Rootstock and Arbuscular Mycorrhiza Combinatorial Effects on Eggplant Crop Performance and Fruit Quality under Greenhouse Conditions

Leo Sabatino [1,*] [ID]**, Giovanni Iapichino** [1,*]**, Beppe Benedetto Consentino** [1] [ID]**, Fabio D'Anna** [1] **and Youssef Rouphael** [2] [ID]

[1] Dipartimento Scienze Agrarie, Alimentari e Forestali (SAAF), Università di Palermo, 90128 Palermo, Italy; beppebenedetto.consentino@unipa.it (B.B.C.); fabio.danna@unipa.it (F.D.)

[2] Dipartimento di Agraria, Università degli Studi di Napoli Federico II, 80055 Portici, Italy; youssef.rouphael@unina.it

[*] Correspondence: leo.sabatino@unipa.it (L.S.); giovanni.iapichino@unipa.it (G.I.); Tel.: +39-091-2386-2252 (L.S.); +39-091-2386-2215 (G.I.)

Abstract: The herbaceous grafting of fruiting vegetables is considered a toolbox for safeguarding yield stability under various distresses and for improving fruit quality. Inoculation with arbuscular mycorrhiza (AM) fungi seems also to be an efficient tool for increasing the assimilation, uptake and translocation of macroelements and microelements, for modulating plant secondary metabolism and for overcoming several forms of plant distress. The present work evaluated the combined effect of grafting the "Birgah" (B) eggplant onto its wild/allied relatives' rootstocks (*Solanum torvum* (T), *S. macrocarpon* (M) and *S. paniculatum* (P)) and AM fungi (*R. irregularis*) on the yield, fruit quality, nitrogen use efficiency, mineral profile, and nutritional and functional quality. The B/T, B/M and B/P grafting combinations significantly increased the marketable fruit and fruit number compared with those in the ungrafted control. Furthermore, irrespective of the grafting combinations, AM fungi significantly enhanced the marketable fruit, fruit number and nitrogen use efficiency (NUE) by 13.3%, 12.7% and 13.3%, respectively compared to those in the untreated control. Exposing the B/T and B/P grafted plants to the +AM treatment significantly increased the ascorbic acid contents by 17.2% and 10.4%, respectively, compared with those in the ungrafted control. Fruits from the combination B/P × +AM had a higher chlorogenic acid content than fruits from the ungrafted control plots. Finally, the B/T × +AM and B/P × +AM combinations decreased glycoalkaloids by 58.7% and 63.7%, respectively, compared with those in the ungrafted control, which represents a highly important target for eggplant fruit healthiness.

Keywords: vegetable grafting; *Solanum melongena* L.; grafting combinations; arbuscular micorrhizal fungi; yield traits; NUE; mineral profile; functional properties

1. Introduction

Eggplant (*Solanum melongena* L.) is a major solanaceous vegetable crop cultivated worldwide [1]. Eggplant originated in the Old World, evolved from *S. insanum*, and it was self-sufficiently domesticated in India and China [2,3]. With a global production around 49.5 Mt, predominantly in Asia [4], eggplant is a relevant source of minerals and vitamins, and in terms of total nutritional value, it has been compared to tomato [1]. *S. macrocarpon* L., also known as African eggplant, and *S. aethiopicum* L., also known as Aethiopicum eggplant, are closely related to *S. melongena* and are mainly distributed in the African continent. In the eggplant cultivation scenario, the Mediterranean Basin represents an important area and Sicily is counted as a secondary eggplant diversification zone [5]. In Italy,

eggplant is grown under either greenhouse or outdoor conditions, and due to the intensive cropping systems commonly used in the vegetable production farms, soilborne diseases and pests widely spread, limiting the yield and growth traits in eggplant [6]. At present, the absence of resistant genotypes, together with the ban on methyl bromide [7], has increased interest in the use of grafted eggplant. There are reports underlining the advantages derived from adopting grafting both in terms of plant biotic/abiotic stress tolerance and yield stability [8–10]. *Solanum torvum* Sw. is the most used rootstock for eggplant as it allows several soilborne diseases to be overcome, such as *Verticilium dahliae* Klebahn, *Ralstonia solanacearum* (Smith) Yabuuchiet al., *Fusarium oxysporum* (Schlechtend:Fr.) f. sp. *Melongenae* Matuoand Ishigami, and *Meloidogyne* spp. root-knot nematodes [6,11]. However, both eggplant's wild and allied relatives and interspecific hybrids are proposed as alternative eggplant rootstocks [12–14]. The use of tomato rootstock is also suggested to improve yield, fruit visual quality and plant vigor in eggplant [15].

Crop rotations and biological diversity long have been the key factors in successful traditional agricultural production systems. Rotation is essential for minimizing the build up of pest and soil borne disease problems. However, intensive greenhouse production often precludes vegetable growers from applying rotation. The lack of rotation and tendency towards monocropping in intensive protected vegetable production systems not only increase the prevalence of insects, soil plant pathogens and nematodes but, along with high nutrient levels, can suppress arbuscular mycorrhizal fungi (AMF). AMF are obligate symbionts between plants and fungi belonging to the monophyletic phylum *Glomeromycota* [16]. AMF symbiosis is extremely important for enhancing the assimilation of key macroelements and microelements (P, Cu and Zn) and improving nutrient uptake and efficiency due to its ability to develop an extended external hypha up to 40–50 times their length [17]. Another prominent and sustainable means of boosting yield is the inoculation of beneficial AMF in specific environments such as soil greenhouses where these fungi are generally suppressed.

Moreover, recent findings demonstrated that AM may modulate plant secondary metabolism, enhancing antioxidant activity as well as the accumulation of antioxidant molecules known as phytochemicals (i.e., carotenoids, flavonoids, glucosinolates and phenolic acids) with health-promoting properties [18]. AM fungi have been reported to not only enhance nutritional status and the quality of the produce but also to be able to reduce several forms of plant distress such as thermal stress [19], salinity stress [20], drought stress [21] and soil heavy metal stress [22–25]. Furthermore, there are reports showing an increased resistance to certain diseases in mycorrhized plants [26]. Although there are several studies on the synergistic effects of grafting and other agronomical or chemical means commonly applied in vegetable crop production [27,28], few experiments have been conducted on the interactive effects of grafting and AM on plant performance and the nutritive value of vegetables. In particular, Kumar et al. [24], studying the combined role of grafting and AM on Cd stress tolerance in tomato, observed that vigorous rootstock such as Maxifort successfully alleviate Cd stress symptoms via several physiological and biochemical mechanisms, such as (i) better nutrient absorption and translocation, (ii) higher synthesis of pigments linked to photosynthetic activity and (iii) better capability of producing enzymes (CAT and APX), proline and key metabolites (phytochelatin, fructans and inulins). Furthermore, Oztekin et al. [29], after studying the effect of grafting and AM on the performance of tomato plants cultivated under different salinity conditions in two growing seasons, conclude that grafting and AM have synergistic effects on tomato plant salinity tolerance. Nevertheless, no specified research has been conducted to study the co-operative effects between using wild and allied eggplant relatives as rootstocks and AM application in improving eggplant crop performance and nutritive value. On the aforesaid basis, the aims of our investigation were to appraise the concerted action of eggplant wild/allied relatives' rootstocks and AM inoculation on the yield, fruit quality and nitrogen use efficiency of "Birgah" eggplant grown under greenhouse conditions. The outcome of this study should provide useful information on the performance of new eggplant rootstocks and on their response to AMF inoculation.

2. Materials and Methods

2.1. Trial Site, Nursery Production and Growing Settings

The investigation was conducted in 2016 at the experimental farm of the University of Palermo. Seeds of the *Solanum torvum*, *Solanum macrocarpon* and *Solanum paniculatum* rootstocks were sown in 44-cell seedling trays, containing pasteurized peat moss (FAP, Padova, Italia), in a protected environment with a day/night temperature cycle of 25 °C/18 °C. Twenty days after rootstock sowing, seeds of the "Birgah" eggplant cultivar were seeded in 104-cell trays and maintained under the same climatic conditions and with the same planting method as the rootstocks. "Birgah" is an eggplant F_1 hybrid belonging to the violet round shape group, and it is one of the most cultivated eggplants, both in open fields and in protected environments, in Sicily. Seventy-five days after planting, all seedlings attained a hypocotyl diameter of at least 2 mm (the minimum recommended for the tube grafting method). The grafting of "Birgah" scions was performed as reported by Sabatino et al. [12,13]. Briefly, the rootstock was cut off, 0.5 cm below the cotyledons, at a 45° angle, and a similar cut was performed on the scion. Care was taken to ensure that the cut surfaces fitted perfectly. To complete the grafting procedure, a plastic clip was attached at the grafting point to guarantee that the correct amount of pressure was applied. The grafted plantlets were placed in a greenhouse equipped with a fog system in order to maintain a humidity of 95% and an air temperature of 20 °C for 7 days. After that, the grafted transplants were subjected, for 3 days, to a slow dropping of the humidity, useful for plant acclimatization. Then, they were ready for transplantation. Ungrafted and self-grafted eggplants were also included.

Prior to transplanting, half of the ungrafted, self-grafted and grafted plantlets were treated with 10 g plant^{-1} of micorrhizal inoculum carrying 40 spores g^{-1} of *Rhizophagus irregularis* (formerly *Glomus intraradices*). All plantlets were transplanted on 9 February, 2016 and maintained till the end of May, 2016 in an unheated greenhouse in Typic Rhodoxeralf soil, characterized by the following soil texture: 46.5% sand, 22.3% silt and 31.2% clay. The soil pH was 7.2. Before the experiment, a brassica crop was cultivated. Mulching with a black polyethylene film of 20 μm was installed. Eggplant plantlets were spaced in order to obtain a plant density of 2 plants m^{-2}. Plants were periodically drip irrigated, receiving 250 kg of nitrogen ha^{-1}, 150 kg of phosphorous pentoxide ha^{-1} and 250 kg of potassium oxide ha^{-1}. The cultivation practices reported by Baixauli [30], suggested for eggplant growth in Mediterranean conditions, were applied regularly subject to crop requirements.

2.2. Yield, Nitrogen Use Efficiency, Nutritional Traits and Functional Compounds

Immediately after harvest, the fruits were weighed and separated into marketable and waste production categories. The number of marketable fruits per plant was recorded, and average fruit weight was also calculated. In total, ten harvests were realized, starting on 21 March, 2016.

A digital penetrometer (Trsnc, Forlì, Italy) was used to determine fruit firmness. Fruit firmness was measured based on three replicates of five fruits per scion/rootstock combination. Each fruit was perforated, using a 6 mm diameter stainless steel cylinder probe, on two opposite sides of the fruit equatorial zone. Firmness was expressed in newtons (N).

Soluble solids content (SSC) was recorded based on three replicates of five fruits and was determined using a refractometer (MTD-045nD, Three-In-OneEnterprises Co. Ltd. Taiwan). Prior to the SSC determinations, the fruit juice was adequately clarified.

Three to five commercially mature fruits for each replicate, from the second and third harvests, were used for the analytical determinations. Each sample consisted of the same quantity of apical, equatorial and distal parts of the fruits. Nutritional and functional determinations were performed on fruits harvested from labelled flowers at the fruit set stage, and all the fruits were harvested 35 days after labelling (fruit commercial maturity stage).

Fruit dry weight was measured via the dehydration of the sample in a heater at 80 °C until a constant weight was achieved. The fruit dry matter percentage was calculated using the fruit fresh and

dry weights. For protein determination, the Kjeldal method was applied. Specifically, sample was exposed to acid-catalyzed mineralization to convert the organic nitrogen into ammoniacal nitrogen. Subsequently, the ammoniacal nitrogen was distilled under alkaline pH. The ammonia produced via the distillation was collected in a boric acid solution and measured through titrimetric dosage. The ammoniacal nitrogen value was multiplied by 6.25.

For Ca, Mg, K and Fe determinations, atomic absorption spectroscopy following wet mineralization was adopted as reported by Morand and Gullo [31]. Phosphorus values were assessed using colorimetry as reported by Fogg and Wilkinson [32].

The nitrogen use efficiency (NUE = yield/N application rate) was calculated and expressed as $t \cdot kg^{-1}$.

The ascorbic acid content was determined as described by Sabatino et al. [33] for tomato. Therefore, the determinations were made with a reflectometer (Merck RQflex∗ 10 m) using Reflectoquant Ascorbic Acid Test Strips. Thus, one gram of eggplant fruit juice was mixed with distilled water to a 10 mL total volume. Subsequently, an appropriate test strip was immersed into the prepared sample then inserted into the meter. The results were expressed as mg of ascorbic acid per 100 g of fresh weight.

The anthocyanin extraction procedure applied was that described by Mennella et al. [34]. The determination was conducted on a lyophilized and powdered sample of 200 mg. The flow rate and absorbance units full scale adopted were 0.8 mL min^{-1} and at 0.1, respectively. For RP-HPLC analyses, purified delphinidin-3-rutinoside (D3R, Polyphenols Laboratories AS, Sandnes, Norway) was used as an external standard with a dissimilar retention time (23.9 min) compared to delphinidin-3-(p-coumaroyl rutinoside)-5-glucoside (nasunin), which was eluted with a longer retention time (25.8 min for cis-nasunin and 26.1 min for trans-nasunin, respectively). Regarding the nasunin determination, in agreement with Lo Scalzo et al. [35], a partially purified standard was used. Total anthocyanins were expressed as mg·100 g^{-1} dry weight (dw), highlighting that the detection limit was 2.00 mg 100 g^{-1} of dw.

For chlorogenic acid determination, the extraction and analysis procedure described by Stommel and Whitaker [36], with slight variations, was applied. Therefore, a binary mobile phase gradient of methanol in 0.01% aqueous phosphoric acid was provided 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^{-1}. The chlorogenic acid quantification, subsequently conducted by RP-HPLC separation, was based on the absorbance at 325 nm relative to the external standard of chlorogenic acid (Sigma-Aldrich, St.Louis, MO) and expressed as mg·100 g^{-1} of dw.

For glycoalkaloids, the extraction method adopted was that reported by Birner [37] with some adjustments. Thus, 0.5 g of lyophilized and powdered flesh tissue sample was extracted with 95% ethanol. For glycoalkaloids (expressed as mg·100 g^{-1}dw) analyses, the method described by Kuronen et al. [38] was applied. Therefore, the analyses were conducted by means of RP-HPLC using purified solasonine and solamargine as the external standards. The detection limit was 0.03 mg 100 g^{-1} of dw.

2.3. Root Colonization Assessment

To evaluate mycorrhizal colonization, the method described by Phillips and Haymann [39] and revised by Torta et al. [40] was adopted. Briefly, three lateral root samples from mychorrhized plants were collected and marked with acid fuchsin. As reported by Kormanik and McGraw [41], mycorrhizal colonization (Mycorrhization Index (MI = % of marked tissue, with respect to the hyaline portion, on the unit of the length of the root)) was evaluated on three fragments, attaining the average value.

2.4. Experimental Design and Statistical Analysis

The trial was organized in a randomized complete block design with 3 replicates per treatment. Each replicate consisted of 10 plants. Consequently, the total number of plants was 300 (10 treatments × 30 plants per treatment = 300 plants). The data were subjected to GLM (General Linear Model)

analysis using the SPSS software package version 20.0. For data expressed in percentages, an arcsin transformation before ANOVA analysis ($\emptyset = \arcsin(p/100)^{1/2}$) was performed. Tukey's HSD test ($p < 0.05$) was used to compare means. Principal component analysis (PCA) was conducted on the whole yield, fruit quality and nitrogen use efficiency data set.

3. Results

3.1. Root Colonization by AM Fungi

AM fungi root colonization was significantly affected by mycorrhizal inoculation (M), whereas the grafting combination (G) did not significantly influence AM root colonization. ANOVA showed no significant interaction (G × M) (Figure S1). Irrespective of the grafting combination, the percentage of root colonization was higher with the inoculated treatment (71.8%) compared to with the non-inoculated one (2.5%) (Figure S1).

3.2. Effect of Grafting Combination and Mycorrhizal Inoculation on Yield, Yield Components and NUE

The marketable yield, number of marketable fruit per plant and percentage of unmarketable production were significantly influenced by the G × M interaction (Table 1).

Table 1. Main effects of the grafting combination and arbuscular mycorrhiza (AM) inoculation on yield and the yield components of greenhouse eggplant.

Treatment	Marketable Yield (t ha^{-1})		Fruits Number (n. plant^{-1})		Fruit Mean Weight (g)		Discarded Production (%)	
Grafting combination (G)								
Birgah ungrafted (B)	36.5	c	6.1	c	505.5	a	6.3	b
Birgah self-grafted (B/B)	37.1	c	6.2	c	501.6	a	6.3	b
Birgah/*S. torvum* (B/T)	48.4	a	8.1	a	500.8	a	5.2	b
Birgah/*S. macrocarpon* (B/M)	40.1	b	6.7	b	500.6	a	10.3	a
Birgah/*S. paniculatum* (B/P)	38.5	bc	6.5	bc	494.7	a	6.3	b
AM fungi (M)								
−AM	37.6	b	6.3	b	499.8	a	9.4	a
+AM	42.6	a	7.1	a	501.4	a	4.4	b
Significance								
G	***		***		NS		***	
M	***		***		NS		***	
G × M	NS		NS		NS		NS	

NS, *** non-significant or significant at $p \leq 0.001$. Data represent mean values of three replicates. Values within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD Test. NS = not significant. +AM, −AM = mycorrhizal and non-mycorrhizal eggplants, respectively.

On the other hand, neither the grafting combination nor mycorrhizal treatment had a significant effect on fruit mean weight (avg. 500.6 g fruit^{-1}). When averaged across mycorrhizal treatments, the marketable yield production reached a maximum value with Brigah grafted onto *S. torvum* (B/T), followed by both the B/M and B/P grafting combinations, while the lowest crop productivity was recorded for ungrafted and self-grafted crops (Table 1). Interestingly, the higher marketable fresh yield observed for eggplants grafted onto *S. torvum* and to a lesser extent onto *S. macrocarpon* and *S. paniculatum*, in comparison to both the ungrafted and self-grafted plants, was associated with an increase in the number of eggplant fruits per plant and not with a change in the mean fruit weight (Table 1). Moreover, the highest discarded production was observed with the B/M grafting combination (Table 1). A significant effect of mycorrhizal treatment on the yield and yield components was also observed (Table 1). Irrespective of grafting combinations, the inoculation of eggplant at the transplantation stage with *R. irregularis* elicited significant increases in the marketable yield and number of fruits per plant of 13.3% and 12.6%, respectively, compared to for the non-inoculated control (Table 1).

Similarly to the effects on the yield and yield components, the nitrogen use efficiency (NUE) for the B/T grafting combination (0.242 t kg^{-1}) was significantly higher, by 31.2%, than that obtained from

the ungrafted and self-grafted eggplant (avg. 0.184 t kg^{-1}; Table 1). Finally, the inoculation of eggplant with *R. irregularis*, when averaging across grafting combinations, induced a significant increase in the NUE, which was 13.3% higher than for the non-inoculated control treatment (Figure 1).
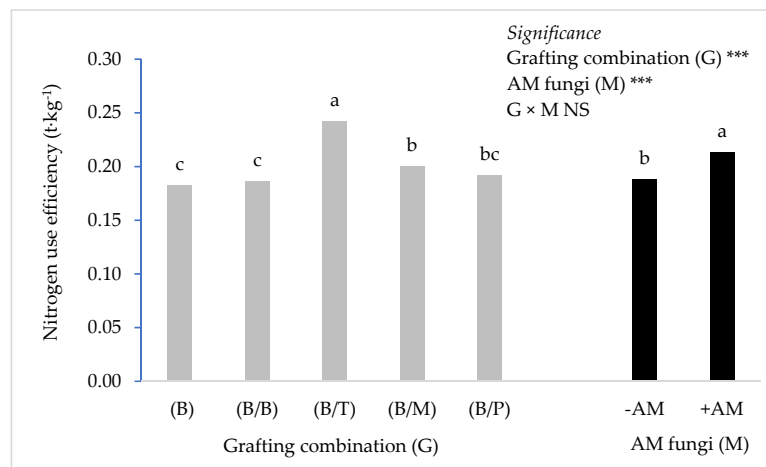

Figure 1. Main effects of grafting combination and AM inoculation on the nitrogen use efficiency (NUE) of greenhouse eggplant. (B): Birgah ungrafted; (B/B): Birgah self-grafted; (B/T): Birgah/*S. torvum*; (B/M): Birgah/*S. macrocarpon*; (B/P): Birgah/*S. paniculatum*.

3.3. Effects of Grafting Combination and Mycorrhizal Inoculation on the Mineral Profile and Nutritional and Functional Quality

Neither the grafting combination nor mycorrhizal inoculation had a significant effect on the fruit dry matter content (DM). The soluble solids content (SSC) and K content of eggplant fruits in the B/T and B/M plots, averaged across mycorrhizal inoculation status, were higher than those for the other grafting combinations (Table 2).

Table 2. Main effects of the grafting combination and AM inoculation on the fruit dry matter (DM) percentage, soluble solids content (SSC) and macromineral content of greenhouse eggplant.

Treatment	Dry Matter (%)		SSC (°Brix)		K (mg 100 g^{-1} dw)		Ca (mg 100 g^{-1} dw)		Mg (mg 100 g^{-1} dw)	
Grafting combination (G)										
Birgah ungrafted (B)	6.0	a	4.3	b	4.3	b	309.2	c	18.0	a
Birgah self-grafted (B/B)	5.9	a	4.3	b	4.3	b	311.5	c	17.0	a
Birgah/*S. torvum* (B/T)	6.0	a	5.1	a	5.1	a	311.4	c	12.3	c
Birgah/*S. macrocarpon* (B/M)	5.9	a	5.1	a	5.1	a	335.5	b	16.8	a
Birgah/*S. paniculatum* (B/P)	5.9	a	4.4	b	4.4	b	347.5	a	14.5	b
AM fungi (M)										
−AM	5.9	a	4.4	b	4.4	b	324.0	a	16.1	a
+AM	6.0	a	4.9	a	4.9	a	322.0	a	15.6	a
Significance										
G		NS		***		***		***		***
M		NS		***		***		NS		NS
G × M		NS		NS		NS		NS		NS

NS, *** non-significant or significant at $p \le 0.001$. Data represent mean values of three replicates. Values within a column followed by the same letter are not significantly different at $p \le 0.05$ according to Tukey's HSD Test. NS = not significant. +AM, −AM = mycorrhizal and non-mycorrhizal eggplants, respectively.

The highest and lowest Ca and mg fruit contents were recorded in the B/P and B/T plots, respectively. Regardless of the grafting combination, the SSC and K fruit contents in the inoculated plots were 11.4% and 11.4% higher than in the non-inoculated ones, respectively. No significant interaction was observed between the grafting combination and mycorrhizal inoculation in terms of the DM percentage; SSC; and K, Ca and mg fruit contents (Table 2).

The grafting combination and mycorrhizal inoculation significantly affected the P and Fe fruit contents, protein content, firmness, ascorbic acid, total anthocyanins, chlorogenic acid and glycoalkaloids (Table 3).

Table 3. Effects of the grafting combination and AM inoculation on the phosphorus (P), iron (Fe), protein, fruit firmness, ascorbic acid, total anthocyanins, chlorogenic acid and glycoalkaloid content of greenhouse eggplant.

Treatment	P (mg 100 g^{-1} dw)		Fe (µg g^{-1})		Proteins (g 100 g^{-1} dw)		Firmness (N)		Ascorbic Acid (mg 100 g^{-1} fw)		Total Anthocyanins (mg 100 g^{-1} dw)		Chlorogenic Acid (mg 100 g^{-1} dw)		Glycoalkaloids (mg 100 g^{-1} dw)	
Birgah ungrafted (B) × −AM	418.07	e	21.13	e	10.77	e	43.5	c	6.51	de	7462.03	d	982.37	b	89.38	b
Birgah ungrafted (B) × +AM	431.43	e	22.73	e	12.93	d	53.2	a	7.27	b	7770.40	c	992.60	b	74.16	c
Birgah self-grafted (B/B) × −AM	419.27	e	25.17	d	10.60	e	43.1	c	6.43	e	7428.13	d	982.71	b	89.13	b
Birgah self-grafted (B/B) × +AM	435.53	e	27.40	c	12.70	d	52.2	a	7.19	bc	7772.43	c	984.99	b	73.27	c
Birgah/*S. torvum* (B/T) × −AM	494.90	d	30.17	b	14.03	c	54.7	a	7.27	b	7154.77	e	757.15	e	44.13	d
Birgah/*S. torvum* (B/T) × +AM	525.70	c	30.70	ab	15.23	a	53.8	a	7.63	a	7393.67	de	823.98	d	36.93	e
Birgah/*S. macrocarpon* (B/M) × −AM	383.67	f	31.43	a	9.87	f	46.4	b	6.71	d	7241.60	e	955.27	c	97.20	a
Birgah/*S. macrocarpon* (B/M) × +AM	382.67	f	31.30	a	9.83	f	52.2	a	7.09	c	7222.50	e	948.57	c	90.33	b
Birgah/*S. paniculatum* (B/P) × −AM	553.23	b	29.33	b	13.07	d	41.5	c	6.67	d	9858.37	b	989.16	b	41.94	d
Birgah/*S. paniculatum* (B/P) × +AM	584.30	a	31.33	a	14.90	b	47.3	b	7.19	bc	11,162.96	a	1027.86	a	32.43	e
Significance																
G	***		***		***		***		***		***		***		***	
M	***		***		***		***		***		***		***		***	
G × M	***		***		***		**		***		***		**		***	

, * significant at $p \leq 0.01$ or significant at $p \leq 0.001$. Data represent mean values of three replicates. Values within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD Test. NS = not significant. +AM, −AM = mycorrhizal and non-mycorrhizal eggplants, respectively.

The inoculation treatment elicited a significant increase in the P fruit content in the B/P and B/T plots compared to in the non-inoculated ones. However, this effect was not apparent with the other grafting combinations.

A similar positive effect was also observed in eggplant grafted onto *S. paniculatum*, since mycorrhizal inoculation increased Fe concentration in inoculated plants compared to in the non-inoculated ones (Table 3). Moreover, eggplant "Birgah" grafted onto *S. torvum* and inoculated with AM fungi displayed the highest total protein value (Table 3). Except for the B/T grafting combination where no significant changes in fruit firmness were observed, the inoculation of eggplant with AM fungi in all the other grafting combinations (ungrafted, B/B, B/M and B/P) incurred a significant increase in fruit firmness (Table 3). Contrarily, for the latter quality parameters (P, Fe, protein and firmness), the beneficial effect of AM fungi inoculation on ascorbic acid in eggplant fruits was apparent with all the grafting combinations tested, with the highest values recorded for "Birgah" grafted onto *S. torvum* (Table 3).

The total anthocyanin and chlorogenic acid contents ranged from 7154 to 11,162 and 757 to 1027 mg 100 g^{-1} dw, respectively. Interestingly, the B/P grafting combination inoculated with AM fungi produced a major amplification of total anthocyanins and chlorogenic acid in comparison to the rest of the treatments (Table 3). Finally, the results indicated that the highest glycoalkaloid content was observed in the non-inoculated B/M grafting combination, whereas the lowest values were recorded in the B/P and B/T combinations inoculated with *R. irregularis* (Table 3).

3.4. Principal Component Analysis of All Agronomic and Qualitative Parameters

The principal component analysis (PCA) on the agronomic and qualitative traits of greenhouse eggplant in response to the grafting combination and mycorrhizal inoculation is reported in Figure 2.
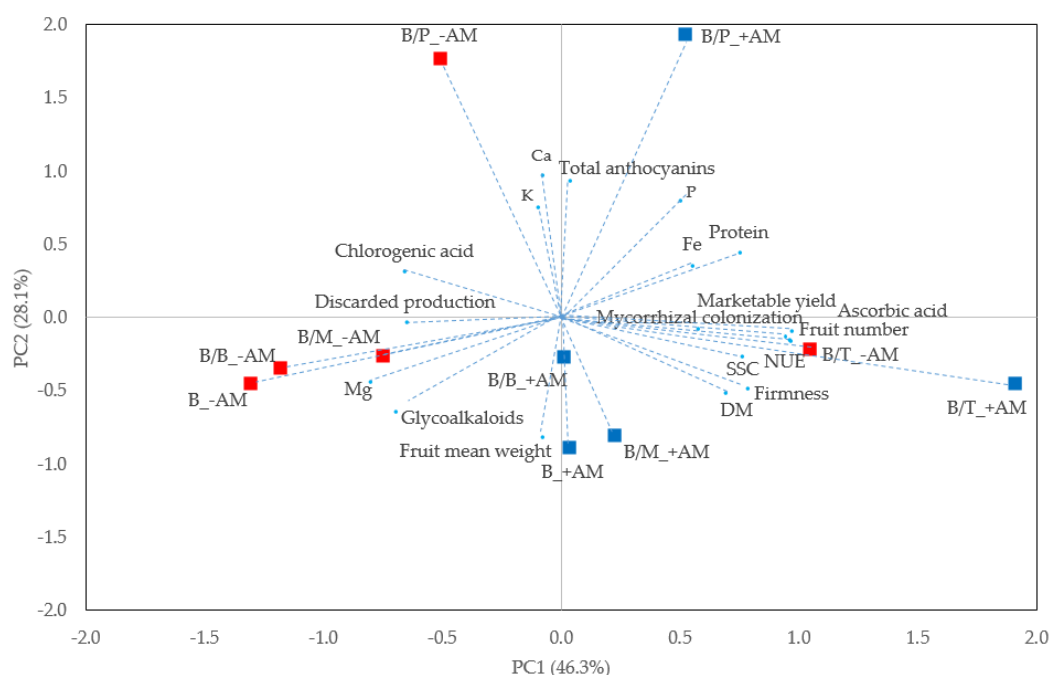

Figure 2. Principal component loading plot and scores of the principal component analysis (PCA) of the yield, yield components, nitrogen use efficiency (NUE), dry matter percentage (DM), protein, firmness, soluble solids content (SSC), minerals (P, K, Ca, mg and Fe), bioactive molecules (ascorbic acid, anthocyanins and chlorogenic acid) and glycoalkaloids of eggplant cv. "Birgah" as a function of grafting combination (ungrafted B and self-grafted B/B, B/T, B/M and B/P) and AM inoculation (+AM and −AM).

The first four PCs (i.e., principal components) were related with eigenvalues higher than 1 and explained 94.7% of the total variance (Table 4).

Table 4. Correlation coefficients for each agronomic and qualitative traits, eigenvalues, variance and cumulative proportions of total variance of the four principal components (PCs).

Variable	PC1	PC2	PC3	PC4
Marketable yield	0.961	−0.157	−0.129	−0.003
Fruit number	0.969	−0.090	−0.156	−0.012
Fruit mean weight	−0.080	−0.813	0.377	−0.041
Discarded production	−0.657	−0.029	−0.727	−0.082
Protein	0.747	0.444	0.360	−0.295
K	−0.097	0.752	−0.394	0.463
P	0.498	0.798	0.193	−0.261
Ca	−0.083	0.972	0.078	0.046
Mg	−0.809	−0.435	0.243	0.290
Fe	0.550	0.359	−0.644	0.345
NUE	0.959	−0.155	−0.130	−0.017
Fruit dry matter	0.691	−0.509	−0.001	−0.001
Firmness	0.780	−0.488	0.088	0.146
SSC	0.757	−0.262	−0.365	0.425
Ascorbic acid	0.940	−0.125	0.210	0.162
Total anthocyanins	0.034	0.936	0.268	0.189
Chlorogenic acid	−0.659	0.319	0.431	0.517
Glycoalkaloids	−0.698	−0.635	−0.155	0.285
Mycorrhizal colonization	0.571	−0.077	0.500	0.636
Eigenvalue	8.793	5.348	2.247	1.601
Variance (%)	46.281	28.148	11.825	8.427
Cumulative (%)	46.281	74.429	86.254	94.681

In the current greenhouse experiment, the loading matrix indicates that variation in Ca and K was most closely aligned with the total anthocyanins, whereas the variation in the glycoalkaloids was not correlated to the SSC (Figure 2). The score plot of the PCA superimposed on the agronomic and qualitative parameters demonstrated a strong clustering of the two mycorrhizal treatments along PC1 (with the exception of *S. torvum* × −AM), with +AM plants concentrating marketable yield, NUE, DM, SSC, mineral composition and ascorbic acid (Figure 2). Particularly, the B/T grafting combination inoculated with AM fungi was positioned on the positive side of PC1 in the lower right quadrant of the PCA score plot, and it exhibited the highest crop performance (high yield, number of fruits and NUE) with premium quality due to high concentrations of DM, SSC and ascorbic acid (Figure 2). Moreover, eggplant grafted onto *S. paniculatum* and inoculated with AM was characterized by higher P, Fe and total anthocyanin content (Figure 2). Finally, non-inoculated ungrafted and self-grafted plants were positioned in the lower left quadrant, characterized by higher glycoalkaloid content (Figure 2).

4. Discussion

In the present work we tested the use of various eggplant grafting combinations and AM fungi, alone or in combination, to boost crop performance and fruit quality in "Birgah" eggplant cultivated in a protected environment. Our results showed that enhancements in terms of yield, yield related traits, NUE, and fruit nutritional and functional quality can be accomplished by harnessing the combined effects of specific grafting combinations and AM fungi. These results are in line with those reported by Sabatino et al. [12,13], who—exploring the effects of using eggplant hybrids and allied species as rootstocks on eggplant yield, plant vigor and overall fruit quality—found that *S. paniculatum* and the interspecific hybrid of *S. melongena* × *S. aethiopicum* gr. *Gilo* provided a higher yield performance and a better fruit quality compared to the ungrafted or self-grafted plants. Similarly, our results are in line with those observed by Oztekin et al. [29], who, studying the influence of AM fungi on salinity

tolerance in tomato grafted plants, stated that AM fungi significantly increased the total and marketable yields as well as the average fruit weight. Several authors [42,43] report that the phytostimulating effect of the AM fungi could be attributed to numerous mechanisms such as enhancing the uptake and translocation of major and trace elements, inducing a more developed root system, improving the water status and photosynthetic efficiency, bolstering the antioxidative defense system, balancing plant hormones, upregulating nutrient transporter action and promoting the production of enzymes such as phosphatases. Therefore, we might hypothesize that the higher yield and yield related traits of mycorrhized plants could have similar explanations. Our outcomes regarding the NUE are in agreement with those reported by Djidonou et al. [44], who—investigating the yield, water status and NUE in field-grown, grafted tomato—found that grafting the "Florida 47" tomato onto "Beaufort" or "Multifort" significantly increased N use. Our findings are also consistent with those of Colla et al. [45], who, studying the influence of selected rootstocks on the plant performance and nitrogen use efficiency of the "Proteo" melon, report that the use of melon grafted onto designated rootstocks would represent a prospective plan for increasing crop productivity and NUE. In the present study, AM fungi increased the NUE of eggplant plants. This accords with the results of Zhu et al. [46], who—evaluating the effects of AM fungi on growth, nitrogen uptake and NUE in wheat—found that AM symbiosis increased NUE. Our findings are also in line with those presented by Liu et al. [47], who, studying the influence of AM on NUE in soybean, concluded that AM fungi play an imperative function in increasing NUE. Likewise, Rouphael et al. [43], in a review, report that plant biostimulants, including microbial plant biostimulants, can enhance NUE. Our study showed that both the *S. torvum* and *S. macrocarpon* rootstocks increased SSC values. This is in congruence with the results of Sabatino et al. [12], who used the "Birgah" eggplant F_1 hybrid as a scion. However, these findings are in contrast to the outcomes reported by Sabatino et al. [13], who tested the "Scarlatti" eggplant F_1 hybrid as an eggplant scion. Furthermore, our results for SSC revealed that AM fungi increase the SSC level in eggplant. However, this is in contrast with previous reports [29] indicating that AM symbiosis does not affect SSC in tomato fruit. Our results on firmness are corroborated by the results of Sabatino et al. [12], who found that *S. torvum* rootstock increased fruit firmness in "Birgah" hybrid and by those reported by Miceli et al. [48], who, assessing the effect of AM fungi and grafting on yield and fruit quality in mini-watermelon, noted that AM symbiosis increases pulp firmness. In our study, at the end of the experiment, the mean mycorrhizal colonization rates were 71.8% and 2.5% in +AM and −AM plants, respectively. There are reports that mycorrhizal hyphae extending into the soil or substrate increase nutrient uptake [43,49] thanks to the mycorrhizal hyphae's capacity to penetrate into very small particles [50]. Our results on fruit mineral composition showed that the B/T, B/M and B/P scion/rootstock combinations improved the K, Ca and Fe fruit contents. Meanwhile, fruits from the B/M grafts revealed a decrease in terms of P content compared to the fruits from the control plots (ungrafted or self-grafted plants). Moreover, the present study showed that AM fungi treatment increased the K fruit content, without affecting the Ca and mg fruit content. In addition, our results highlighted that the B/T and B/P grafts benefit significantly from AM fungi in terms of P and Fe fruit concentrations. These outcomes are in accord with those of Kaya et al. [51] and Oztekin et al. [29], who supposed that a higher fruit mineral (macro and micro) concentration in grafted and mycorrhized plants could be attributed to a positive impact on water and nutrient uptake. Our trial showed that the *S. torvum* and *S. paniculatum* rootstocks increased fruit protein concentrations. This is in agreement with the results of Sabatino et al. [12] but in contrast to those of Sabatino et al. [13], who found no significant differences in terms of fruit protein content between ungrafted, self-grafted or *S. torvum* grafted plants. These results mark the important role played by the scion in terms of nutritional fruit quality in grafted plants. In addition, our results showed that AM fungi augmented the protein concentration in eggplant fruits. This seems to be in accord with the study of Baum et al. [52], who asserted that mycorrhizal inoculation induces a higher accumulation of proteins in onion. Contemporary outcomes exposed that AM symbioses are capable to adjust host plant primary and secondary metabolism, encouraging the synthesis of phytochemicals in the root system and shoots of mycorrhized plants [53]. Our data showed that the *S. torvum* rootstock

significantly increased ascorbic acid in eggplant fruit, whereas fruits from the B/M and B/P grafts did not show significant differences in terms of ascorbic acid compared to the fruits from the control plots, demonstrating that the rootstock plays an imperative role in eggplant fruit quality. This results are partially in accord with those reported by Oztekin et al. [29], who observed that the rootstock factor did not affect vitamin C in tomato fruits, and with those of Miceli et al. [48], who reported that grafting increases fruit ascorbic acid content in mini-watermelon. Conversely, our results on the positive effect of the AM fungi on fruit ascorbic acid content are fully in accord with those of Oztekin et al. [29] and Miceli et al. [48]. There is evidence that the increase in secondary metabolites in mycorrhized plants could be correlated to the higher contents of mineral nutrients [25]. In this regard, overall, our study indicated that AM fungi positively affected total anthocyanins and chlorogenic acid, with the sole exception of with the B/M scion/rootstock combination. Our findings seem to be in line with those reported by Walter et al. [54], who revealed that some graminaceous crops inoculated with AM fungi produce undergo important biochemical modulation leading to phenolic acid accumulation. Our results are in accord with those of other authors [55–57], who, investigating the influence of AM symbiosis on the production of phytochemicals in *Ocimum basilicum*, found higher concentrations of antioxidants when plants were treated with diverse AM fungi species. Baslam et al. [58] remarked that mycorrhized lettuce leaves display higher anthocyanin, carotenoid and phenolic contents than plants from control plots. Castellanos-Morales et al. [59] found that the strawberry mycorrhized plants produced fruits with a higher content of anthocyanidin cyanidin-3-glucoside. Ceccarelli et al. [60] state that artichoke, when treated with two AM fungi species, have increased leaf polyphenolic concentrations and antioxidant activity. Our results on glycoalkaloids support the findings reported by Sabatino et al. [12], who, although not showing a statistically significant effect of the rootstock on glycoalkaloids, highlighted a certain effect of rootstocks on glycoalkaloid content, remarking on the effectiveness of *S. torvum* and *S. paniculatum* in reducing glycoalkaloid concentrations. Furthermore, our results evidenced that +AM treatment had a positive effect on glycoalkaloid reduction which in turn could positively influence human health.

The effectiveness of PCA in interpreting species/cultivar differences across several agronomic and qualitative attributes in response to a wide range of pre-harvest factors such as the genetic material and agricultural practices (i.e., AM inoculation) has been reported previously by several researchers [61–63]. This was also evident in the present study, conducted under protected environment conditions, as the score plot of PCA integrated information on the yield, yield components, NUE, mineral profile and nutritive value of eggplant from five grafting combinations with or without inoculation with AM.

5. Conclusions

In the present work, eggplant wild/allied relatives' rootstocks and AMF significantly interacted, improving the crop performance and quality traits of "Birgah" eggplant. However, the B/T and B/P grafts combined with AMF stood out as producing the best results in terms of the yield traits, NUE, mineral profile, and nutritional and functional quality. Our findings could be useful information for vegetable crop nurseries interested in introducing new eggplant rootstocks that successfully respond when combined with AMF.

Abbreviations

AM	Arbuscular Mycorrhizal
ANOVA	Analysis of Variance
APX	Ascorbate Peroxidase
B	Birgah
B/B	Birgah self-grafted
B/M	Birgah/S. *macrocarpon*
B/P	Birgah/S. *paniculatum*
B/T	Birgah/S. *torvum*
Ca	Calcium
CAT	Catalase
DM	Dry Matter
Fe	Iron
K	Potassium
Mg	Magnesium
NUE	Nitrogen Use Efficiency
P	Phosphorus
PCA	Principal Component Analysis
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
SSC	Soluble Solids Content

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