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TEMPO-SPATIAL DISTRIBUTION AND RELATED FUNCTIONINGS OF ROOT GLOMALIN AND GLOMALIN-RELATED SOIL PROTEIN IN A CITRUS RHIZOSPHERE

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ABSTRACT

Citrus trees in China are mostly cultivated in poor soils, where management of root and soil glomalin are of great significance. The tempo-spatial dynamics of root mycorrhizal colonization, glomalin, sucrose and glucose and soil organic carbon (SOC), total and easily-extractable glomalin-related soil protein (T-GRSP and EE-GRSP), and mean weight diameter (MWD, an indicator of aggregate stability) were studied in the rhizosphere of a 24-yr-old *Citrus unshiu* grafted on *Poncirus trifoliata*. In general, root mycorrhizal colonization, glomalin and GRSP reached maximal values in July and were higher in 0–15cm than in 15–30cm soil layer. Mycorrhizal colonization was positively correlated with T-GRSP and EE-GRSP but negatively with root glucose. There was a relatively low root glomalin concentration (0.047–0.278 mg g^{-1} DW) in citrus rhizosphere. A highly positive correlation of root glomalin and EE-GRSP was with root glucose and root sucrose, suggesting that a certain amount of GRSPs and root glomalin were partly from root glucose and sucrose. SOC but not two GRSP fractions were significantly positively correlated with MWD, and GRSP thus was not the main aggregate binding agent in the soils. In addition, SOC was highly positively correlated with root glomalin and without two GRSPs, resulting in higher contribution of root glomalin to SOC than GRSP. We conclude that root and soil glomalin exhibited highly tempo-spatial distribution patterns and did not contribute aggregate stability.

Key words: Aggregate stability, Citrus, Glomalin, Glomalin-related soil protein, Mycorrhiza, Soil organic carbon

INTRODUCTION

Glomalin is a special *N*-linked glycoprotein combined in hyphae surfaces and spore walls of arbuscular mycorrhizal fungi (AMF) in both soils and roots (Wright and Upadhyaya, 1996; Rosier *et al*., 2008), defined in soils as glomalin-related soil protein (GRSP; Rillig, 2004). In general, glomalin in native state is insoluble and possibly hydrophobic and is high homolog of heat shock protein 60 (Rillig, 2004; Gadkar and Rillig, 2006). Moreover, glomalin may contain 3–5% N and ~37% C (Lovelock *et al*., 2004). Schindler *et al*. (2007) found that GRSP might be not a typical glycoprotein, because GRSP also contained high aromatic C (42–49%) and carboxyl C (24–30%) carbon and low aliphatic C (4–11%) and carbohydrate-type C (4–16%) contents. The amount of C in GRSP represented ca. 4–5% of total soil C in the oldest tropical soils (Rillig *et al*., 2001). Until now, little information is known about the relationships between GRSP and root carbohydrates.

Since GRSP may be located in hyphae surfaces and spore wells of AMF but also in surfaces of roots and soil aggregates, Purin and Rillig (2007) postulated a primary cellular function of GRSP in fungal physiology such as chaperonins and a secondarily environmental function of GRSP in aggregate stability. Most previous studies were conducted in a short time (e.g. one month or three months) to analyze the functions of GRSP in various soils (Violi *et al*., 2008; Bai *et al*., 2009; He *et al*., 2010), whereas the temporal distribution to characteristics of GRSP in a year and its relation to soil C are poorly understood.

Rosier *et al*. (2008) firstly successfully detected root glomalin with Bradford method and ELISA technique and also recommended root glomalin as a tool to quantify mycorrhizal colonization. The work only used *Daucus carota* plants grown under sterile *in vitro* conditions and *Plantago lanceolata* and *Sorghum bicolor* plants grown in a greenhouse, but a field study still needs to be conducted to analyze the root glomalin status. On the other hand, we do not know if the root glomalin is related with root mycorrhizal colonization, root and soil carbohydrates, and GRSPs.

Citrus, one of the important popular fruit trees is highly dependent on mycorrhizal symbiosis in fields. In general, citrus trees are grown in southern regions of China, where soils are poor, especially soil P level. The poor soils severely limit both the expansion of citrus industry areas and high yield and high quality fruit production. Our previous study showed that in citrus orchards, many factors such as root and soil carbohydrates, root mycorrhizal colonization, and soil related enzymes could impact GRSP levels, which had regarded as an useful indicator of soil quality (Wu *et al*.,

2012). The purpose of the present study was to determine tempo-spatial patterns of root mycorrhizal colonization, carbohydrates and glomalin, soil GRSPs and carbohydrates, and aggregate stability in a citrus rhizosphere to answer the following questions: (i) Was root mycorrhizal colonization related to root glomalin in the field? (ii) Was root glomalin related to root glucose, root sucrose, soil carbohydrates, and GRSPs? And (iii) were GRSPs related to SOC and aggregate stability? The expected results will clarify the GRSP functions in citrus orchards and then utilize the functions to improve soil structure and fertility of citrus orchards.

MATERIALS AND METHODS

Experimental site: A citrus orchard was selected as the experimental size located in the Yangtze University campus, Jingzhou, China (30°36′N, and 112°14′E), where the 24-yr-old *Citrus unshiu* trees grafted on trifoliate orange (*Poncirus trifoliata*) were planted. The orchard belongs to the north subtropical humid monsoon climate, with four distinct seasons, 4367–4576 MJ m^{-1} annual total radiation, 1823–1987 h annual sunshine hour, 16.2–16.6ºC annual average temperature, and 1100–1300 mm annual precipitation. The citrus orchard carried out the no-tillage soil management regime of natural grass cover. The physico-chemical properties of the yellow-brown soil from the citrus orchard were as following: pH 6.2, organic matter 9.4 g kg^{-1}, and Olsen-P 16.2 mg kg^{-1}.

Root and soil sampling: The citrus orchard was divided into three blocks (replicates). Soil and fine root samples were collected at random locations along four citrus trees with similar growth vigor. The samples were collected at depth of 0–15cm or 15–30cm soils within a 4m diameter of tree canopy in January, March, May, July, September, and November 2011. Soil samples from four trees per block were well mixed as a composite sample, air-dried, ground, and then sieved with 4mm size for the analysis of water-stable aggregate (WSA) and GRSP. Half of the fine root samples from four trees per block was separately mixed well, cut 1-cm long, and fixed with a FAA solution until analysis of mycorrhizal colonization. The rest of the root samples was stored at -60 ºC until analysis of root carbohydrates.

Variable measurements: The 1-cm root segments were cleared with 10% (w/v) KOH solution at 95ºC for 1.5 h and then stained with 0.05% (w/v) trypan blue dissolved in lactic acid (Phillips and Hayman, 1970). Mycorrhizal colonization was counted as the percent of the mycorrhizal infected root length for the observed total root length.

Root glucose and sucrose concentrations were measured using the method previously described by Wu *et al*. (2011).

Determination of WSA% at 0.25, 0.50, 1.00, and 2.00mm size fractions was followed by the method of Wu *et al*. (2008). Meanwhile, aggregate stability was calculated in terms of the mean weight diameter (MWD, mm) of 0.25–4.00mm WSA as MWD = $\sum_{i=1}^{n} XiWi$, where *Xi* is the diameter of the *i* sieve opening (mm), *Wi* is the proportion of the *i* size fraction of WSA in total sample of WSA, and *n* (=4 in this study) is the number of WSA size fractions.

Easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin-related soil protein (T-GRSP) concentrations were determined using a protocol described by Bedini *et al*. (2009). Root glomalin was measured using the method described by Rosier *et al*. (2008). SOC was measured by the dichromate oxidation spectrophotometric method as described by Rowell (1994).

Statistical analysis: One-way variance (ANOVA) was performed to analyze the data (means ± SE, n = 3). Duncan's multiple range test (P<0.05) was used to compare the significant differences among the means. Pearson correlation coefficients between the variables were calculated by the Proc Corr procedure in SAS (v8.1).

RESULTS

Root mycorrhizal colonizaton: In the citrus trees, the root mycorrhizal colonization varied from 17 to 51% in 0–15cm soil depth and from 14 to 30% in 15–30cm soil depth, and was significantly higher in 0–15cm soil depth than in 15-30cm soil depth (Fig. 1A). Annual root mycorrhizal colonization varied considerably. The root mycorrhizal colonization significantly increased from January to July and then gradually decreased. The highest root colonization was in July, and the lowest in January in 0-15cm soil depth or in November in 15-30cm soil depth.

Root glomalin: Root glomalin concentration ranged from 0.057 to 0.278 mg g^{-1} DW root in 0–15cm depth and from 0.047 to 0.192 mg g^{-1} DW root in 15–30cm depth (Fig. 1B). Root glomalin concentration showed a seasonal dynamic that the minimum was found in March and the maximum in July in 0–15cm soil layer and in May in 15–30cm soil layer. In general, the roots collected from 0–15cm soil depth in annual dynamics recorded significantly higher root glomalin concentration than that from 15–30cm soil depth, except May and September.

Root carbohydrates: Root glucose and sucrose exhibited the significant differences between months in two soil layers (Fig. 2). The highest root glucose concentration in the two soil layers occurred in January and lowest root glucose in March (Fig. 2A). Root sucrose concentration

was found to first decreased and then increased with sampling months from March to November (Fig. 2B). Meanwhile, the highest root sucrose concentrations in the two soil layers were found in November and the lowest root sucrose concentrations in March. Root glucose and sucrose concentrations in 0–15cm soil layer were significantly higher than in 15–30cm soil layer, except a significantly lower difference in July.

Table 1. Pearson's correlation coefficient (*r*) among each variable (*n* = 36)

	Mycorrhizal colonization	Root glomalin	T-GRSP	EE-GRSP	Root glucose	Root sucrose	SOC	MWD
Mycorrhizal colonization	1.000	0.308	0.565**	0.332*	-0.414*	-0.255	0.211	-0.186
Root glomalin		1.000	0.349*	-0.213	0.387*	0.506**	0.351*	-0.232
T-GRSP			1.000	0.475**	-0.161	-0.207	0.076	-0.250
EE-GRSP				1.000	-0.427**	-0.485**	0.032	-0.216
Root glucose					1.000	0.505**	0.314	0.349*
Root sucrose						1.000	-0.010	-0.219
SOC							1.000	0.622**
MWD								1.000

* $P<0.05$. ** $P<0.01$.

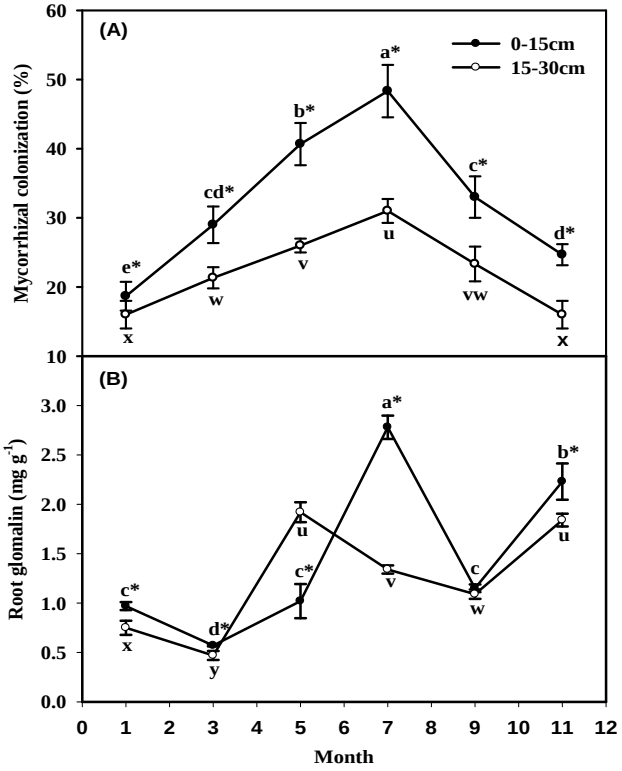

Figure 1. Tempo-spatial distributions of mycorrhizal root colonization (A) and root glomalin (B) in the rhizospheric 0–15 and 15–30cm depth of *Citrus unshiu*. Data (means ± SE, *n*=3) followed by different letters (a, b, c, etc.) above the bars among 0–15cm soil layer, different letters (u, v, w, etc.) above the bars among 15–30cm soil layer, or * above the bars between 0–15cm and 15–30cm soil layer in the same sampling time are significantly different at *P*<0.05.

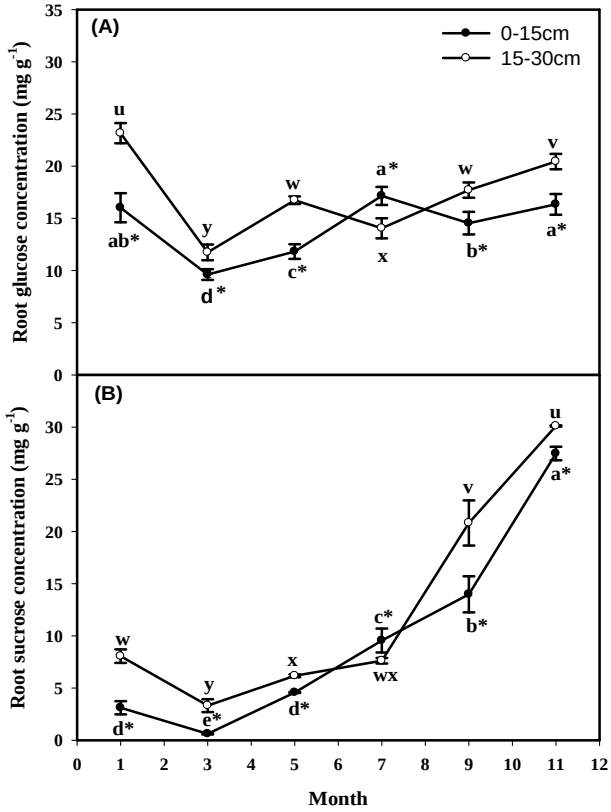

Figure 2. Tempo-spatial distributions of root glucose (A) and root sucrose (B) in the rhizospheric 0–15 and 15–30cm depth of *Citrus unshiu*. Data (means ± SE, *n*=3) followed by different letters (a, b, c, etc.) above the bars among 0–15cm soil layer, different letters (u, v, w, etc.) above the bars among 15–30cm soil layer, or * above the bars between 0–15cm and 15–30cm soil layer in the same sampling time are significantly different at *P*<0.05.

GRSP fractions: GRSP fractions viz. T-GRSP and EE-GRSP in various depth-range categories showed a strong seasonal effect (Fig. 3A, 3B). Comparison of annual GRSP dynamics showed the highest T-GRSP concentration in 0–30cm soil depth in July and the highest EE-GRSP in September in 0–15cm depth and in March in 15–30cm depth. The lowest T-GRSP and EE-GRSP concentrations in 0–15cm soil depth were in May and those in 15–30 soil depth in November. GRSP fractions in annual dynamics were significantly higher in 0–15cm depth than in 15–30cm depth. Average T-GRSP concentration (0-30cm) varied from 0.70 to 1.33 mg g^{-1} DW soil, and EE-GRSP from 0.60 to 0.93 mg g^{-1} DW soil.

Soil organic carbon (SOC): SOC concentration showed significant differences between 0–15cm and 15–30cm soil depths among different months, and significantly higher SOC was found in 0–15cm soil depth (Fig. 3C). Significantly the highest SOC was observed in January in 0–15cm soil depth and in July in 15–30cm soil depth, and the lowest in March in 0–15cm and 15–30cm soil depths.

Mean weight diameter (MWD): MWD in the citrus rhizosphere ranged between 0.11 mm and 0.32 mm at different sampling months and represented significant month dynamics (Fig. 3D). The maximum of MWD always was in January and minimum in March. MWD in the year always was significantly higher in 15-30cm than in 0–15cm soil layer.

Correlation between glomalin and other variables: Correlation analysis showed that root glomalin was significantly positively correlated with T-GRSP, root glucose and sucrose, and SOC (Table 1). T-GRSP was significantly positively correlated with EE-GRSP. EE-GRSP was significantly negatively correlated with root glucose and root sucrose.

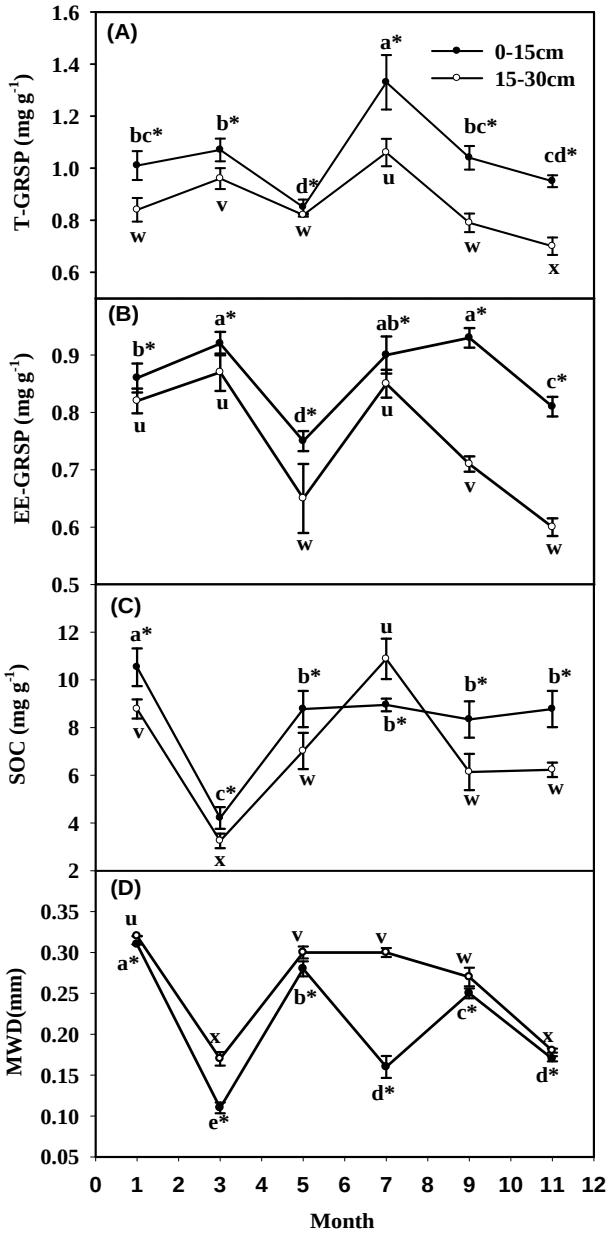

Figure 3. Tempo-spatial distributions of T-GRSP (A), EE-GRSP (B), SOC (C), and MWD (D) of water-stable aggregates in the rhizospheric 0–15 and 15–30cm depth of *Citrus unshiu*. Data (means ± SE, *n*=3) followed by different letters (a, b, c, etc.) above the bars among 0–15cm soil layer, different letters (u, v, w, etc.) above the bars among 15–30cm soil layer, or * above the bars between 0–15cm and 15–30cm soil layer in the same sampling time are significantly different at *P*<0.05. Abbreviations: EE-GRSP, easily extractable glomalin-related soil protein; MWD, mean weight diameter; SOC, soil organic carbon; T-GRSP, total glomalin-related soil protein.

DISCUSSION

Relation between mycorrhizal colonization and glomalin of soil and root: In the present work, higher root mycorrhizal colonization was found in July and also was observed in the topsoil than in the subsoil, which is in agreement with the previous studies in *Citrus unshiu* trees in Wuhan, China (Wu *et al*., 2006) and *Vitis vinifera* in Oregon, USA (Schreiner, 2005). The changes may be dependent on a balance between root and fungal activity which is influenced by various factors including propagule numbers, soil properties, environmental temperature, and root density (Muthukumar *et al*., 2003; Schreiner, 2005).

In the study, EE-GRSP and T-GRSP concentrations were higher in yellow-brown soil of the citrus orchard than those in sandland (Bai *et al*., 2009) but lower than those in grassland (Rillig *et al*., 2006) and rainforest soils (Lovelock *et al*., 2004; Violi *et al*., 2008). Compared with that in 15–30cm soil depth, higher EE-GRSP and T-GRSP concentrations were found in 0–15cm soil depth, which is consistent with the findings of He *et al*. (2010) in the rhizosphere of *Artemisia ordosica* plants. On the other hand, in a year, higher EE-GRSP and T-GRSP concentrations appeared in March and July, and lower in May and November. The significantly positive correlation of EE-GRSP and T-GRSP concentrations with root mycorrhizal colonization implies that temporal distribution pattern of GRSP absolutely depends on mycorrhizal colonization, at least in the present work.

Wright *et al*. (1996) has proposed soil glomalin as an indirect indicator for the analysis of AMF colonization. Rosier *et al*. (2008) further recommended root glomalin as an alternative tool to quantify root mycorrhizal colonization in plants. Since the extraction of root glomalin and soil T-GRSP was used by 50 mM sodium citrate (pH 8.0), it is reasonable that root glomalin was significantly positively correlated with T-GRSP but not with EE-GRSP (Table 1). On the other hand, root glomalin was not significantly linearly correlated with root mycorrhizal colonization in the field-collected citrus materials, providing an evidence that root glomalin did not assess root mycorrhizal colonization of citrus plants. However, a significantly non-linear correlation of root glomalin with root mycorrhizal colonization appeared in carrot roots *in vitro* colonized by *Glomus intraradices* (Rosier *et al*., 2008). It seems that extraction process of root glomalin does not absolutely eliminate the derived proteins either from roots or associated with plant roots below the detection limits of the Bradford method.

Relation between glomalin and root carbohydrates: Arbuscular mycorrhizas, the mutualistic associations, must acquire hexoses (mainly glucose) from the host to the fungal partner (Solaiman and Saito, 1997; Schaarschmidt *et al*., 2007). Our result showed a significantly negative correlation of root mycorrhizal colonization with root glucose but without root sucrose, suggesting that mycorrhizas might consume more root glucose for its development. On the other hand, glomalin contains much carbon because of both its protein and carbohydrate subunits. Moreover, glomalin also locates in surfaces of the root and the intraradical hyphal cell walls of AMF. Therefore, it is reasonable that root glomalin was significantly positively correlated with root glucose and root sucrose. However, we did not know the synthesis pathways of root glomalin in plants but only understand that glomalin is tightly bound within the hyphal wall of AMF and enters into soil via hyphal turnover defined as GRSP (Driver *et al*., 2005). In GRSP fractions, only EE-GRSP but not T-GRSP was significantly negatively correlated with root glucose or root sucrose, implying that a certain amount of GRSPs and root glomalin are partly from root glucose and root sucrose.

Relation between glomalin and soil carbohydrates or aggregate stability: The present result showed that T-GRSP and EE-GRSP were not significantly correlated with SOC. In contrast, He *et al*. (2010) found a significantly positive correlation of T-GRSP or EE-GRSP with SOC. In general, glomalin may contain nearly 37% C (Lovelock *et al*., 2004), which contributes ca. 4–5% of soil total C (Rillig *et al*., 2001). Even then, turnover time of GRSP seems to be relatively slow and might be the range of 6–42 yr (Rillig *et al*., 2001). Therefore, the contribution of GRSP to SOC might be dependent on turnover time of GRSP, environmental conditions, and seasonal fluctuations. Interestingly, our study showed that root glomalin in the present study was significantly positively correlated with SOC. This result indicates that the contribution of root glomalin to SOC was higher than GRSP, at least in the present study.

Previous studies have found the linear correlation of GRSP with aggregate stability in various soils or the curvilinear over a large range of water stability (Wright and Upadhyaya, 1998; Rillig, 2004; Wu *et al*., 2013). Here, we did not find any significant correlation of root and soil glomalin with MWD, suggesting that GRSP is not the main binding agent of soil water-stable aggregates (Rillig *et al*., 2003).

Conclusions: In this study, the dynamics of root mycorrhizal colonization, glomalin, sucrose and glucose, soil T-GRSP, EE-GRSP, SOC, and MWD had highly tempo-spatial patterns. The tempo-spatial distribution pattern of GRSP was related to mycorrhizal colonization. Root glomalin did not assess the level of root mycorrhizal colonization in the field-collected citrus materials, due to the no significantly positive correlation of root glomalin with mycorrhizal colonization. Our results also indicated

that mycorrhizas might consume more root glucose for its development, and a certain amount of GRSPs and root glomalin are partly from root glucose and sucrose. Contribution of root glomalin to SOC was higher than GRSP, at least in the present study. Root and soil glomalin did not contribute to aggregate stability in the citrus rhizosphere.

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