



**Full Length Article**

# Contribution of Arbuscular Mycorrhizas to Glomalin-Related Soil Protein, Soil Organic Carbon and Aggregate Stability in Citrus Rhizosphere

Qiang-Sheng Wu<sup>1\*</sup>, Yong-Ming Huang<sup>1</sup>, Yan Li<sup>1</sup>, Nasrullah<sup>2</sup> and Xin-Hua He<sup>3,4</sup>

<sup>1</sup>College of Horticulture and Gardening, Yangtze University, 88 Jingmi Road, Jingzhou, Hubei 434025, China

<sup>2</sup>Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

<sup>3</sup>Forests NSW, NSW Department of Primary Industries, West Pennant Hills, NSW 2125, Australia

<sup>4</sup>School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia

\*For correspondence: wuqiangsh@163.com

## Abstract

Low soil organic matter (SOM) (5–15 g kg<sup>-1</sup>) and poor physical and chemical soil properties in most of the citrus orchards in the southern regions of China lead to reduce yield and fruit quality. To investigate the contribution of arbuscular mycorrhizas (AMs) to soil carbon pools and aggregate stability, a pot study was designed to analyze glomalin-related soil protein (GRSP), soil organic carbon (SOC), and proportion of different water-stable aggregate (WSA) fractions in rhizosphere of *Citrus tangerina*, *Fortunella margarita* and *Poncirus trifoliata* inoculated with or without an arbuscular mycorrhizal (AM) fungus, *Funneliformis mosseae*. The AM colonization significantly improved all tested plant growth and root morphological traits of five-month-old *C. tangerina* and *F. margarita*, than *P. trifoliata*. Mycorrhizosphere of these three citrus plants showed 19–26% higher easily extractable GRSP (EE-GRSP), 13–20% higher total GRSP (T-GRSP), and 39–325% higher SOC, and 18–72% lower T-GRSP/SOC ratio. Compared with their non-mycorrhizosphere, the mycorrhizosphere of these three citrus plants had significantly higher proportion of WSA at 2.00–4.00, 1.00–2.00, 0.50–1.00, and >0.25 mm size fractions, and 119–217% higher mean weight diameter (MWD, an indicator of aggregate stability). Both EE-GRSP and T-GRSP fractions were significantly positively correlated with SOC, WSA fractions and MWD. The present study indicated a positive contribution of GRSPs to SOC and soil aggregate stability in rhizosphere, irrespectively of citrus genotypes and/or mycorrhization. These results would provide important information on better understanding the contribution of GRSPs to soil organic C dynamics and structure stability. © 2014 Friends Science Publishers

**Keywords:** Arbuscular mycorrhizal fungi; Citrus; Glomalin; Soil organic carbon; Water-stable aggregate

## Introduction

AMs, the most widespread underground symbiosis, are formed between soil arbuscular mycorrhizal fungi (AMF) and roots of ~80% land plants (Smith and Read, 2008). In general, AMF can help the host plant to take up nutrient elements and water from soil, in exchange of photosynthetically fixed carbon (C) for their own activities (Smith and Read, 2008).

AMF produce a glycoprotein glomalin that coats extraradical hyphae (Wright and Upadhyaya, 1996). Approximately 30–40% of the glomalin is constituted by C due to both its protein and carbohydrate subunits (Rillig *et al.*, 2003b). After the death of extraradical hyphae, glomalin enters into soils and is defined as GRSP (Rillig, 2004). In general, GRSP accounted for 4 to 5% of total C and nitrogen (N) source, which was higher than the contribution of microbial biomass C in Hawaiian soils (Rillig *et al.*, 2001). Therefore, GRSP is crucial for the regulation of SOC

and the maintenance of soil fertility (Preger *et al.*, 2007). A few studies have shown a strong positive correlation between GRSP and SOC (Franzluebbers *et al.*, 2000; Cornejo *et al.*, 2008; He *et al.*, 2010), indicating that AMF plays an important role in the soil C cycle. In addition, under elevated CO<sub>2</sub> AMF stimulated the decomposition of SOC in AMF-active zones (Cheng *et al.*, 2012). However, information about the roles of AMF on GRSP and SOC in rhizosphere is lacking, particularly in citrus trees that have poor root system development than most of higher plants.

Soil structure, defined as the size, shape and arrangement of the solid particles and spaces (da Veiga *et al.*, 2009), is usually dependent on the degree of aggregates stability. Meanwhile, soil aggregation is involved in the consolidation of soil particles into microaggregates (<0.25 mm) and further formation of macroaggregates (>0.25 mm) (Borie *et al.*, 2008). Aggregate stability as an indicator of soil structure represents water-holding capacity and SOC storage (Rawlins *et al.*, 2013). Macroaggregates are formed

and stabilized by root, fungal hyphae and GRSP (Nichols and Toro, 2011). Studies have shown that AM hyphae and GRSP contribute to aggregate stability of different size fractions (Wright and Upadhyaya, 1998; Bearden and Petersen 2000; Peng *et al.*, 2013). In contrast, there was a negative correlation between GRSP and percentage of 1.0–2.0 mm water-stable aggregate (WSA) in the rhizosphere of *Stipa tenacissima* in Spain (Rillig *et al.*, 2003a). As a result, the relationship between GRSP and aggregate stability has not been fully understood in the rhizosphere of host plants.

As one of the most important fruit trees in the world, citrus has been widely planted in southern regions of China, where the soil has low soil organic matter (SOM) (5–15 g kg<sup>-1</sup>) and poor physical and chemical properties, thereby leading to reduce yield and quality of citrus fruits (Zhou *et al.*, 2010). Hence, the aims of the present study were to evaluate the contribution of AMs to GRSP, soil C pools, and aggregate stability in rhizosphere of three citrus genotypes, and further to analyze their GRSP contribution to SOC pools and aggregate stability. The generated results would provide important information on improving soil structure and fertility of citrus orchard.

## Materials and Methods

### Experimental Design

The experiment was a 2 × 3 factorial completely randomized design, with three replicates or pots for a total of 18 pots. The first factor was mycorrhizal inoculation without and with *Funneliformis mosseae*. The second factor was citrus genotypes, namely, Kumquat (*Fortunella margarita* Lour. Swingle), Red tangerine (*Citrus tangerina* Hort. ex Tanaka) and Trifoliate orange (*Poncirus trifoliata* L. Raf.).

### Plant Culture

Seeds of Kumquat, Red tangerine, and Trifoliate orange from a Citrus Orchard, Yangtze University, Jingzhou, China were surface sterilized by 70% ethanol for 10 min and then sown in each pot containing 2.8 kg sterilized air-dried ferralsol soils (FAO system) collected in October 2011 from the same orchard. The characteristics of the soil were 6.2 pH, 9.4 g kg<sup>-1</sup> SOM, 123.5 mg kg<sup>-1</sup> available N, 16.2 mg kg<sup>-1</sup> Olsen-P, and 23.6 mg kg<sup>-1</sup> available K.

At sowing time, the inoculated seedlings received 20 g inocula of *F. mosseae* (Nicol. and Gerd.) Gerd. and Trappe in the form of infected roots of *Trifolium repens*, spores, hyphae and river sand. Meanwhile, spore density of the inocula was ~28 spores g<sup>-1</sup>. This AM inoculum was kindly provided by Professor R.-X. Xia of Huazhong Agricultural University, Hubei, China. The non-mycorrhizal seedlings also received both 20 g autoclaved inoculum and 2 mL filtrate (25 µm filter) of mycorrhizal inoculum for other similar microbial communities. After one month, seedlings were thinned to three per pot. The inoculated and

uninoculated plants were grown in a greenhouse from March to August, 2011, where it had 338–982 µmol/m<sup>2</sup>/s photosynthetic photon flux density, 30–35/8–14°C day/night temperature and 65–95% relative air humidity.

### Plant and Soil Sampling

After 143 days, all seedlings were harvested, and the growth performance, including plant height, stem diameter, fresh shoot and root weight were measured. The traits of root systems, including total length, surface area and volume were determined using a WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Soils attached to roots were gently shaken off, air-dried, and sieved (4 mm) for the determination of SOC, easily extractable GRSP (EE-GRSP), total GRSP (T-GRSP), water-stable aggregate (WSA) fractions, and mean weight diameter (MWD).

### Variable Determinations

Root mycorrhizal colonization was determined according to the method of Phillips and Hayman (1970) and was expressed as the percentage of infected root length against observed root length (Wu *et al.*, 2008). Extraction of EE-GRSP or T-GRSP was done with 8 mL 20 mM (pH 7.0) or 50 mM (pH 8.0) citrate buffer at 121°C for 30 or 60 min, and then determined by the Bradford assay with bovine serum albumin as the standard (Wright and Upadhyaya, 1998). Determination of SOC was followed by the chromic acid wet oxidation method (Walkley and Black, 1934).

The 4 mm sieved 30 g dry soil was used to determine the different (0.25, 0.50, 1.00, 2.00, 4.00 mm size) WSA fractions according to Kemper and Rosenau (1986). Mean weight diameter (MWD, mm) on 0.25–4.00 mm size aggregates is sensitive to soil aggregate stability and

calculated as follows as  $MWD = \sum_{i=1}^n XiWi$  (Kemper and

Rosenau, 1986), where,  $Xi$  is the diameter of  $i$  sieve (mm),  $Wi$  is the proportion of the  $i$  size fraction in the total sample mass, and  $n$  is the number of size fractions ( $n = 4$ ).

### Statistical Analysis

Data (means ± SE,  $n = 3$ ) were analysed with variance (ANOVA) with the SAS software (8.1 v). Significant differences in means between treatments were compared with the Duncan's multiple range test at the 5% level. The Pearson's correlation coefficients between GRSPs and SOC or MWD were performed using the Proc Corr's procedure in the SAS.

### Results

Root mycorrhizal colonization with *F. mosseae* in five-month-old citrus plants varied from 29.8 to 54.1% and was significantly higher in *P. trifoliata* than in other two citrus genotypes (Table 1). In general, the inoculation

**Table 1:** Growth traits of three 5-month-old citrus genotypes inoculated with and without an AM fungus *Funneliformis mosseae*

Citrus species	AMF status	Root colonization (%)	Plant height (cm)	Stem diameter (cm)	Root total length (cm)	Root surface area (cm <sup>2</sup> )	Root volume (cm <sup>3</sup> )	Shoot fresh weight (g)	Root fresh weight (g)
<i>Citrus tangerina</i>	AMF	29.8±8.1b,x	9.4±0.9b,x	0.187±0.017ab,x	190±50b,x	32.8±10.6b,x	0.45±0.18b,x	0.42±0.06c,x	0.33±0.06b,x
	Non-AMF	0±0a,y	4.1±1.4c,y	0.149±0.015b,y	122±42b,x	19.4±6.0b,y	0.25±0.07b,y	0.27±0.07c,y	0.12±0.03b,y
<i>Fortunella margarita</i>	AMF	34.4±1.0b,x	8.9±0.2b,x	0.173±0.008b,x	93±34c,x	17.8±5.5c,x	0.29±0.12c,x	0.67±0.06b,x	0.21±0.02c,x
	Non-AMF	0±0a,y	7.2±0.7b,y	0.147±0.007b,y	72±31c,x	11.6±5.0c,y	0.15±0.07c,y	0.48±0.02b,y	0.13±0.02b,y
<i>Poncirus trifoliata</i>	AMF	54.1±7.1a,x	20.5±2.9a,x	0.205±0.013a,x	304±125a,x	50.9±18.1a,x	0.68±0.22a,x	0.92±0.03a,x	0.51±0.03a,x
	Non-AMF	0±0a,y	14.6±0.9a,y	0.207±0.002a,x	230±92a,x	38.8±16.3a,x	0.53±0.25a,x	0.69±0.07a,y	0.49±0.04a,x

Values (means ± SE,  $n = 3$ ) are significantly different ( $P < 0.05$ , DMR test) if followed by different letters between genotypes for the same *F. mosseae* treatment (a, b, c) or between *F. mosseae* treatments for the same genotype (x, y)

with *F. mosseae* significantly increased growth traits, including plant height, stem diameter, shoot and root fresh weight, root total length, root surface area, and root volume in all three citrus genotypes, except stem diameter, root total length, root surface area and volume, and root fresh weight in *P. trifoliata* (Table 1). Meanwhile, significantly higher all growth traits among citrus genotypes generally ranked as *P. trifoliata* > *C. tangerina* > *F. margarita*, no matter whether they were mycorrhizal or not.

Both the T-GRSP and EE-GRSP concentrations in the rhizosphere were generally significantly higher in *C. tangerina* and *F. margarita* than in *P. trifoliata*, irrespective of their mycorrhizal status (Fig. 1a and 1b). Compared to the non-AM seedlings, inoculation with *F. mosseae* significantly increased rhizospheric EE-GRSP and T-GRSP concentrations by 26, 23, and 19% (Fig. 1a) and 13, 20, and 15% (Fig. 1b) in *P. trifoliata*, *C. tangerina*, and *F. margarita*, respectively.

SOC concentrations among genotypes ranked as *F. margarita* > *C. tangerina* > *P. trifoliata*, no matter whether the seedlings were mycorrhizal or not (Fig. 1c). In addition, SOC concentrations were 39% to 325% significantly higher in *F. mosseae* inoculated seedlings than in the non-AM inoculated controls (Fig. 1c). Meanwhile, T-GRSP/SOC ratio patterned as *C. tangerina* > *P. trifoliata* > *F. margarita* between genotypes no matter whether the seedlings were mycorrhizal or not, and as AMF-inoculated > non-AM-inoculated seedlings for each citrus genotype (Fig. 1d).

Compared to the non-AM inoculated seedlings, AMF inoculation significantly increased the proportion of water-stable aggregates in the 2.00–4.00 (WSA<sub>2.00–4.00mm</sub>), 1.00–2.00 (WSA<sub>1.00–2.00mm</sub>), 0.50–1.00 (WSA<sub>0.50–1.00mm</sub>), and >0.25 mm (WSA<sub>>0.25mm</sub>) sizes (Fig. 2a, 2c and 2e), but not in the 0.25–0.50 mm (WSA<sub>0.25–0.50mm</sub>) fraction (Fig. 2d) in the rhizosphere, irrespectively of citrus genotypes. Compared with the non-AM citrus plants, the MWD values were increased by 119, 217, and 127% in *F. mosseae* inoculated *P. trifoliata*, *C. tangerina* and *F. margarita*, respectively (Fig. 2f). In addition, expect a similar proportion of WSA<sub>0.25–0.50mm</sub>, significantly higher all other WSA fractions and MWD between citrus genotypes generally ranked as *F.*

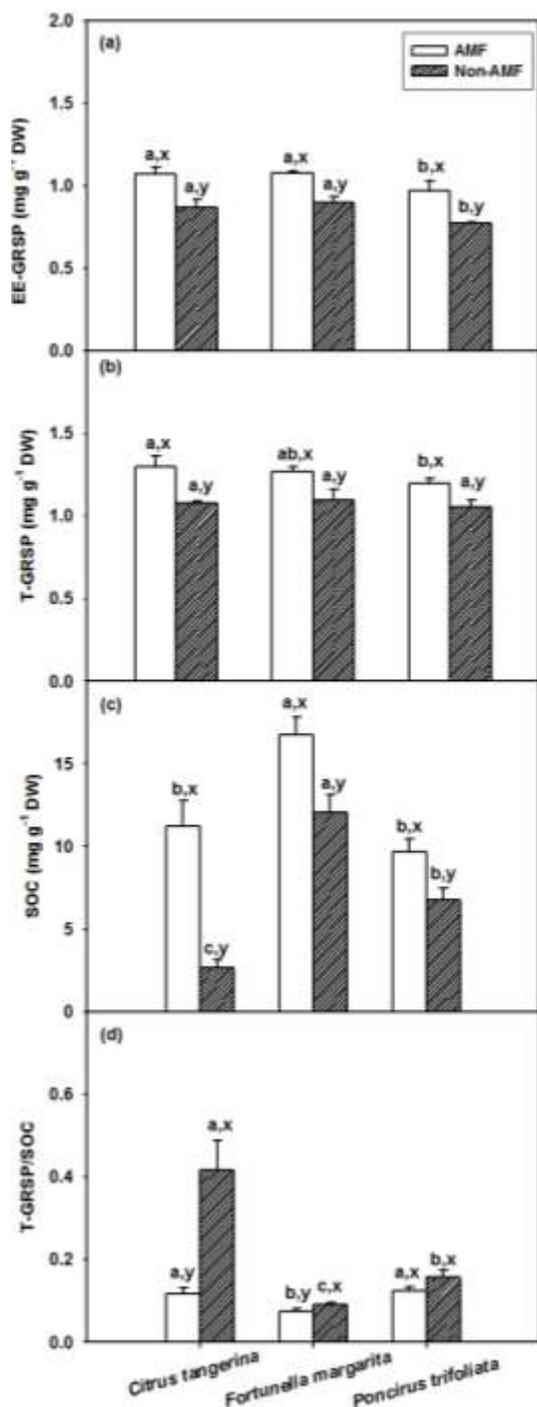
*margarita* > *C. tangerina* > *P. trifoliata* (Fig. 2).

The concentrations of SOC significantly positively correlated with EE-GRSP ( $r^2 = 0.46$ ) or T-GRSP concentrations ( $r^2 = 0.43$ ) (Fig. 3). Meanwhile, all WSA size fractions significantly positively correlated with T-GRSP ( $r^2 = 0.32–0.77$ ) and EE-GRSP ( $r^2 = 0.32–0.78$ ) (Fig. 4a and 4b). In addition, the MWD values significantly positively correlated with EE-GRSP ( $r^2 = 0.89$ ) and T-GRSP concentrations ( $r^2 = 0.72$ ) (Fig. 4c).

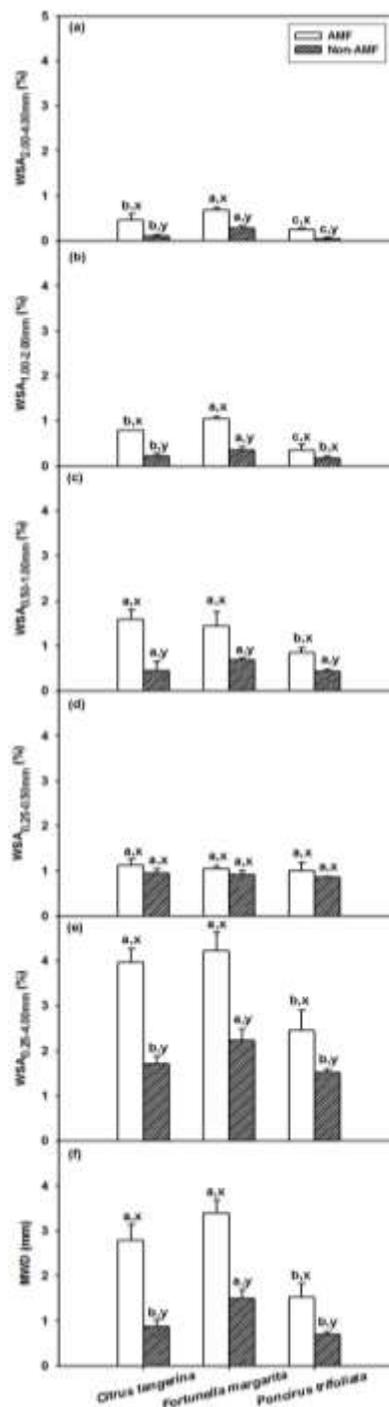
## Discussion

Our results revealed that inoculation with AMF significantly increased the proportion of WSA<sub>2.00–4.00mm</sub>, WSA<sub>1.00–2.00mm</sub>, and WSA<sub>0.50–1.00mm</sub> fractions in citrus rhizosphere, but did not change the WSA<sub>0.25–0.50mm</sub> proportion, irrespectively of citrus genotypes (Fig. 2). Similarly, Sheng *et al.* (2012) also reported that the colonization with *Glomus versiforme* notably increased the proportion of different WSA size fractions in the rhizosphere of pepper and watermelon. Irrespectively of citrus genotypes, significantly higher MWD results were observed in the mycorrhizosphere than the non-mycorrhizosphere. This suggests that mycorrhizal symbiosis can improve aggregate stability by increasing WSA fractions in 2.00–4.00, 1.00–2.00 and 0.50–1.00 mm classes.

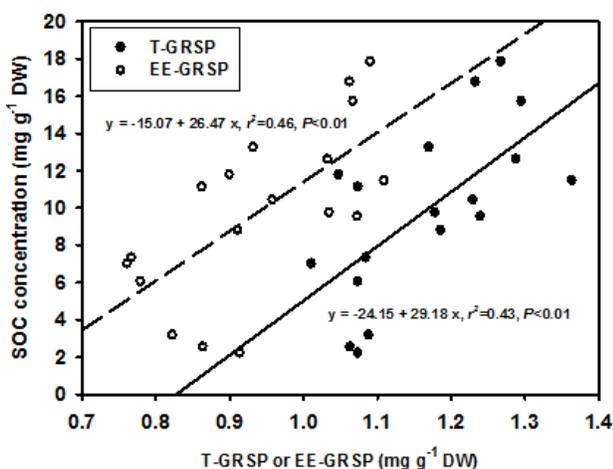
The present results showed that the inoculation with *F. mosseae* significantly increased by 13–20% in T-GRSP and 19–26% in EE-GRSP concentrations in the rhizosphere of these three citrus genotypes, respectively (Fig. 1a and 1b). The present investigation also found significantly higher SOC in the AM than in non-AM citrus genotypes (Fig. 1c). Yang *et al.* (2011) also observed that root mycorrhizal colonization and spore density were significantly positively correlated with SOC. Meanwhile, both T-GRSP and EE-GRSP concentrations showed significantly positive relationships with SOC in this study, which is consistent with other studies on *Argemone subfusiformis*, *Baccharis linearis*, *Oenothera affinis*, and *Polypogon viridis* in Chile and *Chromonaela odorata*, *Combretodendron macrocarpum*, *Disternonanthus benthamianus*, *Piptadeniastrum africanum*



**Fig. 1:** Effects of *F. mosseae* colonization on easily extractable glomalin-related soil protein (EE-GRSP) (a), total GRSP (T-GRSP) (b), soil organic carbon (SOC) (c), and T-GRSP/SOC ratio (d) in the rhizosphere of three 5-month-old citrus genotypes. Values (means  $\pm$  SE,  $n = 3$ ) are significantly different ( $P < 0.05$ , DMR test) if followed by different letters between plant species for the same *F. mosseae* treatment (a, b, c) or between *F. mosseae* treatments for the same genotype (x, y)



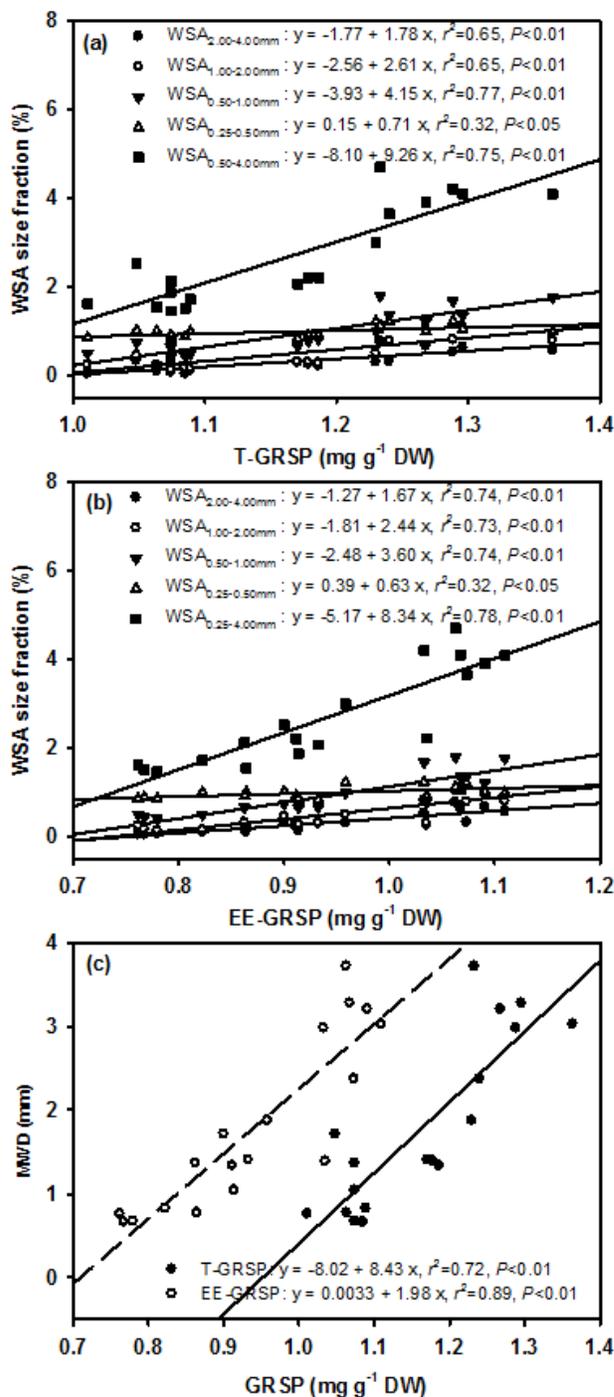
**Fig. 2:** Effects of *F. mosseae* colonization on proportion of water-stable aggregate (WSA) fractions (a–e) at 2.00–4.00, 1.00–2.00, 0.50–1.00, 0.25–0.50 and 0.25–4.00 mm size classes and mean weight diameter (MWD) (f) in the rhizosphere of three 5-month-old citrus genotypes. Values (means  $\pm$  SE,  $n = 3$ ) are significantly different ( $P < 0.05$ , DMR test) if followed by different letters between genotypes for the same *F. mosseae* treatment (a, b, c) or between *F. mosseae* treatments for the same genotype (x, y)



**Fig. 3:** Relationships between easily extractable glomalin-related soil protein (EE-GRSP) (a) or total glomalin-related soil protein (T-GRSP) (b) and soil organic carbon (SOC) (c) in the rhizosphere of three 5-month-old citrus genotypes ( $n = 18$ )

and several crops in Cameroon (Cornejo *et al.*, 2008; Fokom *et al.*, 2012). Since glomalin contributes 4–5% of total soil C and N in soils (Rillig *et al.*, 2001), GRSP may be an important part of SOC pools. In the present study, lower T-GRSP/SOC ratio was found in the mycorrhizal plants than in the non-mycorrhizal plants, which might be due to the fact that GRSP could decrease the decomposition of SOC by physically protecting particulate matter from enzyme activity (Rillig, 2004). However, under elevated  $\text{CO}_2$  conditions, AMF notably increased SOC decomposition (Cheng *et al.*, 2012).

Besides the C contribution, GRSP is positively correlated with soil aggregate stability across various soils (Wright and Upadhyaya, 1998). In the present study, T-GRSP and EE-GRSP were linearly positively correlated with MWD and/or the proportion of the WSA fractions in 2.00–4.00, 1.00–2.00, 0.50–1.00, and 0.25–0.50 mm, suggesting that GRSP plays important role in stabilizing soil aggregates of rhizosphere (Rillig, 2004; Wu *et al.*, 2008; 2012). This is consistent with the results of Wright and Anderson (2000), who observed a highly linear correlation of GRSPs with aggregate stability in winter wheat-fallow cropping system in the central Great Plains, USA. In contrast, other studies on more than arable, pasture, and forest sites reported the negative and sigmoidal correlations of T-GRSP and EE-GRSP with aggregate stability (Rillig *et al.*, 2003a; Spohn and Giani, 2010; Wu *et al.*, 2013). It seems that the contribution of GRSP to aggregate stability depends on hierarchically structured soils, in which soil organic material is the main binding agent (Singh, 2012). In addition, not only GRSP but also mycorrhizal hyphae may contribute the aggregate stability through winding soil particles together (Peng *et al.*, 2013). In terms of a path analysis model Rillig and Steinberg (2002) concluded



**Fig. 4:** Relationships between total glomalin-related soil protein (T-GRSP) (a) or easily extractable glomalin-related soil protein (EE-GRSP) (b) and proportion of water-stable aggregate (WSA) fractions at 2.00–4.00, 1.00–2.00, 0.50–1.00, 0.25–0.50 and 0.25–4.00 mm size classes and mean weight diameter (MWD) (c) in the rhizosphere of three 5-month-old citrus genotypes ( $n = 18$ )

higher aggregate stability contribution from GRSP than from mycorrhizal hyphae. Therefore, further detailed studies

are needed to compare the contribution capability of mycorrhizal hyphae and GRSP to aggregate stability in the citrus rhizosphere.

In summary the present study showed that AM symbiosis in citrus rhizosphere significantly increased growth performance, MWD, proportion of WSA<sub>2.00–4.00mm</sub>, WSA<sub>1.00–2.00mm</sub>, WSA<sub>0.50–1.00mm</sub>, and WSA<sub>>0.25mm</sub> fractions, EE-GRSP, T-GRSP, and SOC concentrations. Meanwhile, GRSPs showed significantly positive contribution to all WSA size fractions, thus providing the stabilization of aggregates in the citrus rhizosphere. These relationships would promote our current understanding of the contribution of GRSP to SOC dynamics and structure stability.

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