horticulturae

MDPI

Article

Easily Extractable Glomalin-Related Soil Protein as Foliar Spray Improves Nutritional Qualities of Late Ripening Sweet Oranges

Lu-Lu Meng [1], Sheng-Min Liang [1], Anoop Kumar Srivastava [2], Yan Li [3], Chun-Yan Liu [1], Ying-Ning Zou [1], Kamil Kuča [4], Abeer Hashem [5], Elsayed Fathi Abd_Allah [6] and Qiang-Sheng Wu [1,4,*]

1 College of Horticulture and Gardening, Yangtze University, Jingzhou 434025, China; lulu_meng1997@163.com (L.-L.M.); Liangsm77@163.com (S.-M.L.); chunyanliu_2009@126.com (C.-Y.L.); 500406@yangtzeu.edu.cn (Y.-N.Z.)
2 ICAR-Central Citrus Research Institute, Nagpur 440033, India; aksrivas2007@gmail.com
3 Xingshan County Special Products Bureau, Yichang 443711, China; liyanliuyiling@126.com
4 Department of Chemistry, Faculty of Science, University of Hradec Kralove, 50003 Hradec Kralove, Czech Republic; kamil.kuca@uhk.cz
5 Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; habeer@ksu.edu.sa
6 Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; eabdallah@ksu.edu.sa
* Correspondence: wuqiangsheng@yangtzeu.edu.cn or wuqiangsh@163.com

Citation: Meng, L.-L.; Liang, S.-M.; Srivastava, A.K.; Li, Y.; Liu, C.-Y.; Zou, Y.-N.; Kuča, K.; Hashem, A.; Fathi Abd_Allah, E.; Wu, Q.-S. Easily Extractable Glomalin-Related Soil Protein as Foliar Spray Improves Nutritional Qualities of Late Ripening Sweet Oranges. *Horticulturae* **2021**, *7*, 228. https://doi.org/10.3390/horticulturae7080228

Academic Editor: Moreno Toselli

Received: 8 July 2021
Accepted: 28 July 2021
Published: 5 August 2021

Abstract: The role of arbuscular mycorrhizal fungi in sweet oranges is well known, but the function of their secondary metabolite, especially the easily extractable glomalin-related soil protein (EE-GRSP), an active fraction of glomalin, is still unclear. The proposed study aimed to analyze the field response of foliar application of exogenous EE-GRSP on tree mycorrhizal development and fruit quality of two sweet orange (*Citrus sinensis* L. Osbeck) varieties viz., Lane Late Navel (LLN) and Rohde Red Valencia (RRV). Application of EE-GRSP significantly increased the root mycorrhizal colonization and soil mycorrhizal hyphal length in both the sweet orange varieties. The external quality of fruits (fruit weight, polar diameter, and equatorial diameter) also improved in response to foliar application of EE-GRSP in both sweet orange varieties. However, EE-GRSP treatment showed no change in fruit soluble solid content, while it increased the Vc content, solids-acid ratio, fructose, glucose, and sucrose content of sarcocarp in the two sweet oranges varieties. The LLN variety treated with EE-GRSP recorded significantly higher N, P, K, Fe, and Si content of sarcocarp as a mark of nutritional quality, while the RRV variety treated with EE-GRSP displayed a higher concentration of nutrients like Cu, Fe, Si, and Zn in the sarcocarp as compared with the corresponding non-treated control. To the best of our knowledge, this is the first report regarding the improvement in fruit quality of late-ripening sweet oranges (especially LLN) in response to foliar application of EE-GRSP as another potential biostimulant.

Keywords: glomalin; mineral composition; mycorrhiza; nutritional quality; sweet orange

1. Introduction

Citrus is an evergreen commercially grown tree with worldwide and substantial trade and tariffs. The Three-Gorge reservoir region in China is an advantageous region for sweet orange cultivation. In recent years, late-ripening varieties of sweet oranges have been planted on a large scale in order to stretch out the ripening period and to solve the problem of the low market price of sweet oranges due to the surplus arrival of fruits in the market.

Arbuscular mycorrhizal fungi (AMF), are a kind of beneficial soil microorganisms that inhabit the rhizosphere of citrus trees [1,2] and colonize the root cells of the host plant, thereby, establishing a mutualistic relationship [3]. Earlier studies have shown that AMF inoculation improved plant growth and nutrient acquisition of potted trifoliate orange and fruit quality of Ponkan mandarin in the field through developed mycorrhizal extraradical hyphae and improved root hairs [4–6]. Another feature of AMF is to enhance drought tolerance in citrus through the regulation of fatty acid composition, polyamine metabolism, root-hair changes, hormone balance, etc. [4,7–9]. However, the field application of AMF in citrus groves is considered impractical and not feasible since in-vitro propagation of AMF on a large scale is still not possible for field application [10].

The spores and mycelium of AMF release a recalcitrant (hydrophobic and heat-stable) glycoprotein, called glomalin deposited into the soil (defined as glomalin-related soil protein, GRSP) [11]. Among them, easily extractable glomalin-related soil protein (EE-GRSP) is comparatively newly produced and more active in GRSP fractions [12]. GRSP is reported to be highly efficient in cementing soil aggregates and maintaining soil aggregate stability, thereby reducing the water and nutrient loss under a stress environment [13,14]. GRSP is a component of soil organic matter, contributing 4–23% of the total soil organic carbon in various soils [15,16], and accounted for the soil nitrogen pool representing 34% of soil total N in citrus orchards [17]. Purified GRSP contained H, O, S, K, P, Ca, Si, Fe, Cu, and Mg elements [18]. The nutrients like K, Ca, and Si are associated with the development of fruit quality [19–21]. For example, foliar K fertilization obviously increased the sugar content of strawberry fruits [19]. Exogenous Si and B treatment elevated the vitamin C content and maintained longer shelf life and flesh firmness of cherry tomato fruits [20,21]. These observations imply that GRSP might have a benefit on fruit quality, however, at present, there is no such direct field evidence.

In the past, we attempted to apply EE-GRSP as a soil/plant regulator in improving plant growth and soil properties in citrus. Exogenous EE-GRSP with a 1/2 strength exhibited positive effects on soil aggregation and plant growth of potted trifoliate orange [22]. Later, Chi et al. [23] observed that trifoliate orange plants treated with EE-GRSP had greater drought tolerance than non-treated plants. Wu et al. [24] observed that exogenous EE-GRSP increased the soil organic carbon content, enhanced soil aggregate stability, and elevated soil phosphatase activities in Satsuma mandarin. Such results provided new insights for more extensive research on exploring GRSP as an important plant/soil regulator in citrus.

Earlier studies have been conducted using AMF inoculation on fruit trees such as citrus, strawberry, and grape to analyze the effect on fruit quality [5,25,26]. The inoculation with *Funneliformis mosseae* and mixed AMF on Ponkan mandarin improved the fruit size, fruit coloration value, and fruit P, Mn, and Fe content to varying degrees [5]. The grape inoculated with *Rhizophagus intraradices* showed greater soluble solids and anthocyanin content of fruits [25]. In strawberry, *Glomus intraradices* improved the phenolic acid content of fruits, such as gallic, *p*-coumaric, ferulic, and ellagic acid [26]. These results showed a positive benefit of AMF on fruit quality. Whether GRSP, a secondary metabolite of AMF, has similar benefits to AMF, is not still known. With these evidence-based benefits (e.g., rich in various mineral elements) of GRSP, we hypothesized that exogenous GRSP could be helpful in improving the nutritional quality of fruits along with other fruit quality parameters. This was the basis of the proposed field experimentation using two late-ripening varieties of sweet oranges, and the information on these lines is very limited.

2. Materials and Methods

2.1. Experimental Setup

The experiment was conducted in a citrus orchard (31°8′ N, 110°46′ E, 200 m) located at Xiakou town, Xingshan county, carrying Lane Late Navel (*Citrus sinensis* L. Osbeck) (LLN) and Rohde Red Valencia orange (*C. sinensis* L. Osbeck) (RRV) grafted on trifoliate orange (*Poncirus trifoliata* L. Raf.) in a 3 × 4 m inter-planting spacing on Xanthi-Udic Ferralsols (FAO system). The experimental site was located in the Three Gorges Region, featuring subtropical continental monsoon climate. The physico-chemical properties of the soil consisted of: pH 5.7, organic matter 13.1 g/kg, Olsen-P 27.1 mg/kg, NH_4OAc-K 156.2 mg/kg, and $KMnO_4$-N 98.3 mg/kg.

The experiment consisted of a 2 × 2 factorial design involving two exogenous EE-GRSP treatments and two sweet orange varieties (LLN and RRV). A total of four treatments replicated five times were arranged in a completely randomized block design. Each block as a replicate had three trees, resulting in a total of 60 trees. The application of EE-GRSP was begun on 8 August 2018. The over-ground part of citrus trees was sprayed with designed EE-GRSP solution (1 L per tree), while the control trees were sprayed with the same amount of 20 mM citrate buffers (pH 7.0). An 8 m distance between treated tree blocks and control tree blocks was maintained to avoid cross-contamination. The spray was repeated three times at fortnightly intervals coinciding with fruit set to fruit developmental stages. The fruits from treated and non-treated trees were harvested at physiological maturity (LLN and RRV harvested on April 15, 2019 and May 10, 2019, respectively). Twenty fruits, uniform in size, free from sunburn, cracks, and diseases were selected from each tree, covering different directions for onward fruit quality analysis. The fine roots and the rhizospheric soil of 5–15 cm soil depth were collected at the commercial maturity of fruits.

2.2. Preparation of Exogenous EE-GRSP Solution

To extract EE-GRSP, the soil from a 28-yr-old Satsuma mandarin (*C. unshiu* Marc. cv. Guoqing No. 1) grafted on trifoliate orange located on the Yangtze University campus (Jingzhou, China) (30°36′ N, 112°14′ E, 36 m) was selected. The soil was classified as the Xanthi-Udic Ferralsols and characterized as pH 6.2, Olsen-P 15.1 mg/kg, and soil organic carbon 9.9 g/kg. The soil was sieved through 4 mm sieve after air-drying. The extraction of EE-GRSP was carried out by following the protocol described by Wu et al. [12]. A 1-g soil sample was mixed with 8 mL 20 mM citrate buffers (pH 7.0), autoclaved for 30 min at 121 °C and 0.11 MPa, and centrifuged at 10,000 g/min for 5 min. Such supernatant was collected continuously and repeatedly up to the amount of 45 L, which was used as the stock solution of exogenous EE-GRSP. The protein concentration (16 mg protein/L) in the stock solution was measured as per Bradford [27] assay. The collected EE-GRSP supernatant was stored at 4 ºC, ready to use if necessary. The original EE-GRSP was diluted with equal volume of 20 mM citrate buffer (pH 7.0), which was prepared as applied exogenous EE-GRSP solution used for field evaluation [22,23].

2.3. Measurements of Variables

Arbuscular mycorrhizal colonization of roots and mycorrhizal hyphal length of soil were assayed by the methods described by Phillips and Hayman [28] and Bethlenfalvay and Ames [29], respectively.

The fruit weight of single fruit was determined by an electronic balance, and the fruit's horizontal and vertical diameter was determined by a digital vernier caliper (111-101B, Guilin Guanglu Measuring Instrument Co., Ltd., Guilin, China). The coloration value of the fruit peel was measured by a colorimeter (CR10, Konica Minolta Inc., Tokyo, Japan). The total soluble solid content was analyzed by a portable refractometer (WYT-4, Shanghai, China). Titratable acidity and vitamin C (Vc) contents in fruit juices were determined using the indicator-based titration method [30] using 0.1 mol/L NaOH-methylene blue indicator and 2,6-dichloroindophenol titration method [31], respectively.

After removing the fruit peel, the fresh sarcocarp was oven-dried, ground, and finally digested in H_2SO_4–H_2O_2 for the analysis of mineral element contents. The concentration of mineral elements such as P, K, Mg, Cu, Fe, Si, and Zn was measured with the help of an ICP Spectrometer (IRIS Advantage 1000, Thermo Jarrel Ash, Franklin, MA, USA). The N content was determined following the procedure as suggested by Zhao et al. [32] using an electrochemical analyzer (Smartchem 200, Scientific Instruments Limited, Weston, FL, USA).

Sucrose, glucose, and fructose contents of oven-dried sarcocarp were determined according to the method described by Wu et al. [33].

2.4. Statistical Analysis

The experimental data were processed using two-factor analysis of variance (ANOVA) in SAS (v8.1) (SAS Institute, Inc., Cary, NC, USA), and Duncan's multiple range test was used to compare the significance between treatments at $p \leq 0.05$.

3. Results and Discussion

3.1. Responses on Mycorrhizal Growth

Both LLN and RRV varieties were colonized by indigenous AMF. Application of exogenous EE-GRSP significantly increased the root AMF colonization of LLN and RRV by 88% and 140%, respectively, with a corresponding increase in soil hyphal length by 188% and 109% (Table 1). Schindler et al. [34] reported that GRSP contained aromatic hydrocarbons (42–49%), carboxyl carbon (24–30%), low fat (4–11%), and typical carbohydrates (4–16%). These C-containing substances are highly beneficial for AMF growth, because AMF requires C from the host plant [35].

Table 1. Application response of exogenous EE-GRSP on root mycorrhizal colonization and mycorrhizal length within the mycorrhizosphere of late-ripening sweet oranges grafted on trifoliate orange.

Sweet Orange Variety	EE-GRSP Treatments	Root AMF Colonization (%)	Soil Mycorrhizal Length (cm/g Soil)
LLN	EE-GRSP	38.02 ± 0.09a	10.02 ± 0.46c
	Non-EE-GRSP	20.23 ± 0.19c	3.48 ± 0.09d
RRV	EE-GRSP	33.89 ± 0.15b	26.20 ± 0.58a
	Non-EE-GRSP	14.10 ± 0.07d	12.51 ± 0.25b
Significance			
EE-GRSP treatments		**	**
Sweet orange varieties		**	**
Interaction		**	**

Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant differences ($p < 0.05$) between treatments. ** $p < 0.01$. LLN and RRV correspond to Lane Late Navel and Rohde Red Valencia, respectively.

3.2. Responses on External Fruit Quality Parameters

Our study showed that foliar treatment of EE-GRSP affected external and internal fruit qualities (Table 2). Compared with non-EE-GRSP-treated trees, EE-GRSP-applied trees exhibited 21.0 and 21.5% significantly higher fruit weight, 10.2 and 5.6% higher fruit polar diameter, and 10.3 and 5.2% higher fruit equatorial diameter of LLN and RRV, respectively, suggesting that exogenous EE-GRSP improved the fruit size, regardless of tested varieties. Sui et al. [36] earlier reported an increase in fruit weight of *Citrus sinensis* following the inoculation of *Funneliformis mosseae*. Since EE-GRSP is a secondary metabolite of AMF [16], an increase in fruit size and weight could be expected with exogenous EE-GRSP treatment. In addition, GRSP contains a K element [18] that promotes peel cell growth and, thus, is supposed to contribute to fruit enlargement. The coloration value of fruits in physiological maturity was enhanced by the treatment with EE-GRSP in RRV, but not in LLN, indicating that the response of fruit coloration to EE-GRSP is predominantly variety-dependent. In addition, the two late-ripening varieties experienced flowering and fruiting together before the commercial harvesting period. The number of flowers and climatic conditions also affect the fruit coloration, resulting in the fluctuation in fruit color development. Thus, more in-depth analysis work is needed to undertake to study the response of EE-GRSP application on the spatio-temporal variation of fruit coloration. Zhang et al. [18] observed 0.28–0.59% K content in purified EE-GRSP in low and high salinity soil. The exogenous EE-GRSP application, therefore, supplies sufficient foliar K. Earlier studies confirmed that foliar application of K as KNO_3 and K_2SO_4 increased the fruit weight and fruit size of Clementine mandarin [37].

3.3. Responses on Internal Fruit Quality Parameters

EE-GRSP also produced a significant response on changes in internal quality parameters of fruits (Table 3). Although foliar EE-GRSP application, to some extent, increased the soluble solid content, the effect was not significant at the 5% level in LLN and RRV varieties (Table 3). However, the LLN variety treated with EE-GRSP recorded a significantly lower titratable acidity in fruits and higher Vc content as compared with non-treated LLN, while, the foliar treatment of EE-GRSP significantly increased the titrable acidity and reduced the Vc content of fruits of RRV. This suggested that exogenous EE-GRSP accelerated the degradation of organic acids (e.g., citric acid) in LLN fruits, while it increased organic acid contents in RRV fruits. The effect on organic acids was dependent on varieties; the underlying mechanism is unknown. We speculated that the mechanism could be related to the change in the enzymes associated with organic acid synthesis or degradation caused by EE-GRSP [38].

Table 2. Application response of exogenous EE-GRSP on the external fruit quality parameters of late-ripening sweet oranges.

Sweet Orange Variety	EE-GRSP Treatments	Fruit Weight (g/Fruit)	Coloration Value	Polar Diameter (mm)	Equatorial Diameter (mm)
LLN	EE-GRSP	228.50 ± 3.61a	78.34 ± 2.84a	74.8 ± 0.9a	78.01 ± 0.65a
	Non-EE-GRSP	188.79 ± 6.81b	79.79 ± 1.88a	67.9 ± 0.8b	70.70 ± 0.68b
RRV	EE-GRSP	157.62 ± 3.53c	73.83 ± 2.64b	65.6 ± 0.8c	65.28 ± 0.64c
	Non-EE-GRSP	129.75 ± 2.99d	70.94 ± 2.09c	62.1 ± 0.4d	62.03 ± 0.71d
Significance					
EE-GRSP treatments		**	*	**	**
Sweet orange varieties		**	**	**	**
Interaction		**	**	**	**

Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant differences ($p < 0.05$) between treatments. * $p < 0.05$; ** $p < 0.01$. LLN and RRV correspond to Lane Late Navel and Rohde Red Valencia, respectively.

An analysis of the changes in solids–acid ratio showed that foliar EE-GRSP application increased solids–acid ratio by 30.4% in LLN without any significant response in RRV. These observations showed that the application of EE-GRSP improved Vc content, titrable acidity, and solids–acid ratio of fruits, depending upon the type of citrus varieties. The purified EE-GRSP is reported to contain a variety of nutrients comprising K, P, S, Cl, Na, Ca, Mg, Zn, Fe, Cu, Si, and Al [18], out of which the role of K in the development of fruit quality and the transport and conversion of assimilates is extensively documented [19,39]. Foliar spray with Ca and Si improved the internal fruit quality of fruits and vegetables [20,21]. Therefore, EE-GRSP, by virtue of different mineral elements, therefore, offers a promising alternative to regulating the internal fruit quality parameters, with some varietal response preferences.

Foliar spray with EE-GRSP significantly increased different forms of sugars, viz., sucrose, fructose, and glucose content by 30.4, 10.2, and 21.4% higher in LLN, with a corresponding increase of 14.6, 15.5, and 33.2% in RRV over control (Table 3). Zhang et al. [8] observed higher leaf chlorophyll concentration coupled with a greater photosynthetic rate of mycorrhizal versus non-mycorrhizal trifoliate orange. The K enrichment of GRSP [18] and superior mycorrhizal development of EE-GRSP-treated sweet orange trees (Table 1), therefore, provided higher sugar concentrations than untreated fruits.

3.4. Responses on Mineral Composition of Fruit Pulp

The foliar application of EE-GRSP altered the concentration of different mineral elements in fruit pulp of LLN and RRV to varying proportions (Table 4). Foliar spray of exogenous EE-GRSP significantly increased N, P, K, Fe, and Si content in fruit pulp of LLN variety by 20.5, 77.7, 9.9, 32.5, and 12.9%, respectively, coupled with the reduction in Cu and Zn content by 16.6 and 12.4%, compared with non-EE-GRSP treatment. While the response of the RRV variety to the application of EE-GRSP showed a distinct increase of 78.0% in Cu, 47.2% in Fe, 39.8% in Si, and 19.0% in Zn content, but was associated with a 32.0% lower N content than non-treated fruits, suggesting a differential nutrient accumulation pattern in two sweet orange varieties in response to foliar application of EE-GRSP. GRSP contains various elements such as N, C, K, Si, and Fe [16–18]. Therefore, the application of exogenous EE-GRSP could deposit some of these elements into the pulp, while the underlying mechanisms are yet not clearly elucidated. Sweet orange trees treated with EE-GRSP had superior mycorrhizal networking inside the root system, which provided a pathway through the hyphal network for developing a parallel nutrient sink for fruits [8]. Earlier studies showed that AMF inoculation promoted the K and Cu concentration of strawberry fruits and the P, Mn, and Fe content of Ponkan mandarin fruits [5,26], providing some valid clues to try out the foliar application of GRSP to stimulate the mycorrhizal development vis-a-vis root system for improved fruit quality. In addition, the weight of single fruit of LLN and RRV treated with EE-GRSP increased significantly, which resulted in a dilution effect on some elements (e.g., Cu in LLN fruits and N in RRV fruits), and thus, the decrease in these elements was observed after EE-GRSP application. Of course, the content of mineral elements in fruits fluctuated with the year and season [40]. The experiment was carried out for one year, and more results on year and citrus varieties are needed to support such observations.

Table 3. Application response of exogenous EE-GRSP on the internal fruit quality parameters of late ripening sweet oranges.

Sweet Oranges	EE-GRSP Treatments	Vc Content (mg/g)	Soluble Solid Content (%)	Titrable Acidity (%)	Solids-Acid Ratio	Sucrose (mg/g DW)	Fructose (mg/g DW)	Glucose (mg/g DW)
LLN	EE-GRSP	4.80 ± 0.03a	13.16 ± 0.31a	0.35 ± 0.04d	38.15 ± 2.27a	135.26 ± 2.03b	462.66 ± 5.36c	86.06 ± 1.96a
	Non-EE-GRSP	4.17 ± 0.04b	12.58 ± 0.48a	0.44 ± 0.06c	29.25 ± 1.01b	103.73 ± 0.90c	419.68 ± 3.38d	70.91 ± 1.50c
RRV	EE-GRSP	3.74 ± 0.04c	10.41 ± 0.44b	2.89 ± 0.02a	3.61 ± 0.16c	154.21 ± 4.54a	613.75 ± 5.17a	81.85 ± 1.49b
	Non-EE-GRSP	4.18 ± 0.03b	10.09 ± 0.66b	2.67 ± 0.05b	3.77 ± 0.26c	134.54 ± 3.90b	531.45 ± 5.96b	61.45 ± 1.28d
Significance								
EE-GRSP treatments		**	*	**	*	**	**	**
Sweet orange varieties		**	**	**	**	**	**	**
Interaction		**	NS	**	**	**	**	**

Data (means ± SD, n = 5) followed by different letters in the column indicate significant differences ($p < 0.05$) between treatments. NS, not significant; * $p < 0.05$; ** $p < 0.01$. LLN and RRV correspond to Lane Late Navel and Rohde Red Valencia, respectively.

Table 4. Application response of exogenous EE-GRSP on the mineral composition of fruit pulp of late-ripening sweet oranges.

Sweet Oranges	EE-GRSP Treatments	N (g/kg)	P (g/kg)	K (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Si (mg/kg)	Zn (mg/kg)
LLN	EE-GRSP	7.47 ± 0.28b	2.47 ± 0.14a	12.88 ± 0.14a	208.90 ± 1.33c	44.72 ± 0.71a	15.38 ± 0.50b	47.15 ± 0.59d
	Non-EE-GRSP	6.20 ± 0.21c	1.39 ± 0.09c	11.72 ± 0.09b	250.60 ± 2.40b	33.75 ± 0.47b	13.62 ± 0.91c	53.83 ± 1.27c
RRV	EE-GRSP	5.69 ± 0.24d	1.53 ± 0.07b	12.88 ± 0.07a	305.07 ± 1.52a	32.92 ± 0.59c	16.84 ± 0.96a	72.67 ± 0.46a
	Non-EE-GRSP	8.37 ± 0.41a	1.60 ± 0.05b	12.86 ± 0.05a	171.42 ± 1.60d	22.37 ± 0.57d	12.05 ± 0.10d	61.09 ± 0.61b
Significance								
EE-GRSP treatments		**	**	*	**	**	**	**
Sweet orange varieties		**	**	*	**	**	**	**
Interaction		**	**	*	**	NS	**	**

Data (means ± SD, n = 5) followed by different letters in the column indicate significant differences ($p < 0.05$) between treatments. NS, not significant; * $p < 0.05$; ** $p < 0.01$. LLN and RRV correspond to Lane Late Navel and Rohde Red Valencia, respectively.

4. Conclusions

Our study first revealed that exogenous EE-GRSP application strongly improved the external fruit quality of two late-ripening varieties of sweet oranges (LLN and RRV), along with the increase in fructose, glucose, and sucrose contents and Si and Fe contents of fruits. The beneficial effect of exogenous EE-GRSP was more pronounced with LLH than with RRV, due to higher root and soil mycorrhizal growth induced by EE-GRSP in the LLH variety. Our field studies provided strong database evidence about the possible role of exogenous EE-GRSP as a natural source of nutrients and other biostimulants as well in improving the nutritional qualities of late-ripening sweet oranges, especially the LLN variety. The extraction process of EE-GRSP is very simple, the materials used, including soils and citric acid buffer, are inexpensive, and the instruments used are mainly refrigerated centrifuges and autoclaves. Therefore, EE-GRSP could be a very high economic value biostimulant in the next few years. The application of EE-GRSP should gain more attention from researchers worldwide to test this concept in other fruits and citrus at different ripening stages.

Author Contributions: Conceptualization, Y.-N.Z. and Q.-S.W.; methodology, L.-L.M., S.-M.L. and Y.L.; data determination and statistical analysis, L.-L.M., S.-M.L., C.-Y.L. and Y.L.; writing—original draft preparation, L.-L.M., S.-M.L., A.K.S. and Q.-S.W.; writing—review and editing, A.H., E.F.A., A.K.S., K.K. and Q.-S.W.; supervision, Q.-S.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Plan in Scientific and Technological Innovation Team of Outstanding Young Scientists, Hubei Provincial Department of Education (T201604) and the Hubei Agricultural Science and Technology Innovation Action Project (Hubei Nongfa [2018] No. 1). The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/134), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data supporting the findings of this study are included in this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. 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]
2. Wu, Q.S.; Sun, P.; Srivastava, A.K. AMF diversity in citrus rhizosphere. *Ind. J. Agric. Sci.* **2017**, *87*, 653–656.
3. Peterson, R.L.; Massicotte, H.B.; Melville, L.H. *Mycorrhizas: Anatomy and Cell Biology*; NRC Research Press: Ottawa, ON, Canada, 2004; pp. 55–80.
4. Zhang, F.; Wang, P.; Zou, Y.N.; Wu, Q.S.; Kuča, K. Effects of mycorrhizal fungi on root-hair growth and hormone levels of taproot and lateral roots in trifoliate orange under drought stress. *Arch. Agron. Soil Sci.* **2019**, *65*, 1316–1330. [CrossRef]
5. Wu, Q.S.; Gao, W.Q.; Srivastava, A.K.; Zhang, F.; Zou, Y.N. Nutrient acquisition and fruit quality of Ponkan mandarin in response to AMF inoculation. *Ind. J. Agric. Sci.* **2020**, *90*, 1563–1567.
6. Yang, L.; Zou, Y.N.; Tian, Z.H.; Wu, Q.S.; Kuča, K. Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings. *Sci. Hortic.* **2021**, *277*, 109815. [CrossRef]
7. Wu, Q.S.; He, J.D.; Srivastava, A.K.; Zou, Y.N.; Kuča, K. Mycorrhizas enhance drought tolerance of citrus by altering root fatty acid compositions and their saturation levels. *Tree Physiol.* **2019**, *39*, 1149–1158. [CrossRef] [PubMed]
8. Zhang, F.; Zou, Y.N.; Wu, Q.S.; Kuča, K. Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environ. Exp. Bot.* **2020**, *171*, 103962. [CrossRef]
9. Zou, Y.N.; Zhang, F.; Srivastava, A.K.; Wu, Q.S.; Kuča, K. Arbuscular mycorrhizal fungi regulate polyamine homeostasis in roots of trifoliate orange for improved adaptation to soil moisture deficit stress. *Front. Plant Sci.* **2021**, *11*, 600792. [CrossRef]
10. Ijdo, M.; Cranenbrouck, S.; Declerck, S. Methods for large-scale production of AM fungi: Past, present, and future. *Mycorrhiza* **2011**, *21*, 1–16. [CrossRef]
11. Holatko, J.; Brtnicky, M.; Kucerik, J.; Kotianova, M.; Elbl, J.; Kintl, A.; Kynicky, J.; Benada, O.; Datta, R.; Jansa, J. Glomalin-truths, myths, and the future of this elusive soil glycoprotein. *Soil Biol. Biochem.* **2020**, *153*, 108116. [CrossRef]
12. 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]

13. Peng, S.L.; Guo, T.; Liu, G.C. The effects of arbuscular mycorrhizal hyphal networks on soil aggregations of purple soil in southwest China. *Soil Biol. Biochem.* **2013**, *57*, 411–417. [CrossRef]
14. Zou, Y.N.; Srivastava, A.K.; Wu, Q.S.; Huang, Y.M. Glomalin-related soil protein and water relations in mycorrhizal citrus (*Citrus tangerina*) during soil water deficit. *Arch. Agron. Soil Sci.* **2014**, *60*, 1103–1114. [CrossRef]
15. Rillig, M.C.; Wright, S.F.; Nichols, K.A.; Schmidt, W.F.; Torn, M.S. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* **2001**, *233*, 167–177. [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. Meng, L.L.; He, J.D.; Zou, Y.N.; Wu, Q.S.; Kuča, K. Mycorrhiza-released glomalin-related soil protein fractions contribute to soil total nitrogen in trifoliate orange. *Plant Soil Environ.* **2020**, *66*, 183–189. [CrossRef]
18. Zhang, Z.H.; Wang, Q.; Wang, H.; Nie, S.M.; Liang, Z.W. Effects of soil salinity on the content, composition, and ion binding capacity of glomalin-related soil protein. *Sci. Total Environ.* **2017**, *581–582*, 657–665. [CrossRef] [PubMed]
19. Bamouh, A.; Bouras, H.; Nakro, A. Effect of foliar potassium fertilization on yield and fruit quality of strawberry, raspberry and blueberry. *Acta Hortic.* **2019**, *1265*, 255–262. [CrossRef]
20. Islam, M.Z.; Mele, M.A.; Baek, J.P.; Kang, H.M. Cherry tomato qualities affected by foliar spraying with boron and calcium. *Hortic. Environ. Biotechnol.* **2016**, *57*, 46–52. [CrossRef]
21. Islam, M.Z.; Mele, M.A.; Choi, K.Y.; Kang, H.M. The effect of silicon and boron foliar application on the quality and shelf life of cherry tomatoes. *Zemdirb. Agric.* **2018**, *105*, 159–164. [CrossRef]
22. 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]
23. 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]
24. Wu, Q.S.; Srivastava, A.K.; Wang, S.; Zeng, J.X. Exogenous application of EE-GRSP and changes in citrus rhizosphere properties. *Ind. J. Agric. Sci.* **2015**, *85*, 802–806.
25. Torres, N.; Giocoechea, N.; Antolin, M.C. Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures. *Agric. Water Manag.* **2018**, *202*, 285–298. [CrossRef]
26. Castellanos-Morales, V.; Villegas, J.; Wendelin, S.; Vierheilig, H.; Eder, R.; Cardenas-Navarro, R. Root colonisation by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria* × *Ananassa duch.*) at different nitrogen levels. *J. Sci. Food Agric.* **2010**, *90*, 1774–1782. [CrossRef]
27. Bradford, M.M. A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **1976**, *72*, 248–254. [CrossRef]
28. Phillips, J.M. 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]
29. Ames, R.N.; Bethlenfalvay, G.J. Mycorrhizal fungi and the integration of plant and soil nutrient dynamics. *J. Plant Nutri.* **1987**, *10*, 1313–1321. [CrossRef]
30. Von Caemmerer, 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. [CrossRef]
31. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, USA, 1991.
32. Zhao, X.Y.; Zhou, S.; Wang, G.; Xing, G.; Shi, W.; Xu, R.K.; Zhu, Z.L. Nitrogen balance in a highly fertilized rice-wheat double-cropping system in Southern China. *Soil Sci. Soc. Am. J.* **2012**, *76*, 1068–1078. [CrossRef]
33. Wu, Q.S.; Lou, Y.G.; 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]
34. 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]
35. 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] [PubMed]
36. Sui, B.Q.; Xu, S.J.; Yue, L.N.; Zeng, M. Effect of the reduction of phosphorus fertilizer and inoculation with AM fungi on the AM formation in the citrus and on the fruit quality. *South Chin. Agric.* **2007**, *1*, 5–7.
37. Hamza, A.; Bamouh, A.; El Guilli, M.; Bouabid, R. Response of 'Cadoux' clementine to foliar potassium fertilization: Effects on fruit production and quality. *Acta Hortic.* **2015**, *1065*, 1785–1793. [CrossRef]
38. Cercos, M.; Soler, G.; Iglesias, D.J.; Gadea, J.; Forment, J.; Talon, M. Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization. *Plant Mol. Biol.* **2006**, *62*, 513–527. [CrossRef] [PubMed]
39. Sun, Q.; Yang, J.; Zhang, S.Y.; Zhang, F.Q.; Ding, S.L. Research progress on relationship between potassium nutrition and photosynthesis physiology and fruit quality of fruit trees. *Guangdong Agric. Sci.* **2006**, *33*, 126–129.
40. Storey, R.; Treeby, M.T. Seasonal changes in nutrient concentrations of navel orange fruit. *Sci. Hortic.* **2000**, *84*, 67–82. [CrossRef]