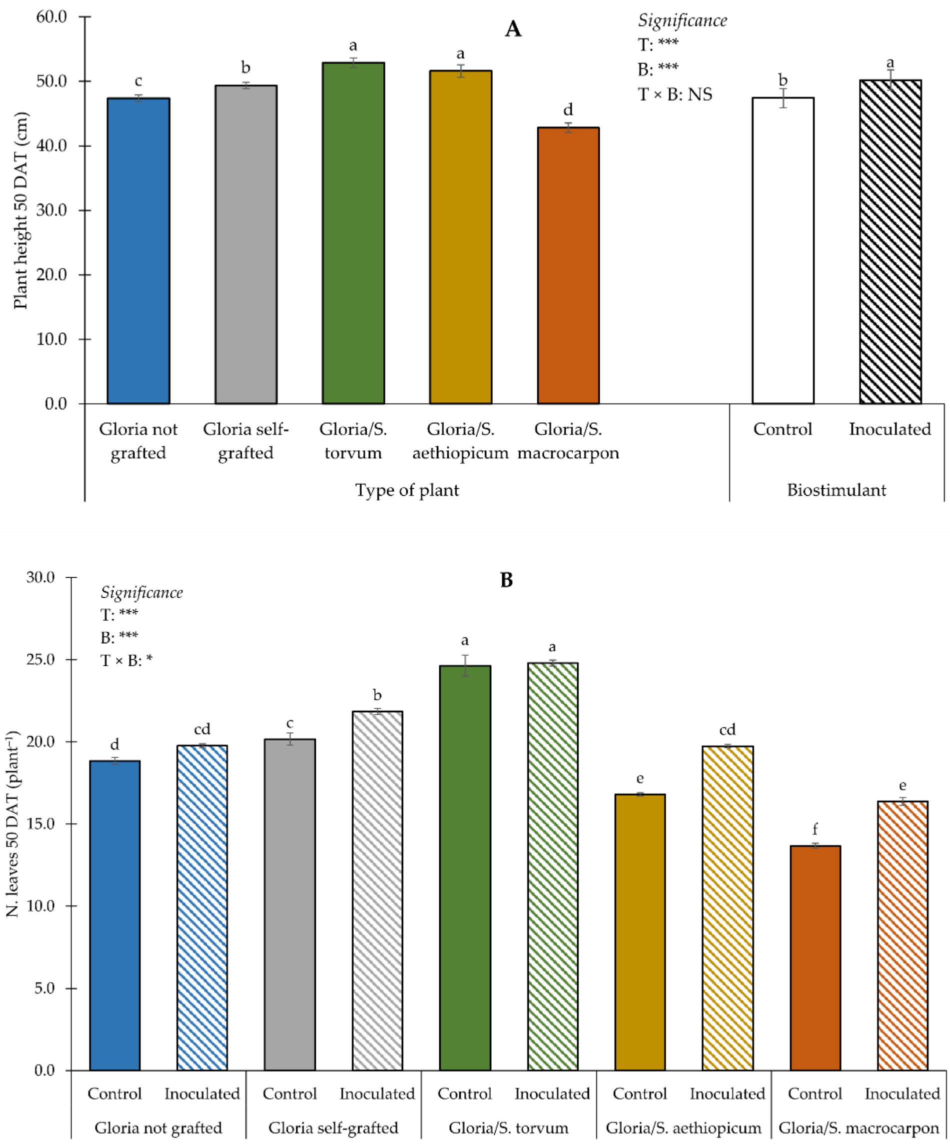


**Figure 1.** Influences of the type of plant and biostimulant application on plant height 50 days after transplanting (DAT) (**A**), and the number of leaves 50 DAT (**B**) of eggplant. Mean values with different letters are significantly dissimilar at $p \leq 0.05$, according to Tukey's test. NS = not significant; * = significant at $p \leq 0.05$; *** = significant at $p \leq 0.001$. Bars indicate the means ± standard error.

Plants grafted onto *S. torvum* had the highest total yield (+26% compared to the not grafted and +18% compared to the self-grafted). Plants grafted onto *S. macrocarpon* showed the lowest total yield. The biostimulant application significantly increased the total yield by 5% compared to the control. Data on the marketable yield followed the trend recognised for the total yield; in particular, plants grafted onto *S. torvum* had marketable yields higher by 31% and 21% compared to the not grafted and self-grafted plants, respectively, whereas the biostimulant treatment increased the marketable yield by 9% compared with the untreated plants. The highest number of marketable fruits was recorded in plants grafted onto *S. torvum* and in those grafted onto *S. aethiopicum*, while the lowest values were assessed in *S. macrocarpon*-grafted plants. Not considering the type of plant, the biostimulant significantly increased the number of marketable fruits. When the plants were grafted onto *S. torvum*, they revealed the highest values in terms of the mean mass of marketable fruits, followed by those grafted onto *S. aethiopicum*. The lowest values were recorded in plants grafted onto *S. macrocarpon*. Averaged from the type of plant, the biostimulant significantly enhanced the mean mass of marketable fruits.

**Table 1.** Influences of the type of plant and biostimulant application on the total yield, marketable yield, number of marketable fruits and mean mass of marketable fruits of eggplant.

| Treatments | | Total Yield (kg Plant$^{-1}$) | | Marketable Yield (kg Plant$^{-1}$) | | Number of Marketable Fruits (no. Plant$^{-1}$) | | Mean Mass of Marketable Fruits (g Fruit$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| Type of plant (T) | Gloria not grafted | 3.8 | c | 3.5 | c | 9.8 | b | 355.3 | b |
| | Gloria self-grafted | 4.1 | c | 3.8 | c | 10.7 | b | 355.9 | b |
| | Gloria/*S. torvum* | 4.8 | a | 4.6 | a | 12.5 | a | 365.9 | a |
| | Gloria/*S. aethiopicum* | 4.4 | b | 4.2 | b | 11.9 | a | 349.0 | b |
| | Gloria/*S. macrocarpon* | 1.7 | d | 1.4 | d | 5.1 | c | 275.0 | c |
| Biostimulant (B) | Control | 3.7 | b | 3.3 | b | 9.6 | b | 336.0 | b |
| | Inoculated | 3.9 | a | 3.6 | a | 10.3 | a | 344.4 | a |
| Significance | | | | | | | | | |
| T | | *** | | *** | | *** | | *** | |
| B | | * | | *** | | ** | | *** | |
| T × B | | NS | | NS | | NS | | NS | |

Values in the same column and with the same letter did not significantly differ at $p \leq 0.05$, according to Tukey's test. NS = not significant; * = significant at $p \leq 0.05$; ** = significant at $p \leq 0.01$; *** = significant at $p \leq 0.001$.

### *3.2. Fruit Dry Matter, Firmness and Soluble Solids Content*

The statistics on fruit dry matter, firmness and SSC did not evidence a significant effect of the T × B interaction (Table 2).

**Table 2.** Influences of the type of plant and biostimulant application on the fruit dry matter, firmness and soluble solids content (SSC) of eggplant.

| Treatments | | Fruit Dry Matter (%) | | Firmness (N) | | SSC (°Brix) | |
|---|---|---|---|---|---|---|---|
| Type of plant (T) | Gloria not grafted | 7.1 | a | −46.8 | a | 4.6 | c |
| | Gloria self-grafted | 7.1 | a | −46.6 | a | 4.3 | d |
| | Gloria/*S. torvum* | 7.1 | a | −46.6 | a | 4.1 | e |
| | Gloria/*S. aethiopicum* | 7.1 | a | −46.8 | a | 4.9 | b |
| | Gloria/*S. macrocarpon* | 7.2 | a | −46.7 | a | 5.2 | a |
| Biostimulant (B) | Control | 7.0 | b | −46.0 | a | 4.5 | b |
| | Inoculated | 7.2 | a | −47.4 | b | 4.7 | a |
| Significance | | | | | | | |
| T | | NS | | NS | | *** | |
| B | | * | | *** | | *** | |
| T × B | | NS | | NS | | NS | |

Values in the same column and with the same letter did not significantly differ at $p \leq 0.05$, according to Tukey's test. NS = not significant; * = significant at $p \leq 0.05$; *** = significant at $p \leq 0.001$.

Regardless of the biostimulant treatment, the type of plant did not influence the fruit dry matter, whereas, disregarding of the type of plant, the biostimulant significantly increased the fruit dry matter by 3% compared to the control. The type of plant had no significant effect on the fruit firmness; conversely, the biostimulant application significantly reduced this parameter. When averaged over the biostimulant treatment, *S. macrocarpon*-grafted plants had the highest SSC values, followed by those grafted onto *S. aethiopicum*. The lowest SSC values were noted in *S. torvum*-grafted plants. Disregarding the type of plant, the inoculation with *A. brasilense* DSM 2298 meaningfully boosted the fruits' SSC.

### *3.3. Minerals, Proteins and NUE*

The ANOVA for the mineral profile did not reveal a significant effect of the interaction between T and B (Table 3).

**Table 3.** Influences of the type of plant and biostimulant application on the mineral profile (phosphorous, potassium, calcium and magnesium) of eggplant fruits.

| Treatments | | P (mg 100 $g^{-1}$ dw) | | K (mg 100 $g^{-1}$ dw) | | Ca (mg 100 $g^{-1}$ dw) | | Mg (mg 100 $g^{-1}$ dw) | |
|---|---|---|---|---|---|---|---|---|---|
| Type of plant (T) | Gloria not grafted | 543.3 | a | 339.7 | b | 108.7 | a | 19.1 | b |
| | Gloria self-grafted | 544.0 | a | 340.0 | b | 109.3 | a | 18.8 | b |
| | Gloria/*S. torvum* | 541.7 | a | 343.6 | b | 109.0 | a | 14.5 | c |
| | Gloria/*S. aethiopicum* | 522.9 | b | 301.9 | c | 98.8 | c | 20.6 | a |
| | Gloria/*S. macrocarpon* | 499.9 | c | 348.2 | a | 106.3 | b | 19.2 | b |
| Biostimulant (B) | Control | 530.6 | a | 334.2 | a | 106.3 | a | 17.4 | b |
| | Inoculated | 530.1 | a | 335.1 | a | 106.5 | a | 19.5 | a |
| Significance | | | | | | | | | |
| T | | *** | | *** | | *** | | *** | |
| B | | NS | | NS | | NS | | *** | |
| T × B | | NS | | NS | | NS | | NS | |

Values in the same column and with the same letter did not significantly differ at $p \leq 0.05$, according to Tukey's test. NS = not significant; *** = significant at $p \leq 0.001$.

The highest P fruit concentration was recorded in the not grafted plants, self-grafted and in *S. torvum*-grafted plants, whereas the lowest values were found in *S. macrocarpon*-grafted plants. Conversely, the biostimulant application had no effect on the P fruit concentration. The highest K values were observed in fruits from plants grafted onto *S. macrocarpon*, with the values higher by 2.5%, 2.4% and 1.5% compared to those observed in the not grafted, self-grafted and *S. torvum*-grafted plants, respectively. Regardless of the type of plant, the biostimulant inoculation did not significantly influence the K concentration in eggplant fruits. Not grafted, self-grafted and *S. torvum*-grafted plants had the highest fruit Ca concentrations, whereas the lowest amounts were appraised in plants grafted onto *S. aethiopicum*. The inoculation with *A. brasilense* DSM 2298 did not affect the Ca concentration. Regardless of the biostimulant application, the highest fruit Mg concentration was shown by plants grafted onto *S. aethiopicum*, whereas the lowest values were assessed in those grafted onto *S. torvum*. Contrariwise, not considering the type of plant, the biostimulant inoculation meaningfully boosted the fruit Mg concentration.

The statistics on proteins underlined a significant influence of the interaction between T and B (Figure 2A), whereas, for NUE, no significant interactive effect was found (Figure 2B).

Fruits from *S. torvum*-grafted plants treated with *A. brasilense* revealed the highest protein concentration, followed by those from plants grafted on the same rootstock but not inoculated. The combination Gloria/*S. macrocarpon* × control had the lowest value. Regardless of the biostimulant, plants grafted onto *S. torvum* had the highest NUE index values, followed by those grafted onto *S. aethiopicum*, which, in turn, highlighted a higher value than the self-grafted or not grafted plants. Conversely, not considering the type of plant, the biostimulant inoculation meaningfully boosted the NUE index.

### *3.4. Fruit Functional Features*

The ANOVA for ascorbic acid (Figure 3A) and glycoalkaloids (Figure 3D) showed a significant effect of the interaction of T × B, while, for chlorogenic acid (Figure 3B) and total anthocyanins (Figure 3C), no interactive effect was found.

Plants grafted onto *S. torvum* and those grafted onto *S. macrocarpon* and inoculated with the PGPB had the highest ascorbic acid concentrations, followed by the combinations Gloria/*S. torvum* × control, Gloria/*S. aethiopicum* × inoculated and Gloria/*S. macrocarpon* × control. The lowest values were appraised in the not grafted or self-grafted control plants. Regardless of the biostimulant application, fruits from plants grafted onto *S. aethiopicum* had the highest chlorogenic acid concentrations, followed by those from plants grafted onto *S. macrocarpon*. The lowest values were recorded on the not grafted or self-grafted plants. Contrariwise, not considering the type of plants, the inoculation with the biostimulant significantly increased the chlorogenic acid concentration compared to the control. When averaged over the biostimulant treatments, fruits from plants not grafted, self-grafted or grafted onto *S. aethiopicum* had the highest total anthocyanins contents, whereas the lowest values were recorded in fruits from *S. macrocarpon*-grafted plants. The biostimulant treatments significantly enhanced the total anthocyanins fruits concentration. Fruits from the combination Gloria/*S. macrocarpon* × control had the highest values, followed by those from the combinations Gloria not grafted × control, Gloria self-grafted × control and Gloria/*S. macrocarpon* × inoculated. The lowest values were appraised in plants grafted onto *S. torvum*, either inoculated or not.

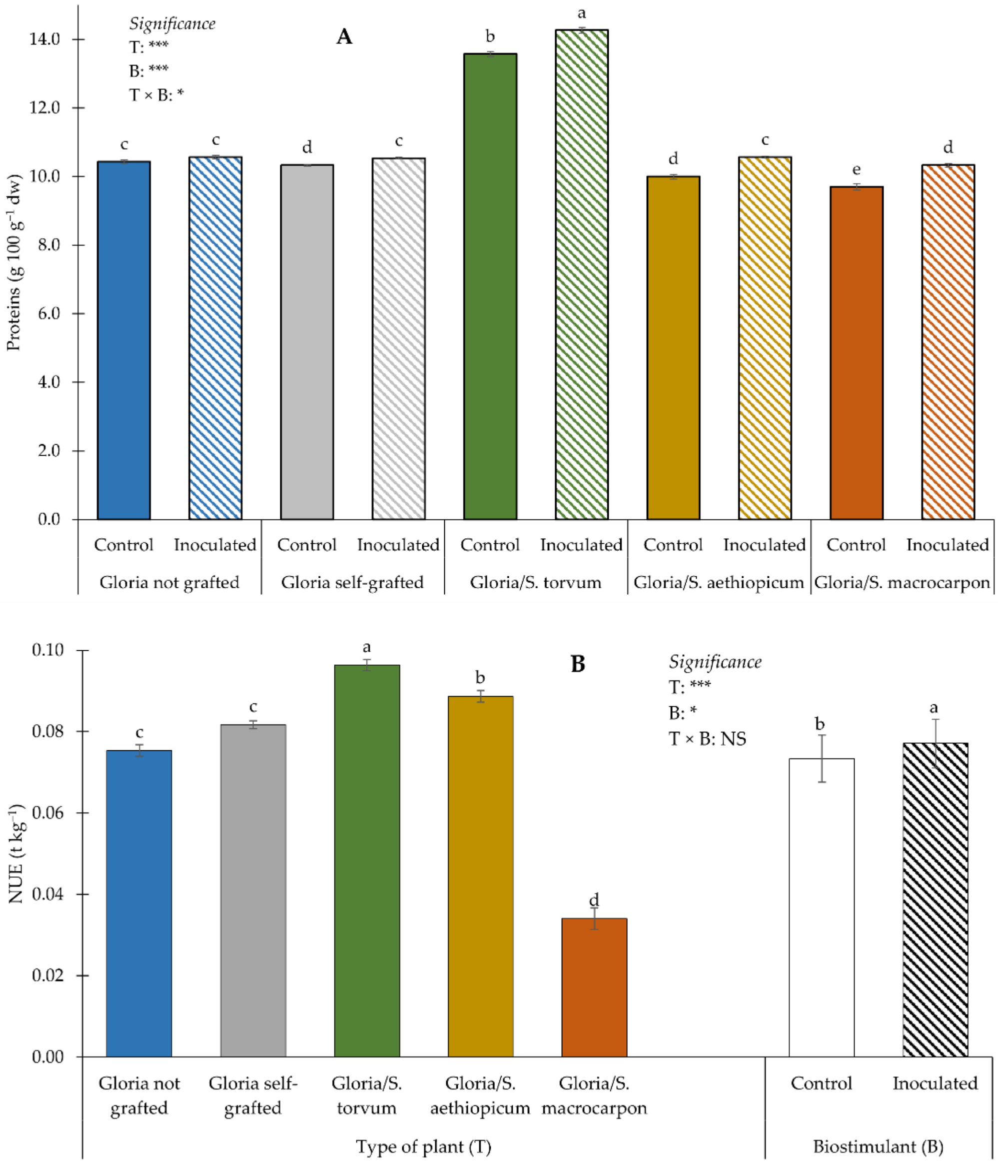


**Figure 2.** Influences of the type of plant and biostimulant application on the fruit protein content (**A**) and nitrogen use efficiency (NUE) (**B**) of eggplant. Mean values with different letters are significantly dissimilar at $p \leq 0.05$, according to Tukey's test. NS = not significant; * = significant at $p \leq 0.05$; *** = significant at $p \leq 0.001$. Bars indicate the means ± standard error.

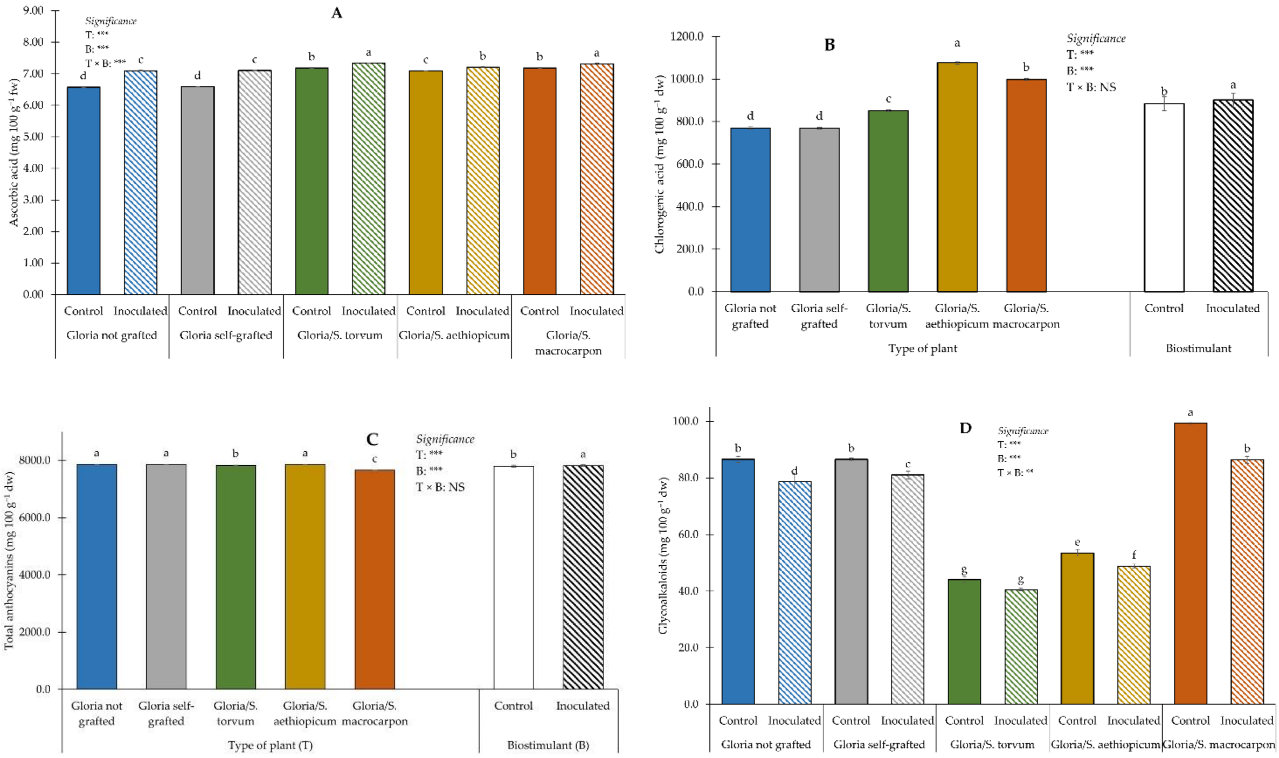


**Figure 3.** Influences of the type of plant and biostimulant application on ascorbic acid (**A**), chlorogenic acid (**B**), total anthocyanins (**C**) and glycoalkaloids (**D**). Mean values with different letters are significantly dissimilar at $p \leq 0.05$, according to Tukey's test. NS = not significant; ** = significant at $p \leq 0.01$; *** = significant at $p \leq 0.001$. Bars indicate the means ± standard error.

## 4. Discussion

In the current research, we appraised the use of different potential rootstocks for eggplant and the inoculation of *A. brasilense* DSM 2298, alone or in combination, to improve the overall plant fitness, plant productivity and fruit quality of 'Gloria' eggplant grown in a greenhouse. The results showed that *S. torvum*- and *S. aethiopicum*-grafted plants had the highest plant heights 50 DAT. Furthermore, our findings also showed that *S. torvum*-grafted plants (inoculated or not) had the highest number of leaves 50 DAT. Our data are coherent with those stated by Sabatino et al. [17], who, studying the potential use of *S. aethiopicum* and the interspecific hybrid of *S. melongena* × *S. aethiopicum* as potential rootstocks for eggplant, found that *S. torvum* and *S. aethiopicum* (accession 1) are the most vigorous rootstocks. Simultaneously, the outcomes revealed that *A. brasilense* DSM 2298 application significantly elicited plant vigour traits. These records entirely agree with those by Consentino et al. [24], who, studying the combinatorial effects of PGPBs and different N-application rates, found that lettuce plants colonised by *A. brasilense* and supplied with suboptimal N rates have the best performances in terms of plant vigour traits. The results are also in agreement with those reported by Gravel et al. [50], who, investigating the influence of diverse PGPB on the growth and yield features of tomatoes, found that plants inoculated with PGPB showed a higher performance in terms of the plant vigour parameters.

Our findings highlighted that *S. torvum*-grafted plants had the highest yield and yield-related traits. Contextually, the *A. brasilense* inoculation proved to be a valid agronomic practice for increasing the yield traits. In this regard, the data are totally in agreement with that of Sabatino et al. [51], who, examining the interactive effect between rootstock and arbuscular mycorrhiza on plant production and fruit features of 'Birgah' $F_1$ eggplant, found that—irrespective of the AM application—*S. torvum*-grafted plants revealed the highest yield. However, considering that plants grafted onto *S. aethiopicum* performed well in terms of yield (higher than 4.0 kg plant$^{-1}$), it seems that the combinatorial use of *S. aethiopicum* rootstock and *A. brasilense* DSM 2298 application could be a feasible alternative to the most common *S. torvum* rootstock. Although the strict actions by which PGPB promote the overall plant growth and yield are not completely acknowledged, Parewa et al. [52] stated that PGPB have an influence on plant growth through a number of actions, such as N fixation, inorganic phosphate solubilisation and the biosynthesis of key hormones.

The current study showed that the type of plant did not significantly influence the fruit dry matter percentage, whereas the *A. brasilense* application improved it. These findings tie in well with those of Bhattacharyya and Jha [53], who described that PGPB inoculation significantly boosts dry matter production in different solanaceous crops (e.g., tomatoes and potatoes). Our results also showed that, although the type of plants did not significantly affect the fruit firmness, an *A. brasilense* application significantly augmented the fruit firmness. Palmer et al. [54] reported that the dry matter concentration is positively related to fruit firmness; therefore, we may assume that our outcomes could be linked to the higher dry matter content recorded in fruits from inoculated plants.

In line with a previous study [16], the results underlined that fruits from plants grafted onto *S. macrocarpon* rootstock had the highest SSC, whereas those from plants grafted onto *S. torvum* rootstock had the lowest, remarking that the SSC trait is also influenced by genotype. Concurrently, our outcomes also displayed that SSC was enhanced by the application of PGPB. Overall, this was in accordance with those reported by Consentino et al. [24], who found that the application of *A. brasilense* exerted a positive action on lettuce SSC. Our outcomes were also coherent with those of Ordookhani and Zare [55], who, studying the effect of PGPBs on the tomato fruit quality, recognised an encouraging influence of the microorganism on fruit SSC.

The fruit mineral profile exhibited that plants grafted onto *S. torvum* had the highest P and Ca concentrations. Plants grafted onto *S. macrocarpon* had the highest K concentration, whereas *S. aethiopicum*-grafted plants revealed the highest Mg concentration. These findings underline the imperative action of the scion–rootstock combination on the nutritional fruit quality in grafted plants. Furthermore, the current research highlighted that the *A. brasilense* DSM 2298 inoculation did not significantly affect the fruit P concentration. In this respect, our results are in line with those of Consentino et al. [24] on lettuce and with those of Hungria et al. [56] on maize and wheat. The findings also pointed out that the K and Ca concentrations were not affected by the biostimulant treatment. These findings are partially in line with those of Sabatino et al. [25], who found that *A. brasilense* DSM 2298 improved the K concentration in leaf tissues; however, it did not influence the Ca concentration in lettuce plants. Our data are in contrast with those of Radhakrishnan and Lee [57], who connected the leaf lettuce K concentration increase to a catalysed metabolism of proteins, enzymes, lipids and nucleic acids. However, these dissimilar findings could be linked to a different plant organ accumulation/distribution. The data revealed that the biostimulant application emphasised the fruit Mg concentration. These outcomes are totally in agreement with those described by different authors [24,56].

The data showed that *S. torvum* rootstock and *A. brasilense* inoculation synergistically enhanced the protein concentrations in eggplant fruits. The findings concur with previous findings reported by Sabatino et al. [51], who found that 'Birgah' eggplant grafted onto *S. torvum* and treated with a microbial biostimulant (arbuscular mycorrhiza fungi) had the highest fruit protein concentrations. Moreover, the outcomes revealed that plants colonised by *A. brasilense* DSM 2298 had higher fruit protein concentrations. As reported by Radhakrishnan and Lee [57], the upsurge in plant N concentration through PGPB action is widely described. de Santi Ferrara et al. [58] reported that nitrogen fixation via microorganisms in soil is the chief motivation for this increase. Specifically, Hungria et al. [56] established that *A. brasilense*, strains Ab-V5 and Ab-V6, had comparable *nif* and *fix* genes that prompted the aptitude to fix atmospheric N.

Although plants grafted onto *S. torvum* rootstock revealed the highest fruit protein concentrations, they also showed the highest NUE index, whereas *S. macrocarpon*-grafted plants had the lowest one. This is in accordance with the higher production of *S. torvum*-

grafted plants (4.8 kg plant$^{-1}$). Averaged from the type of plant, plants treated with *A. brasilense* showed a higher NUE than the control plants. These results agreed with those of Consentino et al. [24] and with those of Zeffa et al. [59], who found that *A. brasilense* improves the NUE of maize via an augmented plants growth.

Antioxidant compounds, such as polyphenols and ascorbic acid, are recognised as promoters of human health, particularly as they relate to noncommunicable diseases (NCDs) [60,61]. In contrast to other nutrients, the plant polyphenol intake deficiency is not associated with precise illnesses, making it problematic to delineate the proper reference intake values for these foodstuff constituents. Our findings showed that the biostimulant application and grafting onto *S. torvum*, *S. aethiopicum* or *S. macrocarpon* improved the fruit ascorbic acid and chlorogenic acid concentrations. Correspondingly, Sabatino et al. [51] detected that *S. torvum*-grafted plants promoted the fruit ascorbic acid content in eggplant. However, Oztekin et al. [62] underlined that grafting does not significantly influence the fruit ascorbic acid concentration in tomatoes. These conflicting outcomes could be ascribed to the genetic diversity of the employed rootstocks [63]. Our findings are also sustained by those of Parewa et al. [52], who reported that the application of PGPB elicits secondary metabolism. Equally, Cappellari et al. [64] found that three PGPB genera triggered phenolic biosynthesis in *Mentha piperita*. There are reports [65,66] that various PGPB activate plant tolerance to phytopathogens through alteration of the secondary metabolism, producing phenolic components. Overall, the increased ascorbic acid and chlorogenic acid concentrations in fruits from grafted plants inoculated with *A. brasilense* are most likely related not only to a modified secondary metabolism but also to a changed plant nutritional status of grafted plants.

Anthocyanins are flavonoids largely found in red and blue fruits and vegetables. As reported by Cassidy [67], anthocyanins, such as other polyphenols, are metabolised by the host and the microbiome to form active metabolites that have anti-inflammatory properties and produce positive vascular effects. In line with other research [13,51], our data indicated that the use of *S. torvum* as a rootstock for eggplant reduced the fruit total anthocyanins concentration. Since—as reported by Manach et al. [68] and Awad et al. [69]—anthocyanins biosynthesis is connected to light exposure and considering that *S. torvum* is one of the most vigorous rootstocks for eggplant, we may assume that the total anthocyanins decline in *S. torvum*-grafted plants is related to a higher leaf shading effect on fruits. Furthermore, our results showed that *A. brasilense* inoculation enhanced the fruit total anthocyanins. These data are in agreement with those of Badar et al. [70], who, studying the effects of different PGPBs on three strawberry cultivars, found that the application of PGPB significantly increased the total anthocyanins concentration. Similar results were found by Lingua et al. [71], who reported an increase in the total anthocyanins concentration in fruits from plants treated with arbuscular mycorrhizal fungi in combination with *Pseudomonas* strains.

The results revealed that specific rootstocks, such as *S. torvum* and *S. aethiopicum*, especially when combined with *A. brasilense* colonisation, significantly decreased the glycoalkaloids concentration in eggplant fruits. The results are in accordance with former findings [25], who showed that microbial biostimulant application and grafting reduced the fruit glycoalkaloids concentration in eggplant. Accordingly, Mystkowska [72] reported that potato plants treated with biostimulants had a lower glycoalkaloids concentration than the control plants. Since suboptimal cultivation conditions increased the glycoalkaloids concentration in potatoes [73], we may suppose that *S. torvum*- and *S. aethiopicum*-grafted plants and those colonised by *A. brasilense* DSM 2298 were less stressed. These assumptions are totally in line with the results of Sabatino et al. [51], who reported that grafting and AM fungi synergistically decreased the fruit glycoalkaloids concentration in eggplant.

## 5. Conclusions

The combined use of sustainable agronomic practices, such as the herbaceous grafting onto specific rootstocks and the biostimulant application, is attaining a reputation in the horticulture sector due to its capacity to boost the yield and quality of vegetable crops.

*S. torvum* and *S. aethiopicum* rootstocks and *A. brasilense* DSM 2298 inoculation considerably acted together, enhancing the plant growth, yield and yield components, as well as nutritional and functional parameters of 'Gloria' $F_1$ eggplant. However, regardless of the type of plant, the microbial biostimulant tested significantly improved the overall plant fitness. The current research could be valuable information for the vegetable plug plant production sector involved in the use of new eggplant rootstocks that effectively react when combined with the microbial biostimulant.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae8080722/s1: Figure S1: Maximum and minimum temperatures recorded during the two cultivation cycles. Table S1: Significance of the three-way ANOVA (type of plant × biostimulant × year).

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