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Biostimulatory Response of Easily Extractable Glomalin-Related Soil Protein on Soil Fertility Mediated Changes in Fruit Quality of Citrus

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Abstract: Arbuscular mycorrhizal fungi secrete glomalin deposited into the soil as glomalin-related soil protein (GRSP), which possess multiple utility to benefit both soil as well as plant health. The present study aimed to assess the effects of the foliar application of an easily extractable GRSP (EE-GRSP) on the fruit quality, soil nutrients, and soil structural changes in three important citrus varieties (Satsuma mandarin Oita 4, Newhall navel orange, and Cocktail grapefruit). The exogenous EE-GRSP significantly elevated root mycorrhizal fungal colonization and soil hyphal length in Newhall and Oita 4 varieties, but without any such response in Cocktail grapefruit variety. The foliar spray of the EE-GRSP improved different external (e.g., pericarp, sarcocarp, and single fruit weight) and internal (e.g., soluble solids, titratable acids, and sugar contents) qualities of fruits to varying magnitudes, depending on citrus variety, with a more prominent effect on Cocktail grapefruit. EE-GRSP-treated fruits of Newhall and Oita 4 were more suitable for processing than non-treated control because of a low fruit hardness. However, no significant effect of the EE-GRSP was observed on the internal quality parameters of Newhall. EE-GRSP-treated citrus trees represented higher soil available nutrients over control, to some extent, especially on Oita 4. The foliar application of the EE-GRSP also increased various GRSP fractions to varying proportions and improved the distribution of water-stable aggregates in the size fraction of 0.25–2 mm, thereby increasing the mean weight diameter, particularly in Newhall and Cocktail grapefruit varieties. These observations provided clues about the stimulatory role of the EE-GRSP through soil structure and nutrient pool-mediated improvements in fruit quality.

Keywords: aggregate; glomalin; mycorrhiza; soil organic carbon; sugar

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1. Introduction

Citrus is an evergreen fruit tree widely cultivated world over. The major citrus species comprise mandarins, oranges, lemons, grapefruits, pummelos, kumquats, limes, citrons, and different hybrids, with high vitamin C, minerals, and carotenoids [1,2]. In China, *Citrus sinensis* cv. Oita 4, an early-ripening variety of Satsuma mandarin, attains maturity for sale in late August and early September [3]. The other sweet orange, such as Newhall navel orange, is the second most widely planted orange group [4], followed by grapefruit (*C. paradisi* Macf.), a hybrid between pummelo and sweet orange, the fourth economically most important citrus fruit global trade and tariff of citrus fruits [5]. Citrus grown on acid soils characterized by low fertility due to kaolinite dominated minerals vulnerable to soil acidification and deterioration in soil structure, thereby causing a gradual decline in orchard productivity [6], coupled with reduced orchard life and inferior fruit quality [7]. As a result, new strategies are needed to be reframed to sustain a citrus production system.

Arbuscular mycorrhizal fungi (AMF) represent a group of soil microbial communities, known to colonize 72% of roots of terrestrial plants, establishing a symbiotic association with host plants to acquire the desired nutrients, enhance resistance to biotic and abiotic stresses and stabilize the soil structure [8–10]. Citrus plants in the field are widely reported possessing fewer root hairs and considered dramatically dependent on AMF facilitating elevated nutrient acquisition [11,12]. The glomalin-related soil protein (GRSP) is a specialized protein produced by extraradical and intraradical hyphae and spores of AMF [13–15]. The GRSP accelerates the stability of soil aggregates and the accumulation of soil organic carbon and sustains the uninterrupted nutrient-supply chain of soil as a growing medium [16,17], besides reducing the toxic effect of heavy metals on plants [18].

In addition to these intrinsic properties, the GRSP is widely reported as a plant growth biostimulant. Wang et al. [19] observed that 1/2 strength of the EE-GRSP stimulated plant growth performance out of tested different strengths of the EE-GRSP to potted trifoliate orange. Later, Chi et al. [20] reported the effect of application of the 1/2-strength EE-GRSP on drought-stressed trifoliate orange seedlings. A significant increase in drought tolerance in EE-GRSP-treated plants was observed, which was associated with the increase in superoxide dismutase activity, gas exchange, and abscisic acid content. The improvement of plant growth by the exogenous EE-GRSP was due to synthesis and transport of endogenous auxins [15]. Meng et al. [21], for the first time, made a successful attempt to report the response of EE-GRSP application on two sweet orange cultivars (Lane Late navel orange and Rohde Red Valencia) for an improvement in external quality (fruit weight and size) and physiological parameters (fructose, glucose, and sucrose concentrations) of fruits. However, the exogenous EE-GRSP showed no significant effects on the soluble solids content, soluble solids-titratable acid ratio, K level, and P level, coupled with decreases in N and vitamin C contents in Rohde Red Valencia variety. These results showed that the exogenous EE-GRSP had a definite positive regulation on citrus fruit quality; however, it depended on citrus species.

In order to expand our understanding on multiple roles of the exogenous EE-GRSP as a biostimulant on citrus fruit quality, we carried out more intensive research in other important citrus varieties, and such responses also warranted to be validated spatially. In this background, we evaluated the response of the exogenous EE-GRSP in three citrus varieties representing different locations with reference to changes in fruit quality and soil properties, to consolidate our earlier observations on the EE-GRSP as a potent biostimulant.

2. Materials and Methods

2.1. Experimental Design

The experiment was laid through a 2×3 factorial design with completely randomized block arrangements. The first factor was represented by the foliar spraying of the exogenous EE-GRSP and non-EE-GRSP; while the second factor consisted of different citrus varieties (Oita 4, Cocktail grapefruit, and Newhall navel orange). Therefore, this experiment had a total of six treatments, each of which was replicated four times, along with three trees per replicate.

2.2. Preparation of the EE-GRSP Solution

The soil samples were collected from the citrus orchard (30°21′22′′ N, 112°8′32′′ E) in the west campus of Yangtze University and sieved following air drying. The EE-GRSPs from these soil samples were extracted using the procedure as suggested by Wu et al. [22].

The 1 g soil samples were extracted with 8 mL of sodium citrate solutions (20 mM, pH 7.0) at 103 kPa and 121 °C for 30 min and centrifuged at 10,000× *g* for 5 min. The supernatant was collected and stored at 4 °C. The above extraction process was then repeated using additional soil. All collected supernatants were well mixed, and the protein concentration in the collected solution was determined using the protocol described by Bradford [23] with bovine serum albumin as the standard. The measured protein concentration was 16.7 mg protein/L. The collected supernatants were stored at 4 °C for no more than 3 days. Before use, the collected supernatants were diluted with an equal volume of 20 mM sodium citrate solution to be used as the exogenous EE-GRSP for the onward response study.

2.3. Experimental Design and Treatments Imposition

Newhall navel orange trees were selected in an orchard (31°34′68″ N, 110°74′95″ E) of Agriculture Bureau, Xingshan, Yichang, China. Cocktail grapefruit trees were selected in an orchard (29°60′63″ N, 114°48′95″ E) of Tongshan, Xianning, China. Oita 4 trees were planted in an orchard (30°71′07″ N, 111°28′57″ E) of Xiling, Yichang, China. All trees used trifoliate orange as a rootstock with a uniform canopy size and an orchard age of 7 years. The Oita 4, Cocktail grapefruit, and Newhall navel orange trees were folia-sprayed with exogenous EE-GRSP solutions to 1 L per tree on 28 July, 1 August, and 15 August 2020, respectively. The application would be conducted again in 5 days and postponed if rainy. A total of three foliar sprays were performed. The control trees were sprayed with the same amount of 20 mM citrate buffers (pH 7.0). The fruits of Oita 4, Cocktail grapefruit, and Newhall navel orange were harvested on 16 September, 5 December, and 15 December 2020 for onward analysis.

2.4. Variable Determinations

Ten fruits from each tree were harvested covering all four directions for the analysis of fruit quality parameters. At the same time, the roots and soils of 5–15 cm soil layers were collected for subsequent analysis.

The weights of the collected fruits were measured with the help of an electronic balance, and the vertical and horizontal diameter of the fruit was measured by a digital vernier caliper. The fruit coloration value, rigidity, and soluble solids content were measured using a colorimeter (CR10, Konica Minolta, Tokyo, Japan), a fruit sclerometer (GY-B, Zhejiang Top Instrument Co., Ltd., Hangzhou, China), and a portable refractometer (WYT-4, Quanzhou Zhongyou Optical Instrument Co., Ltd., Quanzhou, China), respectively. Titratable acids in fruits were determined through the indicator titration method as described by Von and Griffiths [24]. Fructose, glucose, and sucrose contents in fruits were assayed as per the protocol outlined by Wu et al. [25].

Mycorrhizas in root segments with a 1 cm length were stained by the method described by Phillips and Hayman [26]. The hyphal length in the soil was measured by Ames and Bethlenfalvay [27]. Concentrations of the EE-GRSP and the difficultly extractable GRSP (DE-GRSP) in soil were determined following the method as described by Wu et al. [22]. Soil organic carbon (SOC) content was determined using a wet digestion with potassium dichromate oxidation as outlined by Rowell [28]. The distributions of soil water-stable aggregates (WSAs) in different sizes (2–4 mm, 1–2 mm, 0.5–1 mm, and 0.25–0.5 mm) were determined by a wet sieving method [29]. The NO_3-N, NH_4-N, Olsen-P, and NH_4OAc-K in soil were determined using a high-precision Soil Nutrient Detector (HM-TYD, Shandong Hengmei Electronic Technology Co., Ltd., Weifang, China), with standard samples provided by Shandong Macromicro Quantum Technology Co., Ltd. (Weifang, China).

2.5. Data Analysis

The two-factor analysis of variance was carried out using the SAS software (8.1, SAS Institute Inc., Cary, NC, USA), and Bonferroni's post-test was used for significant ($p < 0.05$) differences between the treatments.

3. Results

3.1. Changes in Root Colonization of Mycorrhiza and Hyphal Length in Soil

Compared with the non-EE-GRSP treatment, the foliar application of the EE-GRSP significantly increased the degree of root AMF colonization and the hyphal length in soil in Newhall navel orange trees by 84.08% and 48.11%, respectively, corresponding to 47.30% and 86.21% in Oita 4 trees, respectively; however, no significant difference was observed in Cocktail grapefruit trees (Figure 1a,b). The interaction analysis showed that exogenous EE-GRSP and citrus varieties significantly ($p < 0.05$) interacted with each other to affect the degree of root mycorrhiza colonization and the hyphal length in soil to a greater magnitude.

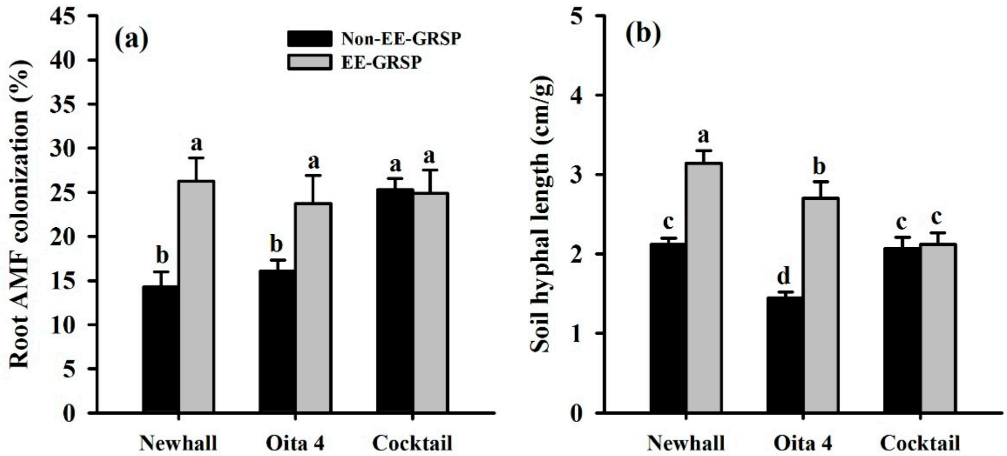

Figure 1. Effects of the foliar spraying of the EE-GRSP on mycorrhizal growth in roots (**a**) and soils (**b**) of citrus plants. Data (means ± SD, $n = 4$) followed by different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: AMF, arbuscular mycorrhizal fungi; EE-GRSP, easily extractable glomalin-related soil protein.

3.2. Changes in External Quality Parameters of Fruits

The foliar spray treatment of the EE-GRSP significantly changed the external fruit quality of all the three citrus varieties (Table 1; Figure 2). The EE-GRSP significantly increased the single fruit weight of Newhall navel orange by 29.58%, compared to the control treatment (Table 1). The exogenous EE-GRSP did not significantly alter the external quality of Oita 4 fruits. Significant increases in pericarp weight, sarcocarp weight, and single fruit weight of EE-GRSP-treated Cocktail grapefruit fruits were observed by 30.81%, 19.65%, and 22.09%, respectively, compared with the treatment without the EE-GRSP. The exogenous EE-GRSP significantly reduced the fruit hardness by 32.48% in Nehwell navel orange, but without any such response in Cocktail grapefruit and Oita 4. A significant ($p < 0.05$) interaction effect of the EE-GRSP and citrus varieties was observed on the fruit hardness and the pericarp weight.

3.3. Changes in Internal Quality Parameters of Fruits

The application of the exogenous EE-GRSP increased the fruit soluble solids contents of three citrus varieties to varying degrees, but Cocktail grapefruit produced the maximum response (Table 2). The foliar spraying of the EE-GRSP also significantly reduced the titratable acid contents of Oita 4 and Cocktail grapefruit fruits by 31.94% and 23.33%, respectively, compared with the control treatment. In addition, the foliar application of the EE-GRSP produced no change in concentrations of fructose, sucrose, and glucose in Newhall navel orange and Cocktail grapefruit fruits, while Oita 4 fruits observed an increased concentration of sucrose by 33.14%, compared with the control treatment. Soluble solids and titratable acid were significantly affected by the interaction effect of the exogenous EE-GRSP and citrus varieties.

Table 1. Effects of the foliar spraying of the easily extractable glomalin-related soil protein (EE-GRSP) on the external quality of citrus fruits.

Varieties	Treatments	Coloration Value	Fruit Hardness (kg × 10^5/cm^3)	Fruit Size (Mm)		Fruit Weight (g FW/Fruit)		
				Longitudinal Diameter	Transverse Diameter	Pericarp	Sarcocarp	Total
Newhall	Non-EE-GRSP	70.57 ± 3.61 a	25.25 ± 2.29 a	76.5 ± 6.5 bc	71.8 ± 5.5 c	60.57 ± 4.63 c	150.08 ± 5.28 cd	210.65 ± 19.57 d
	EE-GRSP	69.84 ± 3.57 a	19.06 ± 1.57 b	84.4 ± 7.8 ab	80.1 ± 7.4 bc	76.78 ± 6.40 c	196.17 ± 16.03 c	272.95 ± 20.76 c
Oita 4	Non-EE-GRSP	54.77 ± 4.12 b	16.34 ± 1.36 bc	54.3 ± 4.6 c	66.2 ± 4.5 c	23.11 ± 2.06 d	70.92 ± 5.75 e	94.03 ± 6.42 e
	EE-GRSP	61.82 ± 4.04 ab	12.31 ± 1.15 c	69.5 ± 5.6 bc	84.4 ± 6.8 bc	30.65 ± 2.90 d	95.32 ± 9.05 de	126.62 ± 11.30 e
Cocktail grapefruit	Non-EE-GRSP	68.59 ± 5.58 a	17.93 ± 1.63 b	86.0 ± 8.6 ab	93.1 ± 7.6 ab	104.91 ± 9.23 b	375.41 ± 31.90 b	480.32 ± 36.13 b
	EE-GRSP	71.32 ± 6.70 a	17.68 ± 1.69 b	101.1 ± 12.2 a	109.4 ± 11.1 a	137.23 ± 11.95 a	449.17 ± 39.88 a	586.40 ± 49.42 a
Significance								
EE-GRSP		*	**	**	**	**	**	**
Varieties		NS	*	*	*	**	*	**
EE-GRSP × Varieties		NS	*	NS	NS	*	NS	NS

Data (means ± SD, n = 4) followed by different letters among treatments indicate significant (p < 0.05) differences. NS, not significant (p < 0.05); *, p < 0.05; **, p < 0.01.

Table 2. Effects of the foliar spraying of the easily extractable glomalin-related soil protein (EE-GRSP) on the internal qualities of citrus fruits.

Varieties	Treatments	Soluble Solids (%)	Titratable Acids (%)	Fructose (mg/g DW)	Sucrose (mg/g DW)	Glucose (mg/g DW)
Newhall	Non-EE-GRSP	12.74 ± 1.77 ab	0.36 ± 0.03 d	235.21 ± 17.67 ab	246.25 ± 21.40 ab	57.32 ± 3.49 ab
	EE-GRSP	14.42 ± 1.29 a	0.35 ± 0.03 d	239.3 ± 13.64 a	258.55 ± 24.12 ab	59.92 ± 5.38 a
Oita 4	Non-EE-GRSP	8.96 ± 0.75 c	0.72 ± 0.05 a	162.42 ± 14.14 b	201.4 ± 14.14 bc	44.62 ± 3.67 bc
	EE-GRSP	9.24 ± 0.87 c	0.49 ± 0.02 c	209.07 ± 18.04 ab	268.15 ± 22.62 a	54.28 ± 4.24 ab
Cocktail grapefruit	Non-EE-GRSP	9.88 ± 0.70 bc	0.60 ± 0.03 b	220.35 ± 19.68 a	184.7 ± 14.14 c	42.90 ± 2.82 c
	EE-GRSP	13.70 ± 1.24 a	0.46 ± 0.02 c	253.82 ± 21.95 a	225.17 ± 18.70 abc	51.00 ± 3.25 abc
Significance						
EE-GRSP		**	**	*	**	*
Varieties		*	*	*	*	*
EE-GRSP × varieties		*	**	NS	NS	NS

Data (means ± SD, n = 4) followed by different letters among treatments indicate significant (p < 0.05) differences. NS, not significant (p < 0.05); *, p < 0.05; **, p < 0.01.

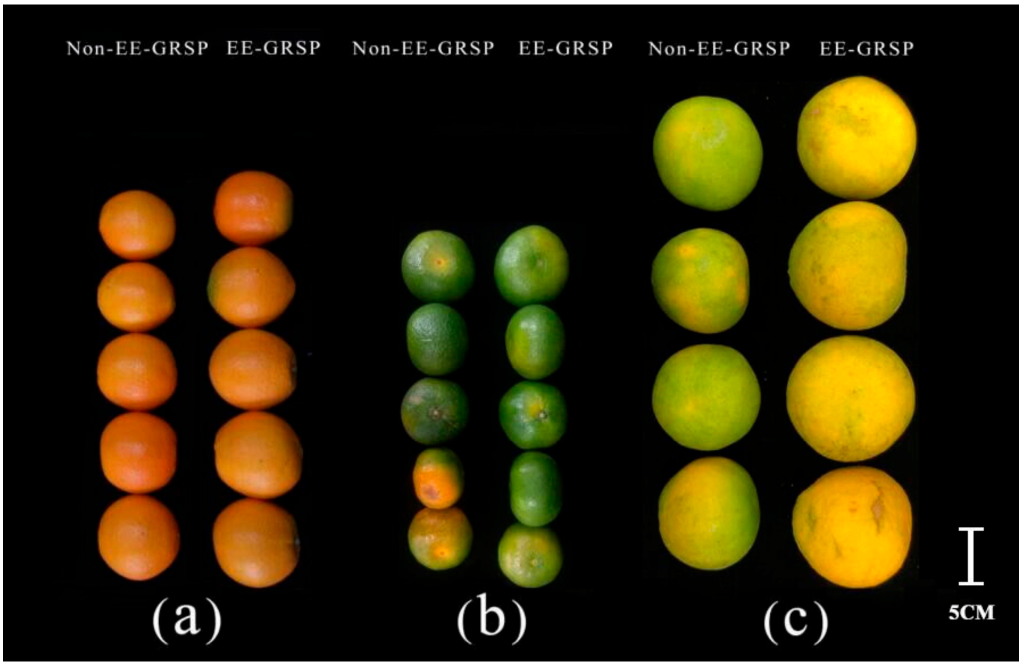

Figure 2. Changes in the external quality of Newhall navel orange (**a**), Satsuma mandarin Oita 4 (**b**), and Cocktail grapefruit (**c**) after the foliar spraying of easily extractable glomalin-related soil protein (EE-GRSP).

3.4. Changes in Soil Available Nutrients

Compared with the control treatment, the foliar application of the EE-GRSP significantly improved the pool of available nutrients within the citrus rhizosphere (Table 3). Soil NO_3-N and NH_4OAc-K concentrations in Newhall navel orange trees were significantly increased with the foliar treatment of the EE-GRSP by 134.00% and 26.15%, respectively. Soil NH_4-N, NO_3-N, Olsen-P, NH_4OAc-K, and SOC contents in Oita 4 trees had significant increases following the EE-GRSP treatment by 32.77%, 224.18%, 85.08%, 82.67%, and 27.22%, respectively, while in Cocktail grapefruit trees soil SOC contents were significantly increased with the foliar spray of the EE-GRSP by 37.83%, along with no significant changes in NH_4-N, NO_3-N, Olsen-P, and NH_4OAc-K. These responses produced significant interactions between EE-GRSP treatments and citrus varieties.

Table 3. Effects of the foliar spraying of the EE-GRSP on soil available nutrient levels of citrus plants.

Varieties	Treatments	NH_4-N (mg/kg)	NO_3-N (mg/kg)	Olsen-P (mg/kg)	NH_4OAc-K (mg/kg)	SOC (mg/g)
Newhall	Non-EE-GRSP	56.80 ± 4.51 c	88.01 ± 5.65 de	170.30 ± 12.74 b	378.93 ± 21.21 b	7.56 ± 0.48 d
	EE-GRSP	44.62 ± 3.23 c	205.94 ± 14.14 b	152.03 ± 9.89 b	478.03 ± 35.18 a	8.48 ± 0.63 d
Oita 4	Non-EE-GRSP	133.34 ± 9.26 b	78.37 ± 5.74 d	93.94 ± 5.65 c	277.63 ± 26.88 c	12.86 ± 0.91 c
	EE-GRSP	177.03 ± 14.39 a	254.06 ± 22.36 a	173.86 ± 13.20 b	507.16 ± 45.25 a	16.36 ± 1.41 b
Cocktail grapefruit	Non-EE-GRSP	136.16 ± 8.61 b	101.11 ± 8.07 cd	253.46 ± 16.26 a	253.66 ± 16.26 c	15.36 ± 0.70 bc
	EE-GRSP	147.89 ± 12.19 b	124.53 ± 9.89 c	244.33 ± 21.21 a	228.20 ± 19.79 c	21.17 ± 2.24 a
Significance						
EE-GRSP		**	**	**	**	**
Varieties		*	**	*	**	**
EE-GRSP × Varieties		*	**	**	**	*

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. NS, not significant ($p < 0.05$); *, $p < 0.05$; **, $p < 0.01$. Abbreviations: EE-GRSP, easily extractable glomalin-related soil protein; SOC, soil organic carbon.

3.5. Changes in Concentrations of Soil GRSP Fractions

Significant changes in concentrations of soil GRSP fractions were observed in response to the foliar spray of the EE-GRSP (Table 4). Compared with the control treatment, the foliar spray of the EE-GRSP significantly increased soil EE-GRSP concentration by 40.91% and 45.95% in Newhall navel orange and Oita 4 trees, respectively, without any significant difference in Cocktail grapefruit trees. The application of the EE-GRSP also increased DE-GRSP concentrations by 70.83% and 38.00% in Oita 4 and Cocktail grapefruit trees, respectively, along with no significant difference in Newhall navel orange trees, while the T-GRSP concentrations were significantly increased by 28.99% in Newhall navel orange trees, 38.81% in Oita 4 trees, and 21.85% in Cocktail grapefruit trees with the foliar spray of the EE-GRSP. Additionally, DE-GRSP concentration was significantly affected by the interactions of EE-GRSP treatment and citrus varieties.

Table 4. Effects of the foliar spraying of the EE-GRSP on soil GRSP levels of citrus plants.

Varieties	Treatments	EE-GRSP (mg/g)	DE-GRSP (mg/g)	T-GRSP (mg/g)
Newhall	Non-EE-GRSP	0.44 ± 0.02 de	0.25 ± 0.02 c	0.69 ± 0.04 d
	EE-GRSP	0.62 ± 0.04 bc	0.26 ± 0.03 c	0.89 ± 0.05 c
Oita 4	Non-EE-GRSP	0.37 ± 0.02 e	0.24 ± 0.02 c	0.67 ± 0.03 d
	EE-GRSP	0.54 ± 0.03 cd	0.41 ± 0.05 b	0.93 ± 0.08 c
Cocktail grapefruit	Non-EE-GRSP	0.69 ± 0.05 ab	0.50 ± 0.03 b	1.19 ± 0.06 b
	EE-GRSP	0.76 ± 0.04 a	0.69 ± 0.04 a	1.45 ± 0.09 a
Significance				
EE-GRSP		**	**	**
Varieties		**	**	**
EE-GRSP × varieties		NS	*	NS

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. NS, not significant ($p < 0.05$); *, $p < 0.05$; **, $p < 0.01$. Abbreviations: DE-GRSP, difficultly extractable glomalin-related soil protein; EE-GRSP, easily extractable glomalin-related soil protein; GRSP, glomalin-related soil protein; T-GRSP, total glomalin-related soil protein.

3.6. Changes in Soil WSA Distribution and Aggregate Stability

Compared with the control treatment, the foliar spray of the EE-GRSP significantly increased the percentages of WSA distribution in the sizes of 0.5–1 mm and 0.25–0.5 mm in Newhall trees contributing by 74.15% and 208.02%, respectively (Table 5). Exogenous EE-GRSP treatment significantly increased WSA percentage in the size of 0.5–1 mm in Oita trees by 59.44%. While in Cocktail grapefruit trees, the application of the EE-GRSP increased the WSA percentages in the sizes of 1–2 mm and 0.5–1 mm fractions by 27.30% and 133.24%, respectively. The application of the EE-GRSP resulted in significant increases in MWD of the citrus rhizosphere by 15.10% and 24.19% higher on Newhall and Cocktail grapefruit, respectively. The WSA percentages in the sizes of 2–4 mm, 1–2 mm and 0.5–1 mm were significantly interacted by both the EE-GRSP and citrus varieties.

Table 5. Effects of the foliar spray of the EE-GRSP on distribution of soil WSAs and MWD in citrus plants.

Varieties	Treatments	Percentage of WSAs (%)				MWD (mm)
		2–4 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	
Newhall	Non-EE-GRSP	65.98 ± 3.53 ab	7.60 ± 0.42 bc	4.41 ± 0.07 c	1.87 ± 0.21 c	2.45 ± 0.21 bc
	EE-GRSP	80.43 ± 7.07 a	6.01 ± 0.35 c	7.68 ± 0.42 b	5.76 ± 0.41 b	2.82 ± 0.14 a
Oita 4	Non-EE-GRSP	47.84 ± 4.94 c	14.24 ± 1.27 a	9.59 ± 0.74 b	10.49 ± 0.70 a	1.57 ± 0.07 e
	EE-GRSP	52.82 ± 7.07 bc	15.24 ± 0.70 a	15.29 ± 1.41 a	12.21 ± 2.12 a	1.93 ± 0.12 de
Cocktail grapefruit	Non-EE-GRSP	58.8 ± 3.53 bc	6.74 ± 0.21 c	3.52 ± 0.35 c	5.26 ± 0.14 b	2.15 ± 0.07 cd
	EE-GRSP	65.85 ± 5.65 ab	8.58 ± 0.41 b	8.21 ± 0.49 b	7.29 ± 0.56 b	2.67 ± 0.10 ab

Table 5. *Cont.*

Varieties	Treatments	Percentage of WSAs (%)				MWD (mm)
		2–4 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	
Significance						
EE-GRSP		**	**	**	**	**
Varieties		NS	NS	**	**	*
EE-GRSP × Varieties		*	*	*	NS	NS

Data (means ± SD, n = 4) followed by different letters among treatments indicate significant (p < 0.05) differences. NS, not significant (p < 0.05); *, p < 0.05; **, p < 0.01. Abbreviations: EE-GRSP, easily extractable glomalin-related soil protein; MWD, mean weight diameter; WSAs, water-stable aggregates.

4. Discussion

The application of the exogenous EE-GRSP distinctly increased the root colonization of AMF and the hyphal length in soil in Newhall navel orange and Oita 4 trees, along with no significant difference in Cocktail grapefruit trees, suggesting that such varying magnitudes of response were highly dependent upon citrus varieties. Meng et al. [21] earlier observed improvements in root colonization of AMF and hyphal length in soil in two late-ripening sweet orange varieties (Lane Late navel and Rohde Red Valencia) in response to the foliar spray of the EE-GRSP. In fact, the EE-GRSP contained a variety of C-containing compounds and mineral elements (e.g., Fe and Mg), with the C content up to 1.01 ± 0.19 mg/g [15,16]. Such mineral element contents of the EE-GRSP are instrumental towards chlorophyll formation, thus facilitating the synthesis of photosynthates and onward transfer to root mycorrhizal pool, thus promoting the mycorrhizal formation in roots and soil [30].

Our study showed that the repeated EE-GRSP spray on fruits showed quite varying effects on the external quality of citrus fruits. Compared with the control treatment, the foliar spray of the EE-GRSP did not improve the fruit coloration values of three citrus varieties. Meng et al. [21] reported an improvement in fruit coloration value in Rohde Red Valencia, but not Lane Late navel orange. This suggests that the EE-GRSP effects on fruit coloration are dependent on citrus varieties. On the other hand, the foliar spray of the EE-GRSP improved fruit weight, depending on citrus varieties, which was evident from the improved total fruit weight on Newhall navel orange and the improved pericarp, sarcocarp, and total fruit weight on Cocktail grapefruit. Liu et al. [15] observed an increase in auxin content (indoleacetic acid and indole butyric acid) in leaves in response to the application of the exogenous EE-GRSP. Such an increase in auxin levels after the foliar spray of the EE-GRSP may be a reason for promoting the improvement of fruit size and weight. In our study, EE-GRSP-treated trees showed relatively lower fruit hardness on Newhall and Oita 4, but not Cocktail grapefruit, suggesting that some EE-GRSP-applied citrus fruits are more suitable for processing, whereas more field experiments are needed to confirm the effect.

The results of the present study also showed that the foliar spray of the EE-GRSP did not alter the internal quality of Newhall fruits, partially improved the internal quality (titratable acids and sucrose contents) of Oita 4 fruits but significantly improved soluble solids and titratable acids contents of Cocktail grapefruit fruits, suggesting the role of the EE-GRSP in fruit quality development. A similar result was earlier obtained with the response of the foliar spray of the EE-GRSP in Lane Late navel orange fruits [21]. Previously, it was also observed that purified GRSP contained elements like K, Mg, Ca, and Si, highly conducive for the formation of photosynthates and the transport of assimilates to fruits, thus improving the internal quality of fruits [14,21,31]. Wu et al. [31] further analyzed the effects of EE-GRSP treatment on the metabolism of starch and sucrose in citrus fruits by transcriptomic analysis. They found 15 different candidate genes implicated in the metabolism of starch and sucrose, with sucrose phosphate synthase 4 (a sucrose synthase) and β-fructofuranosidase (a sucrose cleavage enzyme) being induced by the exogenous EE-GRSP. Thus, the foliar spraying of the EE-GRSP might facilitate the synthesis of sucrose and its onward decomposition into glucose and fructose in fruits. However, underlying molecular mechanisms are not so well understood and have to be yet investigated.

Our results showed that the exogenous EE-GRSP triggered an increase in the concentrations of the EE-GRSP, the DE-GRSP, and the T-GRSP in citrus rhizosphere to varying degrees, especially on Oita 4, which is consistent with the results of Liu et al. [17]. The SOC has important roles in soil formation, fertility transformations, and soil physicochemical properties, of which the GRSP is an essential component [16,32]. The study showed that the exogenous application of the EE-GRSP resulted in significant increases in SOC in Oita 4 and Cocktail grapefruit trees, not in Newhall navel orange trees, indicating an important contribution of the EE-GRSP towards the buildup of SOC pools, depending upon citrus species. The application of the EE-GRSP is believed to enhance carbon sequestration in soil ecosystems by protecting unstable compounds in soil aggregates, thereby restricting the decomposition of accumulated organic matter [33].

The present results also indicated that the application of the exogenous EE-GRSP elevated soil NO_3-N and NH_4OAc-K contents in Newhall trees and increased soil NH_4-N, NO_3-N, Olsen-P, and NH_4OAc-K contents in Oita 4, along with no significant change in Cocktail grapefruit. Therefore, soils with high fertility promoted the tree growth and fruit quality in addition to prolonging the orchard life [34–36]. Earlier studies revealed that enhanced activities of various soil enzymes (phosphatase, polyphenol oxidase, and peroxidase) were involved in nutrient mineralization and the nutrient biogeochemical cycle, thus promoting the accumulation of nutrients [37]. It remains to be seen how such nutrient pool of soil affects the nutrient-partitioning citrus plants for elevated quality production of citrus.

Soil aggregates are used as an indicator of soil structure [4]. The exogenous EE-GRSP increased the percentages of different WSAs to varying degrees, depending upon citrus varieties. The change in WSAs resulted from an increase in the pool of GRSP and SOC following the EE-GRSP treatment, playing a glue in cementation of soil aggregates. On the other hand, MWD is an indicator of soil aggregate stability [33]. The exogenous EE-GRSP promoted the MWDs in Newhall and Cocktail grapefruit varieties, indicating an improvement of soil structure in the citrus rhizosphere. In short, the foliar application of the EE-GRSP promoted the distribution of WSAs, especially the WSA in the size of 0.5–1 mm, through the increased buildup of GRSP fractions and SOC, collectively imparting a favorable effect towards better root growth and consequently an improved quality of citrus fruits.

5. Conclusions

Our experiment supported the role of the EE-GRSP as a biostimulant improving the quality of citrus fruits, especially in Cocktail grapefruit, through changes in WSA and soil fertility parameters, aided further through mycorrhizal growth within the rhizosphere, a setup for elevated citrus performance. More details about soil−water relations and the contribution of the EE-GRSP towards recalcitrance of SOC over time could add better a scientific interpretation of such EE-GRSP-mediated plant responses.

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References

1. Lado, J.; Gambetta, G.; Zacarias, L. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. *Sci. Hortic.* **2018**, *233*, 238–248. [CrossRef]
2. Duru, S.; Hayran, S.; Gul, A. The analysis of competitiveness of Mediterranean countries in the world citrus trade. *Med. Agric. Sci.* **2022**, *35*, 21–26. [CrossRef]
3. Cao, M.A.; Zhang, F.; Abd_Allah, E.F.; Wu, Q.S. Mycorrhiza improves cold tolerance of Satsuma orange by inducing antioxidant enzyme gene expression. *BIOCELL* **2022**, *46*, 1959–1966. [CrossRef]
4. Cheng, X.F.; Xie, M.M.; Li, Y.; Liu, B.Y.; Liu, C.Y.; Wu, Q.S.; Kuča, K. Effects of field inoculation with arbuscular mycorrhizal fungi and endophytic fungi on fruit quality and soil properties of Newhall navel orange. *Appl. Soil. Ecol.* **2022**, *170*, 104308. [CrossRef]
5. Uzun, A.; Gulsen, O.; Yesiloglu, T.; Aka-Kacar, Y. Distinguishing grapefruit and pummelo accessions using ISSR markers. *Czech J. Genet. Plant.* **2010**, *46*, 170–177. [CrossRef]
6. Srivastava, A.K.; Hota, D.; Dahat, S.; Sharma, D. Citrus nutrition: An Indian perspective. *Ann. Plant Soil Res.* **2022**, *24*, 1–15. [CrossRef]
7. Cao, S.; Yang, S.; Gong, B.; Han, J.; Liao, W.; Zeng, B.; Luo, S.; Zhang, W. Effect of organic-inorganic fertilizer combined with alkaline materials soil-fruit improvement of citrus orchard. *China Fruits* **2022**, *3*, 44–49.
8. Wu, Q.S.; Srivastava, A.K.; Zou, Y.N. AMF-induced tolerance to drought stress in citrus: A review. *Sci. Hortic.* **2013**, *164*, 77–87. [CrossRef]
9. Bonfante, P. The future has roots in the past: The ideas and scientists that shaped mycorrhizal research. *New Phytol.* **2018**, *220*, 982–995. [CrossRef]
10. Cheng, S.; Zou, Y.N.; Kuca, K.; Hashem, A.; Abd_Allah, E.F.; Wu, Q.S. Elucidating the mechanisms underlying enhanced drought tolerance in plants mediated by arbuscular mycorrhizal fungi. *Front. Microbiol.* **2021**, *12*, 809473. [CrossRef]
11. Wu, Q.S.; Sun, P.; Srivastava, A.K. AMF diversity in citrus rhizosphere. *Ind. J. Agric. Sci.* **2017**, *87*, 653–656.
12. Yang, L.; Zou, Y.N.; Tian, Z.H.; Wu, Q.S.; Kua, K. Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings. *Sci. Hortic.* **2021**, *277*, 109815. [CrossRef]
13. Magurno, F.; Malicka, M.; Posta, K.; Wozniak, G.; Lumini, E.; Piotrowska-Seget, Z. Glomalin gene as molecular marker for functional diversity of arbuscular mycorrhizal fungi in soil. *Biol. Fertil. Soils* **2019**, *55*, 411–417. [CrossRef]
14. Barna, G.; Makó, A.; Takács, T.; Skic, K.; Füzy, A.; Horel, Á. Biochar alters soil physical characteristics, arbuscular mycorrhizal fungi colonization, and glomalin production. *Agronomy* **2020**, *10*, 1933. [CrossRef]
15. Liu, R.C.; Gao, W.Q.; Srivastava, A.K.; Zou, Y.N.; Kuča, K.; Hashem, A.; Abd_Allah, E.F.; Wu, Q.S. Differential effects of exogenous glomalin-related soil proteins on plant growth of trifoliate orange through regulating auxin changes. *Front. Plant Sci.* **2021**, *12*, 745402. [CrossRef]
16. He, J.D.; Chi, G.G.; Zou, Y.N.; Shu, B.; Wu, Q.S.; Srivastava, A.K.; Kuča, K. Contribution of glomalin-related soil proteins to soil organic carbon in trifoliate orange. *Appl. Soil Ecol.* **2020**, *154*, 103592. [CrossRef]
17. Liu, R.C.; Zou, Y.N.; Kuča, K.; Hashem, A.; Abd_Allah, E.F.; Wu, Q.S. Exogenous glomalin-related soil proteins differentially regulate soil properties in trifoliate orange. *Agronomy* **2021**, *11*, 1896. [CrossRef]
18. Rillig, M.C. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.* **2004**, *84*, 355–363. [CrossRef]
19. Wang, S.; Wu, Q.S.; He, X.H. Exogenous easily extractable glomalin-related soil protein promotes soil aggregation, relevant soil enzyme activities and plant growth in trifoliate orange. *Plant Soil Environ.* **2015**, *61*, 66–71. [CrossRef]
20. Chi, G.G.; Srivastava, A.K.; Wu, Q.S. Exogenous easily extractable glomalin-related soil protein improves drought tolerance of trifoliate orange. *Arch. Agron. Soil. Sci.* **2018**, *64*, 1341–1350. [CrossRef]
21. Meng, L.L.; Liang, S.M.; Srivastava, A.K.; Li, Y.; Liu, C.Y.; Zou, Y.N.; Wu, Q.S. Easily extractable glomalin-related soil protein as foliar spray improves nutritional qualities of late ripening sweet oranges. *Horticulturae* **2021**, *7*, 228. [CrossRef]
22. Wu, Q.S.; Li, Y.; Zou, Y.N.; He, X.H. Arbuscular mycorrhiza mediates glomalin-related soil protein production and soil enzyme activities in the rhizosphere of trifoliate orange grown under different p levels. *Mycorrhiza* **2015**, *25*, 121–130. [CrossRef] [PubMed]
23. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **1976**, *72*, 248–252. [CrossRef]
24. Von, C.S.; Griffiths, H. Stomatal responses to CO_2 during a diel Crassulacean acid metabolism cycle in *Kalanchoe daigremontiana* and *Kalanchoe pinnata*. *Plant Cell Environ.* **2009**, *32*, 567–576.
25. Wu, Q.S.; You, G.L.; Li, Y. Plant growth and tissue sucrose metabolism in the system of trifoliate orange and arbuscular mycorrhizal fungi. *Sci. Hortic.* **2015**, *181*, 189–193. [CrossRef]

26. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]
27. Ames, R.N.; Bethlenfalvay, G.J. Mycorrhizal fungi and the integration of plant and soil nutrient dynamics. *J. Plant. Nutr.* **1987**, *10*, 1313–1321. [CrossRef]
28. Rowell, D.L. *Soil Science: Methods and Applications*; Longman Scientific & Technical: Harlow, UK, 1994; p. 350.
29. Nimmo, J.R.; Perkins, K.S. Aggregate stability and size distribution. In *Methods of Soil Analysis: Part 4*; Soil Science Society of America, Inc.: Madison, WI, USA, 2002; pp. 317–328.
30. Gavito, M.E.; Jakobsen, I.; Mikkelsen, T.N.; Mora, F. Direct evidence for modulation of photosynthesis by an arbuscular mycorrhiza-induced carbon sink strength. *New Phytol.* **2019**, *223*, 896–907. [CrossRef]
31. Wu, H.H.; Srivastava, A.K.; Li, Y.; Zou, Y.N.; Hashem, A.; Abd_Allah, E.F.; Wu, Q.S. Transcriptomic analysis of late-ripening sweet orange fruits (*Citrus sinensis*) after foliar application of glomalin-related soil proteins. *Agriculture* **2021**, *11*, 1171. [CrossRef]
32. Lovelock, C.E.; Wright, S.F.; Clark, D.A.; Ruess, R.W. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *J. Ecol.* **2004**, *92*, 278–287. [CrossRef]
33. Schindler, F.V.; Mercer, E.J.; Rice, J.A. Chemical characteristics of glomalin-related soil protein (GRSP) extracted from soils of varying organic matter content. *Soil Biol. Biochem.* **2007**, *39*, 320–329. [CrossRef]
34. Srivastava, A.K.; Singh, S. Citrus decline: Soil fertility and plant nutrition. *J. Plant Nutr.* **2009**, *32*, 197–245. [CrossRef]
35. Zhao, Z.; Chu, C.; Zhou, D.; Sha, Z.; Wu, S. Soil nutrient status and the relation with planting area, planting age and grape varieties in urban vineyards in shanghai. *Heliyon* **2019**, *5*, e02362. [CrossRef] [PubMed]
36. Srivastava, A.K.; Wu, Q.S.; Mousavi, S.M.; Hota, D. Integrated soil fertility management in fruit crops: An overview. *Int. J. Fruit Sci.* **2021**, *21*, 413–439. [CrossRef]
37. Srivastava, A.K.; Paithankar, D.H.; Venkataramana, K.T.; Hazarika, B.; Patil, P. INM in fruit crops: Sustaining quality production and soil health. *Ind. J. Agric. Sci.* **2019**, *83*, 379–395.