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# *Solanum aethiopicum* gr. *gilo* and Its Interspecific Hybrid with *S. melongena* as Alternative Rootstocks for Eggplant: Effects on Vigor, Yield, and Fruit Physicochemical Properties of Cultivar 'Scarlati'

Leo Sabatino <sup>1,\*</sup>, Giovanni Iapichino <sup>1</sup>, Giuseppe Leonardo Rotino <sup>2</sup>, Eristanna Palazzolo <sup>1</sup>, Giuseppe Mennella <sup>3</sup> and Fabio D'Anna <sup>1</sup>

<sup>1</sup> Dipartimento Scienze Agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy; giovanni.iapichino@unipa.it (G.I.); eristanna.palazzolo@unipa.it (E.P.); fabio.danna@unipa.it (F.D.)

<sup>2</sup> Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria CREA-GB, Centro di Ricerca Genomica e Bioinformatica, Via Paullese, 26836 Montanaso Lombardo (LO), Italy; giuseppeleonardo.rotino@crea.gov.it

<sup>3</sup> Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria CREA-OF, Centro di Ricerca Orticoltura e Florovivaismo, Via Cavallegeri, 84098 Pontecagnano-Faiano, Italy; giuseppe.mennella@crea.gov.it

\* Correspondence: leo.sabatino@unipa.it; Tel.: +39-329-801-6975

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**Abstract:** Grafting is generally considered effective in ameliorating vegetable crop tolerance to biotic and abiotic stresses. The use of interspecific hybrid as rootstock for eggplant may represent a valid alternative approach to enhance eggplant performance. However, studies on the effects of different rootstocks on eggplant plant vigor, yield, and fruit quality traits often show conflicting results. Thus, an experiment was performed in two spring–summer growing seasons (2014 and 2015) by grafting eggplant 'Scarlati' F<sub>1</sub> hybrid on two accessions of *S. aethiopicum* gr. *gilo* and on the interspecific hybrid *S. melongena* × *S. aethiopicum* gr. *gilo* in comparison to the most common eggplant rootstock *S. torvum*. Results indicate that *S. melongena* × *S. aethiopicum* gr. *gilo* interspecific hybrid and *S. torvum* improved grafting success, plant vigor, early flowering and yield in 'Scarlati' F<sub>1</sub> scion. All rootstocks tested did not negatively influence fruit apparent quality traits and fruit quality composition. Moreover, fruit glycoalkaloids content remained below the recommended threshold value. These findings suggest that the use of *S. melongena* × *S. aethiopicum* gr. *gilo* interspecific hybrid as rootstock may be a good alternative to the most commonly used *S. torvum*.

**Keywords:** wild eggplant relative; interspecific hybrid; scion/rootstock combination; plant vigour; yield; fruit quality attributes

## 1. Introduction

Eggplant (*Solanum melongena* L.) is the world's sixth most important vegetable after tomato, watermelon, onion, cabbage, and cucumber [1] and the most important *Solanum* crop native to the Old World [2]. Sicily is one of the most important production areas within the Mediterranean Basin that is considered a secondary center of diversification [3]. Soilborne diseases arising from continuous cropping are major problems that limit productivity in eggplant [4]. Therefore, improving soilborne resistance is one of the major scientific and economic challenges in eggplant. Grafting has been attempted as an effective mean to control soilborne diseases and abiotic stresses while simultaneously

not adversely affect plant growth or even to improve vigor and yield [4]. Among the abiotic stresses that affect vegetable crops, salinity continues to be a main factor in reducing vegetable crop yield and profits in many arid and semi-arid regions. In this respect, Colla et al. [5] demonstrated the effectiveness of grafting to improve salinity tolerance in cucumber. However, the use of specific rootstocks may offer many other advantages such as drought resistance [6] or heavy metal tolerance [7].

*Solanum torvum* Sw. is the rootstock commonly used for grafting, especially in the most intensive protected cultivation. However, its use has been limited due to lack of rapid and homogeneous synchronized seed germination [8]. Studies have shown that grafting can affect yield and fruit quality in eggplant [9,10]. Kyriacou et al. [11], in their review, reassessed that grafting itself and the prevalence of particular type of commercial rootstocks influence vegetable fruit quality and, partially, storability. They also reported that grafting significantly affects morphometric traits, textural characteristics, sweetness and acidity, as well as functional compounds in eggplant. However, according to Kyriacou et al. [11], current reports on the changes conferred by grafting on eggplant fruit quality provide conflicting information probably due to the environment in which experiment were run. In particular, they stressed both possible rootstock–scion interactions, underscoring graft combinations and different stemming from failure to standardize fruit harvest maturity. In this respect, Gisbert et al. [9] and Sabatino et al. [10] found that the use of interspecific hybrid rootstocks derived from compatible crosses of eggplant with related species can be a valuable approach to improve/preserve eggplant production.

*Solanum aethiopicum* gr. *gilo* (scarlet eggplant), a close relative species of *S. melongena*, has been considered a noticeable resource for eggplant genetic improvement and as potential rootstock. It presents traits of interest, including resistance to *F. oxysporum* f. sp. *melongenae*, *R. solanacearum* [12,13], and root-knot nematodes [14]. Since scarlet eggplant is a cultivated species, it does not display those undesirable traits that are commonly present in the wild relatives of eggplant such as small fruit production, presence of prickles, and high saponins and glycoalkaloids concentrations [15]. A great genetic and morphological variability has been evidenced in *S. aethiopicum* [16]; therefore, it is particularly relevant to characterize the accessions for the presence and expressivity of the traits of interest before starting a breeding program. Interspecific sexual and somatic hybrids between *S. melongena* and *S. aethiopicum* gr. *gilo* have been produced and although they present a high degree of sterility, recombination between the two genomes has been demonstrated [17] and backcross generations to *S. melongena* with useful introgressions of *S. aethiopicum* gr. *gilo* obtained [13,18,19]. The aim of this work was to explore the possibility to use two accessions of *S. aethiopicum* gr. *gilo* and an initial highly fertile introgression line (BC<sub>1</sub>) from the somatic hybrid between *S. melongena* and *S. aethiopicum* gr. *gilo* as rootstock for eggplant. The introgression line was resistant to *Fusarium oxysporum* f.sp. *melongenae* and is the founder of the series of improved eggplant lines resistant to *Fusarium* wilt carrying the resistant locus *RfoSa1* [18]. The effects of these three genetic materials when used as rootstock, on vigor, yield, and fruit quality traits of eggplant 'Scarlati' F<sub>1</sub> hybrid were evaluated. The results are compared with those obtained from ungrafted, self-grafted, and *S. torvum* rootstock grafted plants. Therefore, our aim was also to validate the assumption that using interspecific hybrid rootstocks may be a good approach to enhance eggplant performance because the presence of the eggplant genome together that one of the wild/allied species may improve the overall affinity between rootstock and scion exploiting the advantage of grafting.

## 2. Materials and Methods

### 2.1. Plant Material

The eggplant F<sub>1</sub> hybrid 'Scarlati' (black cylindrical shape) (EnzaZaden, The Netherlands) was used as the scion cultivar as well as the rootstock and ungrafted control. Five rootstocks were evaluated, which included materials corresponding to the three species *S. melongena* (F<sub>1</sub> hybrid 'Scarlati'), *Solanum torvum*, and *Solanum aethiopicum* gr. *gilo* (two accessions, named accession 1 and accession 2), and one interspecific hybrid of *S. melongena* × *S. aethiopicum* gr. *gilo*, which is a double haploid line

obtained from another culture of the tetraploid backcrosses from the somatic hybrid eggplant cv Dourga(+) *S. aethiopicum* gr. *gilo* accession 2 with a tetraploid plant of the eggplant line DR2 [13,18]. The materials used as rootstocks originated from the germplasm collection of the Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria CREA-GB, Centro di Ricerca Genomica e Bioinformatica (Montanaso Lombardo, Italy).

## 2.2. Seedlings Production and Grafting

For the production of the grafted plant material, on 10 February 2014 and 9 February 2015, *S. aethiopicum* gr. *gilo* and *S. torvum* rootstock seeds were planted in 40-cell seedling trays (cell volume of 83 cm<sup>3</sup>), under a temperature regime of 25 °C/18 °C (day/night) in a propagation greenhouse. After 20 days, seeds of the F<sub>1</sub> 'Scarlati' eggplant were planted in 104-cell trays (cell volume of 33 cm<sup>3</sup>) under the same temperature regime and planting method as the rootstocks. Due to the faster germination and growth, the *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock was sown simultaneously to the F<sub>1</sub> 'Scarlati' hybrid. Trays were watered manually every day to maintain the substrate at water holding capacity. Seventy-five days after the sowing of *S. torvum* and *S. aethiopicum* gr. *gilo* (accessions 1 and 2), all seedlings had reached an adequate diameter for grafting. The eggplant cultivar Scarlati was grafted onto 'Scarlati' rootstocks (self-grafted), *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1 and accession 2), and *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks using the tube grafting method described by Lee [20] and modified by Miceli et al. [21]. Plants at the 3–4 leaf stage were used as rootstocks. The grafted plantlets were misted, incubated within a plastic tunnel in a greenhouse, and maintained at a temperature of 20 °C and a humidity rate of 95% for 7 days. After 7 days, the grafted plantlets were acclimatized to the natural conditions of the greenhouse by slowly dropping the humidity (RH 70–80%) for 3 days, until they were ready for transplant.

## 2.3. Cultivation Conditions

'Scarlati' plants ungrafted, self-grafted, and grafted onto *S. melongena*, *S. torvum*, and accession 1 and 2 of *S. aethiopicum* gr. *gilo* rootstocks were transplanted on 5 May 2014 and 4 May 2015 on a Typic Rhodoxeralf soil in the experimental farm of the Department of Agricultural, Alimentary and Forest Sciences (SAAF) (longitude 13°19' E, latitude 38°09' N), of the University of Palermo, Italy. The field trials were conducted in a sandy clay loam soil (46.5% sand, 22.3% silt, 31.2 clay) at pH 7.2 in a completely randomized design with 4 replications of 10 plants (40 plants per treatment). In both years, the preceding crop was cauliflower. Plants were spaced 1.0 m between rows and 0.5 m apart within the row and drip irrigated. Fertilization was applied with drip irrigation throughout the growing cycle and consisted of 250 kg nitrogen ha<sup>-1</sup>, 150 kg phosphorous pentoxide ha<sup>-1</sup>, and 250 kg potassium oxide ha<sup>-1</sup> (Yara, Oslo, Norway). Standard horticultural practices for eggplant production in the Mediterranean environment were adopted [22].

Average daily temperature during the experimental period from May to August of 2014 and 2015 was obtained from the meteorological station of the experimental farm of the Department SAAF (Figure S1). In terms of temperatures, the weather during the experimental period in 2014 and 2015 was similar to the long term average. Nevertheless, the average monthly temperatures showed the highest negative deviation in June (1.8 and 2.2 °C in 2014 and 2015, respectively).

## 2.4. Grafting Success, Biometric Parameters, Yield, and Apparent Fruit Quality Evaluation

Grafting success was recorded after two weeks from grafting and was calculated on 100 grafted seedlings for each rootstock used. Plant height and root collar diameter (via a digital caliper) at 50, 80, and 110 days after transplanting (DAT), number of leaves at 50 DAT, and aboveground biomass produced at the end of fruit harvest (including total yield and vegetative part produced (weight of the plant at the end of harvests plus vegetative part removed by pruning after plant branching)) were determined. First flower formation (expressed as DAT) were also recorded.

Immediately after harvesting fruits were weighed. Total yield ( $\text{kg plant}^{-1}$ ), marketable yield ( $\text{kg plant}^{-1}$ ), and number of marketable fruits per plant were collected. Average marketable fruit weight (g) was calculated.

Color ( $a^*$  and  $b^*$  parameters -CIELab) was measured on four replications of five fruits per rootstock–scion combination. The records were taken on two opposite point of eggplant fruit skin (equatorial zone) by a colorimeter (Chroma-meter CR-400, Minolta Corporation, Ltd., Osaka, Japan). Hue angle ( $H^\circ$ ) was calculated as follows:  $H^\circ = \arctan(b^*/a^*)$ .

Fruit firmness was determined by measuring its resistance to the plunger of a digital penetrometer (Trsnc, Italy). Each fruit was subjected to a compression in two opposite point in the equatorial part using a 6 mm diameter stainless steel cylinder probe. The mean peak force was calculated in Newton (N).

Apparent fruit quality traits of 'Scarlati' eggplant fruit were measured in four replications of ten representative commercially mature fruits from non-grafted and self-grafted plants, and from plants derived from 'Scarlati' scions grafted onto *S. torvum* and accession 1 and 2 of *S. aethiopicum* gr. *gilo* hybrid rootstocks. Fruit length/width ratios were calculated. Several traits were measured in an arbitrary scale according to the European Eggplant Genetic Resources Network (EGGNET) descriptors [23]. These traits included fruit curvature (1 = none; 9 = U-shaped), fruit cross section (1 = circular; 9 = very irregular), fruit calyx length (1 = very short (>10%); 9 = very long (>75%)), and fruit calyx prickles (0 = none; 9 = very many (>30)). In addition to these EGGNET descriptors, seed index (0 = none; 5 = very many (>80) seeds visible in a longitudinal fruit section) was measured.

#### 2.5. Pulp Browning, Soluble Solid Content, and Chemicals

The colorimeter was also used to determine the lightness of fruit pulp by measuring  $L^*$  value (0 = black and 100 = white). Fruits were sectioned in the equatorial part and the color of the pulp was measured immediately after cutting ( $L_0$ ) and after 30 min ( $L_{30}$ ) in two areas (central and lateral) of the section. The oxidation potential was estimated using Larrigaudiere et al. [24] method with little modifications as in part suggested by Concellòn et al. [25]. The oxidation potential was expressed as  $\Delta L_{30} = (L_{30} - L_0)$ .

Sampling for the quality analysis of the fruits was carried out as described by Sabatino et al. [10] and Sabatino et al. [26]. Thus, 3–5 commercially mature fruits for each replication from the second and third harvest were used; only healthy fruits were chosen. Care was taken to ensure that each sample contained the same percentage weight of apical, middle, and distal parts of the fruits. Qualitative fruit characteristic analyses were conducted on fruits harvested from labeled fruits (the flowers were labeled at the fruit set stage) and all fruits were harvested after 35 days from labeling (fruit commercial maturity stage).

Sample of the fruit pulp were squeezed by hand with a garlic squeezer. The juice was filtered and soluble solids content (SSC) was measured using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd. Taiwan).

Fruit dry matter percentage was determined in samples dried at 105 °C until constant weight as  $100\% \times (\text{dry weight}/\text{fresh weight})$ .

Proteins, metals, total anthocyanins, chlorogenic acid, and glycoalkaloids were determined only in the second trial (2015). Protein concentration was determined from N content obtained from the Kjeldahl method. In particular, a sample rate was subjected to acid-catalyzed mineralization to turn the organic nitrogen into ammoniacal nitrogen. The ammoniacal nitrogen was then distilled in an alkaline pH. The ammonia formed during this distillation was collected in a boric acid solution and determined through titrimetric dosage. The protein concentration was reported as  $N \times 6.25$ . Phosphorus content were assessed using colorimetry [27]. Ca, Mg, and K were determined using atomic absorption spectroscopy following wet mineralization [28].

Polyphenols were extracted and analyzed according to Stommel and Whitaker [29] with minor modifications. The analyses were performed through a Waters E-Alliance HPLC system constituted

by a 2695 separations module with quaternary pump, auto sampler, and a 2996 photodiode array detector; data were acquired and analyzed with Waters Empower software on a personal computer. A binary mobile phase gradient of methanol in 0.01% aqueous phosphoric acid was used according to this procedure: 0–15 min, linear increase from 5 to 25% methanol; 15–28 min, linear increase from 25 to 50% methanol; 28–30 min, linear increase from 50 to 100% methanol; 30–32 min, 100% methanol; 32–36 min, linear decrease from 100 to 5% methanol; 36–43 min, 5% methanol. The flow rate was 0.8 mL/min. Quantification of chlorogenic acid (CA), carried out 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). The results were expressed as mg·100 g<sup>-1</sup> of fw.

The extraction and the analysis of anthocyanins were carried out on 200 mg of lyophilized and powdered peel as reported in Mennella et al. [30]. Briefly, the chromatographic separations were performed at a flow rate of 0.8 mL/min and at 0.1 absorbance units full scale (AUFS). Purified delphinidin-3-rutinoside (D3R, Polyphenols Laboratories AS, Sandnes, Norway) was used as external standard in RP-HPLC analyses, with a different retention time (23.9 min) compared to delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin), that was eluted at a longer retention time (25.8 min for cis-nasunin and 26.1 min for trans-nasunin, respectively). As for nasunin quantification, a partially purified standard was used according to Lo Scalzo et al. [31]. The results were expressed as mg·100 g<sup>-1</sup> of peel fw; the limit of detection was 2.00 mg·100 g<sup>-1</sup> of peel dw.

Glycoalkaloids were extracted as described by Birner [32] with some modifications. Glycoalkaloid extraction was detected from 0.5 g lyophilized samples and powdered flesh tissue by 95% ethanol. The analyses were carried out by means of RP-HPLC according to Kuronen et al. [33] using partially purified solasonine and solamargine as the external standard. The data were expressed as mg·100 g<sup>-1</sup> fw; the limit of detection was 0.03 mg·100 g<sup>-1</sup> of dw.

## 2.6. Statistical Analysis

Data were statistically analyzed by a two-way analysis of variance (ANOVA) (year (Y) × rootstock (R)) using SPSS software package version 14.0 (StatSoft, Inc., Chicago, IL, USA). Proteins, metals, total anthocyanins, chlorogenic acid and glycoalkaloids, which were determined only in the second trial (2015), were analyzed via one-factor analysis of variance (ANOVA) using a fixed-effects model for the effect of rootstock treatment. For data expressed in percentage, the arcsin transformation before ANOVA analysis [ $\varnothing = \arcsin(p/100)^{1/2}$ ] was applied. To separate treatment means within each measured parameter, Tukey HSD test was performed at  $p = 0.05$ .

## 3. Results

The interaction between rootstock and year was always not significant; therefore, for the sake of simplicity it was omitted from the tables and graphs. Similarly, the factor year was statistically significant only for first flower formation and is reported only in the correspondent table.

### 3.1. Grafting Success and Plant Biometric Parameters

Rootstock significantly affected grafting success, number of leaves at 50 DAT and aboveground biomass (Table 1).

**Table 1.** Year and rootstock effects on grafting success and plant biometric parameters of ‘Scarlati’ F<sub>1</sub> scion.

Treatments	Grafting Success (%)	No. Leaves 50 DAT (No.)	Aboveground Biomass (kg)	First Flower Formation (DAT)
Rootstock				
‘Scarlati’ ungrafted	-	17.5 b	4.5 ab	53.1 a
<i>S. torvum</i>	99.3 a	24.3 ab	5.1 a	49.0 d
‘Scarlati’ self-grafted	99.6 a	25.4 a	4.9 a	52.9 ab
Hybrid <i>S. melongena</i> × <i>S. aethiopicum</i>	99.0 a	21.1 ab	4.6 ab	49.3 d
<i>S. aethiopicum</i> (accession 1)	93.6 b	27.3 a	3.7 c	51.9 bc
<i>S. aethiopicum</i> (accession 2)	89.1 b	17.6 b	4.0 bc	51.5 c
Year				
2014	96.5 a	22.3 a	4.4 a	52.0 a
2015	95.8 a	22.1 a	4.5 a	50.6 b
Significance				
Rootstock	***	**	***	***
Year	NS	NS	NS	***

DAT, days after transplanting. Values within a column and a year followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey HSD Test). The significance is designated by asterisks as follows: \*, statistically significant differences at  $p$ -value below 0.05; \*\*, statistically significant differences at  $p$ -value below 0.01; \*\*\*, statistically significant differences at  $p$ -value below 0.001; NS = not significant.

The tube grafting method proved highly efficient with success percentages that ranged from 89.1 to 99.6% among the materials used (Table 1). No significant differences were found in terms of success rate among ‘Scarlati’, *S. torvum*, and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks, which showed percentages of graft success ranging from 99% (*S. melongena* × *S. aethiopicum* gr. *gilo* hybrid) to 99.6% (‘Scarlati’). In contrast, *S. aethiopicum* gr. *gilo* (accessions 1 and 2) had a significantly lower percentage of success (93.6 and 89.1%, respectively) with respect to the other rootstocks (Table 1). No visible disaffinity signals at the grafting zone were observed for any rootstock–scion combination.

The mean number of leaves at 50 DAT varied between 17.5 and 27.3 leaves per plant for the ‘Scarlati’ ungrafted and *S. aethiopicum* gr. *gilo* (accession 1) rootstock, respectively (Table 1). ‘Scarlati’ scions grafted onto *S. melongena* (‘Scarlati’) and *S. aethiopicum* gr. *gilo* (accession 1) rootstocks had a higher number of leaves at 50 DAT than those ungrafted or grafted onto *S. aethiopicum* gr. *gilo* (accession 2). However, eggplant plants grafted onto *S. torvum* and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid did not significantly differ from those grafted onto the other rootstocks tested.

Aboveground biomass produced ranged from 5.1 kg for ‘Scarlati’ grafted on *S. torvum* rootstock to 3.7 kg for those grafted on *S. aethiopicum* gr. *gilo* (accession 1) rootstock (Table 1). Plants grafted onto *S. torvum* rootstock did not significantly differ compared to those grafted onto *S. melongena* (self-grafted plants), whereas the lowest aboveground biomass values were collected from eggplant plants grafted onto *S. aethiopicum* gr. *gilo* (accessions 1 and 2) (3.7 and 4.0 kg).

Regardless of the rootstock, the second trial (2015) gave the shortest time of first flower formation (50.6 DAT), whereas, irrespective of the year, plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid and *S. torvum* gave the shortest time of first flower formation (49.3 and 49.0 DAT, respectively). ‘Scarlati’ ungrafted had the longest first flower formation (53.1 DAT) (Table 1).

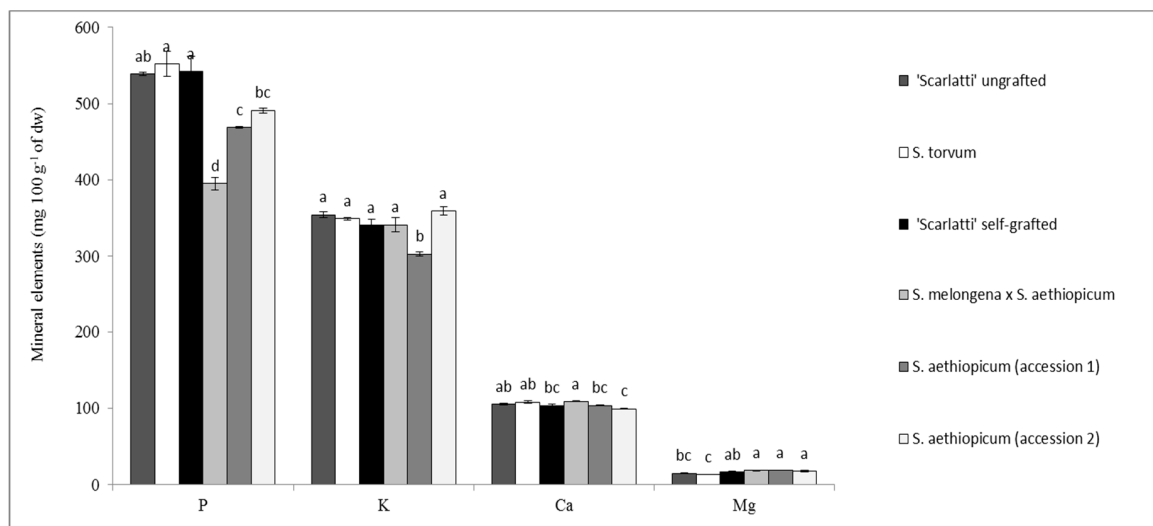
Rootstock significantly influenced plant height at 50, 80, and 110 DAT (Figure 1).

Plants grafted onto *S. aethiopicum* gr. *gilo* (accession 1) had the highest plant height, whereas plants grafted onto *S. aethiopicum* gr. *gilo* (accession 2) had the lowest values (Figure S2).

At 80 DAT, no significant differences were found in plant height among ‘Scarlati’, *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1), and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks. *S. aethiopicum* gr. *gilo* (accession 2) had a significantly lower plant height value with respect to the other rootstocks (Figure 1). However, plant height at 50 and 110 DAT, in ‘Scarlati’ F<sub>1</sub> grafted onto

*S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks did not significantly differ from those grafted onto *S. aethiopicum* gr. *gilo* (accession 2).

Rootstock significantly influenced root collar at 50, 80, and 110 DAT (Figure S3). Plants grafted onto *S. aethiopicum* gr. *gilo* (accession 2) and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks had the lowest root collar values, whereas plants grafted on *S. aethiopicum* gr. *gilo* (accession 1) had the highest value (Figure S3).



**Figure 1.** Mineral elements of ‘Scarlati’ eggplant fruits produced from ungrafted, self-grafted, and grafted onto *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1), *S. aethiopicum* gr. *gilo* (accession 2), and *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks. Bars with different letters are significant by Tukey HSD Test ( $p < 0.05$ ).

### 3.2. Yield

Rootstock significantly influenced total yield, marketable yield and number of marketable fruits (Table 2).

**Table 2.** Rootstock effects on yield traits of ‘Scarlati’ F<sub>1</sub> scion.

Treatments	Total Yield Plant <sup>-1</sup> (kg)	Marketable Yield Plant <sup>-1</sup> (kg)	No. Marketable Fruits Plant <sup>-1</sup> (No.)	Average Fruit Weight (g)
Rootstock				
‘Scarlati’ ungrafted	3.4 ab	3.3 ab	14.5 a	231.3 ns
<i>S. torvum</i>	3.8 a	3.6 a	16.6 a	216.8 ns
‘Scarlati’ self-grafted	3.9 a	3.6 a	16.2 a	223.5 ns
Hybrid <i>S. melongena</i> × <i>S. aethiopicum</i>	3.4 ab	3.2 ab	15.1 a	213.9 ns
<i>S. aethiopicum</i> (accession 1)	2.7 c	2.5 c	9.1 b	330.1 ns
<i>S. aethiopicum</i> (accession 2)	3.1 bc	2.9 bc	14.1 a	207.5 ns
Significance				
Rootstock	***	**	***	NS

Values within a column and a year followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey HSD Test). The significance is designated by asterisks as follows: \*, statistically significant differences at  $p$ -value below 0.05; \*\*, statistically significant differences at  $p$ -value below 0.01; \*\*\*, statistically significant differences at  $p$ -value below 0.001; NS = not significant.

Total yield ranged between 2.7 kg plant<sup>-1</sup> for ‘Scarlati’ grafted on *S. aethiopicum* gr. *gilo* (accession 1) and 3.9 kg plant<sup>-1</sup> for those grafted onto *S. melongena* (self-grafted plants) (Table 2). No significant differences were found in the total yield among ‘Scarlati’ and *S. torvum* rootstocks, which had total yield that ranged from 3.8 kg plant<sup>-1</sup> (*S. torvum*) to 3.9 kg plant<sup>-1</sup> (‘Scarlati’ self-grafted). *S. aethiopicum*

gr. *gilo* (accessions 1 and 2) had a significantly lower total yield (2.7 and 3.1 kg plant<sup>-1</sup>, respectively) than the other rootstocks (Table 2). However, plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* rootstock and ‘Scarlati’ ungrafted plants did not significantly differ in total yield compared to the plants grafted onto ‘Scarlati’ and *S. torvum* rootstocks. Data collected on marketable yield supported the trend established for total yield (Table 2). Plants with *S. aethiopicum* gr. *gilo* (accession 1) as rootstock had a significantly lower fruit number in comparison to those grafted onto ‘Scarlati’, *S. torvum*, and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid, and not different from those grafted onto *S. aethiopicum* gr. *gilo* (accession 2) (Table 2). In addition, no differences were found among plants grafted onto ‘Scarlati’, *S. torvum*, *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid, and *S. aethiopicum* gr. *gilo* (accession 2) for number of marketable fruits plant<sup>-1</sup> which produced a significantly higher number of fruits than accession 1 of *S. aethiopicum* gr. *gilo*. No significant differences among treatments were found for the average fruit weight (Table 2).

### 3.3. Apparent Fruit Quality

Rootstock significantly influenced fruit length and fruit calyx length parameters (Table S1). Fruit from plants grafted on *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock were significantly more elongated than those from plants grafted onto ‘Scarlati’ rootstock or from ungrafted ‘Scarlati’ plants (Table S1), which in turn had more elongated fruits than those from plants grafted onto *S. aethiopicum* gr. *gilo* (accession 2).

Fruit calyx length in ‘Scarlati’ scion grafted onto *S. aethiopicum* gr. *gilo* × *S. melongena* hybrid rootstock was significantly higher than in plants grafted onto *S. torvum*; however, fruits from self-grafted and ungrafted ‘Scarlati’ plants did not significantly differ neither from fruits from plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid nor from those from plants grafted onto *S. torvum*. No significant differences among rootstocks tested were found for fruit width (average of 4.7 cm), fruit length–width ratio (average of 3.9), fruit curvature (average of 1.0), fruit cross-section (average of 7.1), fruit calyx prickles (average of 0.2), or seeds index (average of 1.1) (Table S1).

### 3.4. Intrinsic and Extrinsic Fruit Quality

Hue color parameter in fruits from plants grafted onto *S. torvum* and *S. aethiopicum* gr. *gilo* (accession 1) had the highest values (360.3 and 360.2, respectively) (Table 3).

**Table 3.** Rootstock effects on Hue°, fruit dry matter, firmness, and soluble solids content (SSC) in ‘Scarlati’ F<sub>1</sub> scion.

Treatments	H°	Fruit Dry Matter (%)	Firmness (N)	SSC (°Brix)
Rootstock				
Scarlati’ ungrafted	360.1 b	7.3 ns	60.0 ns	4.5 b
<i>S. torvum</i>	360.3 a	7.2 ns	43.2 ns	3.9 c
Scarlati’ self-grafted	360.1 b	6.8 ns	44.2 ns	4.1 c
Hybrid <i>S. melongena</i> × <i>S. aethiopicum</i>	360.0 b	6.8 ns	49.0 ns	4.6 ab
<i>S. aethiopicum</i> (accession 1)	360.2 ab	6.9 ns	50.6 ns	4.6 ab
<i>S. aethiopicum</i> (accession 2)	360.1 b	6.8 ns	38.8 ns	4.9 a
Significance				
Rootstock	***	NS	NS	***

Values within a column and a year followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey HSD Test). The significance is designated by asterisks as follows: \*, statistically significant differences at  $p$ -value below 0.05; \*\*, statistically significant differences at  $p$ -value below 0.01; \*\*\*, statistically significant differences at  $p$ -value below 0.001; NS = not significant.

Treatments tested had no effects on fruit dry matter and firmness (Table 3).

Rootstock significantly influenced SSC (Table 3). The highest value of SSC (4.6, 4.9 and 4.6 °Brix, respectively) were found in plants grafted onto *S. aethiopicum* gr. *gilo* (accessions 1 and 2) and *S.*



*melongena* × *S. aethiopicum* gr. *gilo* hybrid, whereas the lowest value (3.9 and 4.1 °Brix, respectively) were observed in plants grafted onto *S. torvum* and ‘Scarlati’ (Table 3).

‘Scarlati’ scions grafted onto *S. aethiopicum* gr. *gilo* (accession 1) and *S. melongena* × *S. aethiopicum* gr. *gilo*, had the highest values of L<sub>0</sub> central area (Table 4), while fruits from plants grafted onto *S. aethiopicum* gr. *gilo* (accession 2) rootstocks had the lowest value.

**Table 4.** Rootstock effect on pulp lightness measured immediately after cutting (L<sub>0</sub>) in the central and lateral area and the fruit browning ( $\Delta L_{30}$ ) of the lateral area in ‘Scarlati’ F<sub>1</sub> scion.

Treatments	L <sub>0</sub> Central Area	L <sub>0</sub> Lateral Area	$\Delta L_{30}$ Lateral Area
Rootstock			
‘Scarlati’ ungrafted	82.4 bc	80.6 b	2.5 bc
<i>S. torvum</i>	82.9 b	81.0 b	2.3 c
‘Scarlati’ self-grafted	82.5 b	80.6 b	2.5 bc
Hybrid <i>S. melongena</i> × <i>S. aethiopicum</i>	83.5 ab	81.5 ab	2.7 ab
<i>S. aethiopicum</i> (accession 1)	84.8 a	82.9 a	2.7 ab
<i>S. aethiopicum</i> (accession 2)	80.7 c	78.9 c	2.9 a
Significance			
Rootstock	***	***	***

Values within a column and a year followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey HSD Test). The significance is designated by asterisks as follows: \*, statistically significant differences at  $p$ -value below 0.05; \*\*, statistically significant differences at  $p$ -value below 0.01; \*\*\*, statistically significant differences at  $p$ -value below 0.001; NS = not significant.

Data collected for L<sub>0</sub> lateral area supported the trend established for L<sub>0</sub> central area (Table 4).

*S. aethiopicum* gr. *gilo* (accessions 1 and 2) and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks induced the highest  $\Delta L_{30}$  lateral area values (Table 4). While, *S. torvum* rootstock induced the lowest one.

ANOVA for total anthocyanins was not statistically significant (Table 5).

**Table 5.** Rootstock effect on total anthocyanins, glycoalkaloids, chlorogenic acid, and proteins in ‘Scarlati’ F<sub>1</sub> scion.

Treatments	Total Anthocyanins (mg·100 g <sup>-1</sup> of fw)	Glycoalkaloids (mg·100 g <sup>-1</sup> of fw)	Chlorogenic Acid (mg·100 g <sup>-1</sup> of fw)	Proteins (g·100 g <sup>-1</sup> of fw)
Rootstock				
‘Scarlati’ ungrafted	60.6 ns	8.4 a	21.7 ab	1.0 ab
<i>S. torvum</i>	43.0 ns	1.0 b	40.3 a	1.0 ab
‘Scarlati’ self-grafted	47.8 ns	2.7 b	34.1 a	1.0 ab
Hybrid <i>S. melongena</i> × <i>S. aethiopicum</i>	70.8 ns	8.6 a	4.4 b	0.9 bc
<i>S. aethiopicum</i> (accession 1)	80.0 ns	3.7 b	40.0 a	1.1 ab
<i>S. aethiopicum</i> (accession 2)	106.4 ns	1.9 b	17.9 ab	0.8 d
Significance				
Rootstock	NS	*	**	***

Values within a column and a year followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey HSD Test). The significance is designated by asterisks as follows: \*, statistically significant differences at  $p$ -value below 0.05; \*\*, statistically significant differences at  $p$ -value below 0.01; \*\*\*, statistically significant differences at  $p$ -value below 0.001; NS = not significant; fw = fresh weight.

The highest glycoalkaloids content was observed in fruits from ungrafted plants and in those from plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks (Table 5).

Fruits from ‘Scarlati’ grafted on *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1), and ‘Scarlati’ self-grafted plants had the highest chlorogenic acid content, whereas, fruits from plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock showed the lowest value (Table 5).

We found that protein content in fruits from plants grafted onto *S. aethiopicum* gr. *gilo* (accession 1) was slightly higher than those from ‘Scarlatti’ ungrafted, self-grafted, and *S. torvum* grafted plants. The lowest protein content was detected in fruits from plants grafted onto *S. aethiopicum* gr. *gilo* (accession 2) (Table 5).

Rootstock significantly affected mineral content (Figure 1).

Ungrafted, self-grafted, and *S. torvum* rootstock grafted plants had the highest fruit P content (538, 551, and 541 mg·100 g<sup>-1</sup> of dw, respectively). The lowest fruit P content value (395.4 mg·100 g<sup>-1</sup> of dw) was found in *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock (Figure 1). Fruits harvested from plants grafted on *S. aethiopicum* gr. *gilo* (accession 1) rootstock had a significantly lower K content (302.9 mg·100 g<sup>-1</sup> of dw) in comparison to those grafted onto ‘Scarlatti’, *S. torvum*, *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid, *S. aethiopicum* gr. *gilo* (accession 2), and ‘Scarlatti ungrafted’ (341.0, 349.0, 341.3, 359.1, and 354.4 mg·100 g<sup>-1</sup> of dw, respectively) (Figure 1). Moreover, no significant differences were found among plants grafted onto ‘Scarlatti’, *S. torvum*, *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid, *S. aethiopicum* gr. *gilo* (accession 2), and ‘Scarlatti ungrafted’ for fruit K content. ‘Scarlatti’ grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid gave the highest fruit Ca content (108.9 mg·100 g<sup>-1</sup> of dw), whereas fruits from plants grafted *S. aethiopicum* gr. *gilo* (accession 2) gave the lowest Ca fruit content (99.4 mg·100 g<sup>-1</sup> of dw) (Figure 1). ‘Scarlatti’ grafted onto *S. aethiopicum* gr. *gilo* (accessions 1 and 2) and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid had the highest Mg content (19.3, 18.1, and 18.6 mg 100 g<sup>-1</sup> of dw, respectively) (Figure 1), whereas the lowest Mg contents were found in fruits from ‘Scarlatti’ grafted on *S. torvum* (13.8 mg·100 g<sup>-1</sup> of dw).

#### 4. Discussion

Nowadays, both a high yield performance and fruit quality attributes (intrinsic and extrinsic) are very important due to the functional role of vegetables in the human diet [29].

Along with approaches such as genetic improvement and optimization of the cultivation conditions, herbaceous grafting technique represents a toolbox for securing or increasing yield stability and fruit quality with or without stress conditions [26,34–37]. In this paper, we investigated the effects of *S. aethiopicum* gr. *gilo* (accessions 1 and 2) versus the interspecific hybrid of *S. melongena* × *S. aethiopicum* gr. *gilo* on plant performance and fruit quality attributes of ‘Scarlatti’ eggplant F<sub>1</sub> hybrid (black cylindrical shape). All the results are compared with those obtained from non-grafted, self-grafted, and *S. torvum* rootstock grafted plants.

Grafting success is an important concern when using new scion–rootstock combinations [37]. Generally, Solanaceous crops are grafted by cleft or tube grafting methods [4,20,38]. In our experiment using the tube grafting technique, grafting success rates of 89.1% for *S. aethiopicum* gr. *gilo* (accession 1), 93.6% for *S. aethiopicum* gr. *gilo* (accession 2), and over 99% for grafting onto ‘Scarlatti’, *S. torvum*, and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid, were observed. Our findings are in accord with those obtained by Bletsos et al. [4] and Rahman et al. [39], who reported a high grafting success rate of eggplant cultivars grafted onto *S. torvum*. Although, *Solanum aethiopicum* gr. *gilo* is a close relative species of *S. melongena* [16], from our results it appears that graft incompatibility might exist especially when ‘Scarlatti’ is grafted onto *S. aethiopicum* gr. *gilo* accession 2. Thus, we may assume that the genotype is a crucial aspect for the identification of a suitable rootstock as significant differences may exist between different accessions of a species. Regarding the *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock, our outcomes are in accord with those obtained by Gisbert et al. [9], who reported a high graft success rate when interspecific hybrids of eggplants were used as rootstocks. Our results are also consistent with those of Sabatino et al. [10], who reported a high graft success rate of eggplant ‘Birgah’ F<sub>1</sub> hybrid (violet globose shape) grafted onto *S. melongena* × *S. aethiopicum* rootstock, confirming the capacity of this interspecific hybrid to give good results with two different types of common eggplant. In fact, Birgah is one of the violet F<sub>1</sub> hybrid belonging to a local Sicilian fruit typology that has been subjected to lower breeding improvement efforts when compared to elongated black-purple typelike ‘Scarlatti’, which is world-wide diffused especially in the most industrialized countries. Our experiment confirms

that rootstock might play a major role on plant biometric parameters. Plant height, which may be considered an indicator of vigor was highest in plants with *S. aethiopicum* gr. *gilo* (accession 1), *S. torvum*, and interspecific hybrid *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks and lowest in those plants with *S. aethiopicum* gr. *gilo* (accession 2) rootstock grafts. Therefore, vigor of the rootstock is important in conferring scion vigor. In the absence of grafting incompatibility signals, grafted plants may also develop more rapidly, hence contributing to earliness. In our work, plants grafted onto *S. torvum* and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstocks flowered earlier than those grafted on the other rootstocks tested. Our results are consistent with those obtained by Gisbert et al. [9] and Sabatino et al. [10], who reported that plant vigor is positively related to fruit earliness. Increased earliness has also been reported for other fruiting vegetables such as melon grafted onto *Cucurbita* rootstock [40,41]. In our experiment, grafting has proved a useful technique to enhance eggplant ‘Scarlati’ F<sub>1</sub> hybrid yield traits. We also found that plants grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid rootstock had higher total and marketable yield plant<sup>-1</sup> than plants grafted onto *S. aethiopicum* gr. *gilo* (accessions 1 and 2), confirming that this latter rootstock has little value for enhancing eggplant yield. Conversely, interspecific hybrid of *S. melongena* × *S. aethiopicum* gr. *gilo* rootstock demonstrated great improvements in agronomic performance due to grafting. However, in our study grafting did not increase the number of marketable fruits. These findings are different from those of Gisbert et al. [9] and Sabatino et al. [10], who reported that eggplants grafted onto interspecific hybrids produced consistently more fruits per plant than ungrafted ones. However, despite in the present work grafting did not significantly improve the number of fruits per plant, the data recorded in our experiment supported the trend established in the experiments by Gisbert et al. [9] and Sabatino et al. [10]. On the other hand, these findings seem to be consistent with those obtained by Maršič et al. [42], who reported that, in the second trial, grafting did not increase the number of fruit per plant in eggplant ‘Galine’ F<sub>1</sub> (eggplant cylindrical shape). Therefore, it seems that the number of fruit per plant is a yield component influenced by the genetic background of the rootstock scion combination.

Apparent visual quality is an important criterion when consumers decide to purchase eggplant fruits. In our study, rootstocks affect fruit length and fruit calyx length. Our results are in accord with those obtained by Gisbert et al. [9], who hypothesized that fruit shape changes are probably due to changes in the concentration of growth regulators induced by the rootstock. Our findings on apparent visual quality are partially different from those of Sabatino et al. [10], who reported that rootstock significantly influences fruit width but has no significant effect on calyx length. Thus, it seems that, although fruit shape in eggplant is highly heritable and under genetic control [43,44] and even though rootstocks may affect cultivar fruit shape parameters due to changes in the concentration of growth regulators [9], rootstock may differently affect specific fruit shape characters in relation to the scion genotype.

Our results demonstrated that rootstock may enhance H° color parameter of skin eggplant fruit. Our outcomes are in contrast with those of Moncada et al. [45], who revealed that *S. torvum* rootstock decreased H° parameter in ‘Black Bell’, ‘Black Moon’, and ‘Longo’ eggplants. Thus, it seems that fruit skin color parameters are differently affected by scion–rootstock combination. Moreover, in our study, fruits from plants grafted onto *S. torvum* and *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid had the highest H° color parameter value, which is an important commercial and qualitative prerequisite in eggplant fruit. Eggplant ranks among the top 10 vegetables in terms of antioxidant fruit activity. Accordingly to these data, a high fruit pulp browning potential could be expected [46,47]. Mishra et al. [46], King et al. [48], and Prohens et al. [49] revealed that eggplant cultivars differed in their extents of post-cut browning, which could be due to variations in the PPO activity or level of soluble phenolics. Our results on pulp browning are consistent with the findings of Sabatino et al. [10], who reported little or no effect of grafting by using *S. torvum* rootstock. However, we also found that fruits from plant grafted onto *S. aethiopicum* gr. *gilo* (accessions 1 and 2) revealed the highest value in terms of  $\Delta L_{30}$ .

Mennella et al. [19] revealed that chlorogenic acid is the major monomeric phenolic compound in eggplant fruits. Although few differences were found in fruit composition traits, higher fruit chlorogenic acid content was found in fruits harvested from plants with 'Scarlati', *S. torvum*, *S. aethiopicum* gr. *gilo* (accessions 1 and 2) rootstocks, and ungrafted plants in comparison to fruits harvested from 'Scarlati' scions grafted onto *S. melongena* × *S. aethiopicum* gr. *gilo* hybrid. This higher chlorogenic acid concentration may be an additional indication of stress in this rootstock–scion combination, as stress conditions induce accumulation of phenolics [50,51]. Our results are dissimilar from those of Sabatino et al. [10], who reported no differences in terms of fruit chlorogenic acid content among rootstocks tested, but are consistent with those of Sabatino et al. [26] who reported that grafting significantly increased phenolic concentration in fruits from grafted eggplant landraces only in three out of four genotypes. Our outcomes on phenolic content are also consistent with those of Maršič et al. [42] who demonstrated that grafting significantly increased phenolic concentration in fruits from a grafted eggplant landrace as opposed to commercial varieties. Moreover, Toppino et al. [52] discovered environment-specific QTLs associated to the amount of chlorogenic acid. Accordingly, it seems that fruit phenolic content in eggplant is a quality trait also affected by scion–rootstock interactions. Mennella et al. [19], Friedman and McDonald [53], and Friedman [54] observed that genetic, environment, and growing conditions may affect glycoalkaloids concentration in potato, tomato, and eggplant. In addition, Jones and Fenwick [55] and Krits et al. [56] assessed that the level of total glycoalkaloids in potato tubers should not exceed 200 mg·kg<sup>-1</sup> of fw (or 200 mg·100 g<sup>-1</sup> of dw). Glycoalkaloids concentration ranged from 0.98 to 8.64 mg·100 g<sup>-1</sup> of fresh weight. Rootstock affected glycoalkaloids concentration in eggplant; however, it is important to remark that glycoalkaloids amount in fruits from grafted plant remained below the recommended threshold value. Our findings are in contrast with those of Sabatino et al. [10], who observed no significant differences in terms of glycoalkaloids concentration in 'Birgah' F<sub>1</sub> eggplant fruits from plants grafted onto several potential rootstocks including hybrids and allied species.

Generally, changes in overall fruit composition between grafted and ungrafted plants were reported in tomato [57] and pepper [58]. Our results are in accord with those of Sabatino et al. [10], who found some differences in fruit quality attributes among fruits from ungrafted or self-grafted plants.

## 5. Conclusions

After the ban of methyl bromide use for soil fumigation, the soilborne diseases arising from continuous cropping are major issues that negatively affect productivity in eggplant. In this scenario, grafting is an effective tool to control vegetable crop stresses. Nowadays, *S. torvum* and tomato hybrids are the main eggplant rootstocks commercially used. However, investigations on the influence of new rootstocks on biometric parameters, yield performance, and fruit quality characteristics of different eggplant groups can provide further advances in this specific grafting field. In the present study, the interspecific hybrid of *S. melongena* × *S. aethiopicum* gr. *gilo* exhibited high grafting compatibility and high yield performance. These findings, together with the absence of negative effects on apparent 'Scarlati' F<sub>1</sub> hybrid fruit quality and composition, demonstrate that this interspecific hybrid may be a viable rootstock alternative to *S. torvum*.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/5/223/s1>, Figure S1: Monthly meteorological data from May to August of 2014 and 2015 from the meteorological station of the experimental farm of the Department of Agricultural, Alimentary and Forest Sciences, University of Palermo, Italy, Figure S2: Plant height evolution of 'Scarlati' eggplant plant ungrafted, self-grafted and grafted onto *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1), *S. aethiopicum* gr. *gilo* (accession 2) and *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks. Bars indicate the standard error of the mean. ANOVA analysis for plant height at 50, 80, and 110 DAT showed the following significance: \*\*\* (statistically significant differences at *p*-value below 0.001), \*\*\* and \* (statistically significant differences at *p*-value below 0.05), respectively, Figure S3: Root collar evolution of 'Scarlati' eggplant plant ungrafted, self-grafted and grafted onto *S. torvum*, *S. aethiopicum* gr. *gilo* (accession 1), *S. aethiopicum* gr. *gilo* (accession 2) and *S. melongena* × *S. aethiopicum* gr. *gilo* rootstocks. Bars indicate the standard error of the mean. ANOVA analysis for root collar at 50, 80, and 110 DAT showed the

following significance: \*\*\* (statistically significant differences at  $p$ -value below 0.001), \*\*\* and \*\*\*, respectively. Table S1: Rootstock effects on apparent fruit quality in 'Scarlati' F1 scion.

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