INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–1057/2013/15–3–603–606
http://www.fspublishers.org

Full Length Article

Relationships between Glomalin-Related Soil Protein in Water-Stable Aggregate Fractions and Aggregate Stability in Citrus Rhizosphere

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Abstract

Despite glomalin-related soil protein (GRSP) as a stable and persistent glycoprotein liked to arbuscular mycorrhizal fungi is highly correlated with aggregate stability of various soils, its characteristics in water-stable aggregate fractions and function with aggregate stability are not known. GRSP at 0–15 and 15–30 cm depth in a yellow-brown soil was determined from rhizosphere of 23-year-old *Citrus unshiu* trees grafted on *Poncirus trifoliata* in central China. Easily extractable-GRSP (EE-GRSP) ranged 0.45–0.62 mg/g and total-GRSP (T-GRSP) 0.61–1.07 mg/g dry soil within all water-stable aggregate fractions (0.25, 0.50, 1.00, 2.00 and 4.00 mm). GRSP significantly decreased with the decrease of water-stable aggregate size and soil depth, and significantly negatively linearly correlated with percentage of 0.50–1.00 mm size water-stable aggregate. Mean weight diameter (an indicator to aggregate stability) was significantly higher at 15–30 cm than at 0–15 cm depth. Mean weight diameter at 0–30 cm depth was significantly negatively linearly correlated with EE-GRSP in 0.50–1.00 mm size water-stable aggregate and T-GRSP in 2.00–4.00 mm size water-stable aggregate, but positively with EE-GRSP in 0.25–0.50 mm size water-stable aggregate. These results suggested that GRSP exhibited a certain distributive characteristic in water-stable aggregate fractions as EE-GRSP mainly localized in 0.25–1.00 mm size water-stable aggregate fraction, while T-GRSP in 2.00–4.00 mm size water-stable aggregate fraction. © 2013 Friends Science Publishers

Keywords: Aggregates; Arbuscular mycorrhizal fungi; Easily extractable glomalin-related soil protein; Mean weight diameter; Total glomalin-related soil protein

Introduction

Glomalin, a stable and persistent glycoprotein, is released by hyphae and spores of arbuscular mycorrhizal fungi (AMF) (Wright and Upadhyaya, 1996; Driver *et al*., 2005). Glomalin in soil is assessed as glomalin-related soil protein (GRSP), which is an alkaline-soluble protein liked to AMF (Rillig, 2004; Vodnik *et al*., 2008). GRSP contains 37% carbon and 3–5% nitrogen, and contributes to the storage of soil carbon (3%) and nitrogen (5%) and to soil structure stabilization as a biochemical binding agent in soil particle aggregation (Purin and Rillig, 2007; Fokom *et al*., 2012).

GRSP strongly correlates with soil aggregate stability, which depends on GRSP concentrations in water-stable aggregates (WSA) size fractions (Rillig, 2004; Wright *et al*., 2007). A linear relationship under low GRSP, while a curvilinear relationship beyond a certain “saturation” GRSP concentration, was suggested between GRSP and aggregate stability (Rillig, 2004). All these studies were conducted in natural or crop soils, whereas no studies were from fruit orchid soils particularly in citrus rhizosphere, where GRSP was quite low since citrus is an underdeveloped root hair fruit plant (Wu *et al*., 2012). Additionally, many of citrus trees in China are cultured in extremely poor soils, and thus improvement of soil aggregates is a looming problem.

The objective of this study is to analyze the distributive characteristics of GRSP and its correlation with aggregate stability in WSA size fractions in a *Citrus unshiu* (cv. Guoqing 1 grafted on *Poncirus trifoliata*) rhizosphere.

Materials and Methods

Experimental Site

The study was conducted in a citrus orchard on Yangtze University, China (30°36′N, 112°14′E). The area has the north subtropical humid monsoon climate, with four distinct seasons, plenty of rain, suitable light, and a long frost-free period. The annual total radiation is 4367–4576 MJ/m, the annual sunshine hour 1823–1987 h, the average annual temperature 16.2–16.6ºC, and the annual precipitation 1100–1300 mm. The citrus orchard was carried out by a no-tillage soil management with natural grass cover.

To cite this paper: Wu, Q.S., X.H. He, M.Q. Cao, Y.N. Zou, S. Wang and Y. Li, 2013. Relationships between glomalin-related soil protein in water-stable aggregate fractions and aggregate stability in *Citrus Rhizosphere. Int. J. Agric. Biol*., 15: 603–606

Soil Sampling

Four similar 23-year-old citrus trees from each of three blocks (replicates) were randomly selected from the orchard. About 500 g yellow-brown soils were collected at 0–15 or 15–30 cm depth within a 2 m radius of tree canopy in September 2011. The physical-chemical properties of the soil were pH 6.2, organic matter 9.4 g/kg, and Bray-P 16.23 mg/kg. Soil samples from 4-tree/block were mixed as one composite sample, air-dried, ground, and then sieved (4 mm) for the analysis of WSA and GRSP.

Variable Determinations

Determinations of WSA (0.25, 0.50, 1.00, 2.00 and 4.00mm size), aggregate stability, easily extractable-GRSP (EE-GRSP) and total-GRSP (T-GRSP) were carried out following Kemper and Rosenau (1986) and Wright and Upadhyaya (1996). Meanwhile, aggregate stability was calculated by the mean weight diameter (MWD, mm) of 0.25–4.00mm aggregates 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 in the total sample mass, *n* (=4 in this study) is the number of size fractions.

Statistical Analysis

One-way ANOVA was performed to analyze the data (means ± SE, n = 3). Fisher's Protected Least Significant Differences (*LSD*, $P<0.05$) were used to compare the means. Pearson correlation coefficients between GRSPs and other variables were calculated using Proc Corr procedure in SAS (v8.1).

Results

Significantly higher distribution of water-stable aggregates (WSA, %) in the soil profile ranked as 0.25–0.50 mm > 2.00–4.00 mm > 0.50–1.00 mm > 1.00–2.00 mm at 0–15 cm depth and 0.25–0.50 mm ≈ 0.50–1.0 mm > 2.00–4.00 mm > 1.00–2.00 mm at 15–30 cm depth (Fig. 1a). Both the $WSA_{0.50–1.00\ mm}$ and $WSA_{1.00–2.00\ mm}$ were significantly lower at 0–15 cm than at 15–30 cm depth, whereas $WSA_{1.00–2.00\ mm}$ was significantly lowest at the two soil depths.

A range of 0.45–0.62 mg/g EE-GRSP and 0.61–1.07 mg/g T-GRSP was found in the WSA fractions at 0–30 cm soil depth (Fig. 1b, c). A significantly higher T-GRSP for all measured WSA fractions was at 0–15 cm than that at 15–30 cm soil depth. Significantly higher EE-GRSP in WSA fractions ranked as $WSA_{0.25–0.50\ mm}$ < $WSA_{0.50–1.00\ mm}$ = $WSA_{1.00–2.00\ mm}$ = $WSA_{2.00–4.00\ mm}$ at 0–15 cm, whereas $WSA_{0.25–0.50\ mm}$ = $WSA_{0.50–1.00\ mm}$ = $WSA_{1.00–2.00\ mm}$ < $WSA_{2.00-4.00\ mm}$ at 15–30 cm (Fig. 1b). Meanwhile, T-GRSP at 0–15 cm and 15–30 cm soil depth generally decreased with the decrease of WSA size (Fig. 1c), and T-GRSP at 0–15 cm soil depth was significantly higher than at 15–30cm soil depth.

The MWD values were significantly higher at 15–30cm than at 0–15 cm depth (Fig. 2). EE-GRSP significantly positively correlated with T-GRSP in the $WSA_{0.50–1.00\ mm}$ only (Fig. 3a), but not with other WSA sizes at 0–30 cm soil depth (data not shown). Meanwhile, EE-GRSP and T-GRSP of $WSA_{0.50–1.00\ mm}$ fraction significantly negatively correlated with only $WSA_{0.50–1.00\ mm}$ (Fig. 3b). For the 0–30 cm soil depth MWD was significantly positively related to EE-GRSP in the $WSA_{0.25–0.5\ mm}$ fraction (r^2=0.90, $P<0.05$), negatively to EE-GRSP in the $WSA_{0.50–1.00\ mm}$ (r^2=0.81, $P<0.05$) or T-GRSP in the $WSA_{2.00–4.00\ mm}$ (r^2=0.77, $P<0.05$) (Fig. 3c) and neutrally related to EE-GRSP and T-GRSP in all other WSA sizes (data not shown).

Discussion

Our study showed that the citrus rhizosphere displayed lower $WSA_{0.50–1.00\ mm}$ and $WSA_{1.00–2.00\ mm}$ at the 0–15 cm depth than at the 15–30 cm depth, which might relate to tillage, erosion, and the subsequent loss of organic matter at the 0–15 cm depth (Neufeldt *et al*., 1999). In the present study, EE-GRSP concentrations ranged from 0.45 to 0.62 mg/g DW soil in the WSA fractions at 0–30 cm soil depth, and T-GRSP from 0.61 to 1.07 mg/g DW soil. These results differed from 0.3–0.6 mg/g EE-GRSP and 0.5–0.8 mg/g T-GRSP at 0–40 cm soil depth in the same citrus orchid (Wu *et al*., 2012). These might result from different sampling seasons as September in this study versus May in our previous study (Wu *et al*., 2012), since significant variation of GRSPs occurred seasonally in an intermountain grassland in western Montana, US (Lutgen *et al*., 2003).

In general, EE-GRSP and T-GRSP concentrations at 0–15 cm and 15–30 cm soil depth decreased with the decrease of WSA sizes in the citrus orchard, which is consistent with results from 0–5 cm depth in a US Acrisols under no tillage (Wright *et al*., 2007). As a biochemical agent GRSP can bind micro-aggregates into macro-aggregates in soil particles (Purin and Rillig, 2007; Fokom *et al*., 2012), resulting in a distinctive distribution pattern in WSA sizes. In contrast, no differences in GRSPs were found in $WSA_{<6.00\ mm}$ fractions at 0–5 cm depth under chisel, moldboard and disk tillage (Wright *et al*., 2007), as well as in a cropland, a permanent pasture and a forest soil in Germany (Spohn and Giani, 2010). These results suggested that GRSP distributions in WSA fractions might be different in different soil ecosystems and under different soil managements. A significantly higher T-GRSP for all measured WSA fractions at 0–15 cm than at 15–30 cm soil depth might also indicate higher mycorrhizal hyphae and spore biomass at the topsoil than at the subsoil (Harner *et al*., 2011).

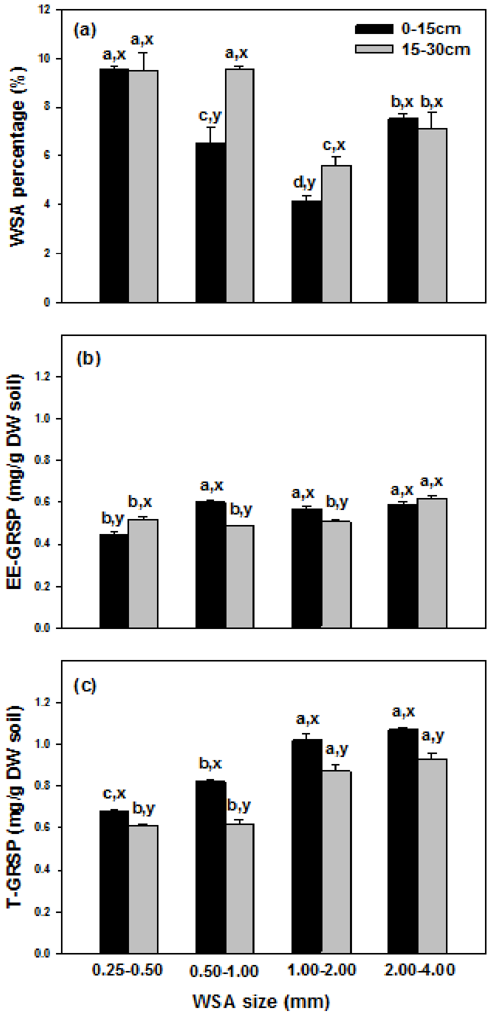

Fig. 1: Aggregation percent and EE-GRSP and T-GRSP concentration of different size WSA in rhizospheric 0–15 and 15–30 cm soil depth of *Citrus unshiu*. Data (means ± SE, *n*=3) followed by different letters above the bars are significantly different between WSA fractions for the same soil depth (a, b, c) or between soil depths for the same WSA fraction (x, y) at $P < 0.05$ against the Least Significant Differences. Abbreviations: EE-GRSP, easily extractable glomalin-related soil protein; T-GRSP, total glomalin-related soil protein; WSA, water-stable aggregate

A significantly positive correlation of EE-GRSP with T-GRSP in the $WSA_{0.50–1.00\ mm}$ only indicated that EE-GRSP might only relate to T-GRSP in a certain WSA fraction, though EE-GRSP relates to T-GRSP at 0–15 cm (Wright and Upadhyaya, 1998) or 0–40 cm soil depth (Wu *et al*., 2012). In addition, both EE-GRSP and T-GRSP in the $WSA_{0.50\text{-}1.00\ mm}$ fraction at the 0–30 cm soil depth significantly negatively correlated with percentage of the $WSA_{0.50\text{-}1.00\ mm}$ fraction, but not correlated with other WSA fractions (data not shown), which supported the hypothesis of Rillig (2004).

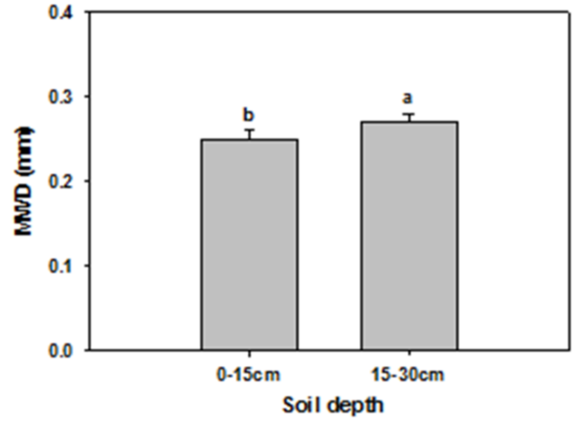

Fig. 2: Mean weight diameter (MWD) of the water-stable aggregate in rhizospheric 0–15 and 15–30 cm depth of *Citrus unshiu*. Data (means ± SE, *n*=3) followed by different letters (a, b) above the bars are significantly different at P<0.05

The higher MWD value at 0–15 cm soil depth indicated a better aggregate stability at 0–15 cm soil depth, rather than at 15–30 cm soil depth, in this citrus orchid. In addition, for the 0–30 cm soil depth MWD significantly positively correlated with EE-GRSP in $WSA_{0.25–0.5\ mm}$ fraction, negatively with EE-GRSP in $WSA_{0.50–1.00\ mm}$ or T-GRSP in $WSA_{2.00–4.00\ mm}$, and neutrally to EE-GRSP and T-GRSP in all other WSA sizes (data not shown). However, a negative correlation of EE-GRSP or T-GRSP with the percentage of $WSA_{1.00–2.00\ mm}$ was found under a *Stipa tenacissima* canopy in Spain (Rillig *et al*., 2003), but not under the citrus canopy in this study. Positively linear correlation of GRSP with aggregate stability was generally found in a variety of soils (Rillig, 2004; Rillig and Mummey, 2006; Fokom *et al*., 2012). In addition, a sigmoidal correlation of GRSP with aggregate stability was found in sandy hydromorphic soils of Northwest Germany (Spohn and Giani, 2010). Such correlations might indicate that GRSP was not the main aggregate binding agent in the soil (Rillig *et al*., 2003). These varied results suggested that the relationship between GRSP and WSA could be positive, negative or neutral. Interestingly, all the three relationships had been displayed in our citrus rhizosphere, implying that different GRSP fractions could provide variant roles in aggregate stability, e.g. EE-GRSP in $WSA_{0.25–1.00\ mm}$, while T-GRSP in $WSA_{2.00–4.00\ mm}$ fraction.

In conclusion, (i) both EE-GRSP and T-GRSP concentrations decreased with the decrease of WSA sizes, and higher GRSP in the WSA fraction generally was at 0–15 cm soil depth and coarser soil particles ($WSA_{2.00–4.00\ mm}$) than at 15–30 cm soil depth and fine particles ($WSA_{0.25–0.50\ mm}$). (ii) Contributions of GRSP to aggregate stability may relate to WSA fractions in this citrus rhizosphere: positively to EE-GRSP in $WSA_{0.25–0.5.00\ mm}$, negatively to EE-GRSP in $WSA_{0.50–1.00\ mm}$ and of T-GRSP in $WSA_{2.00–4.00\ mm}$, and neutrally to GRSPs in other tested WSA size fractions.

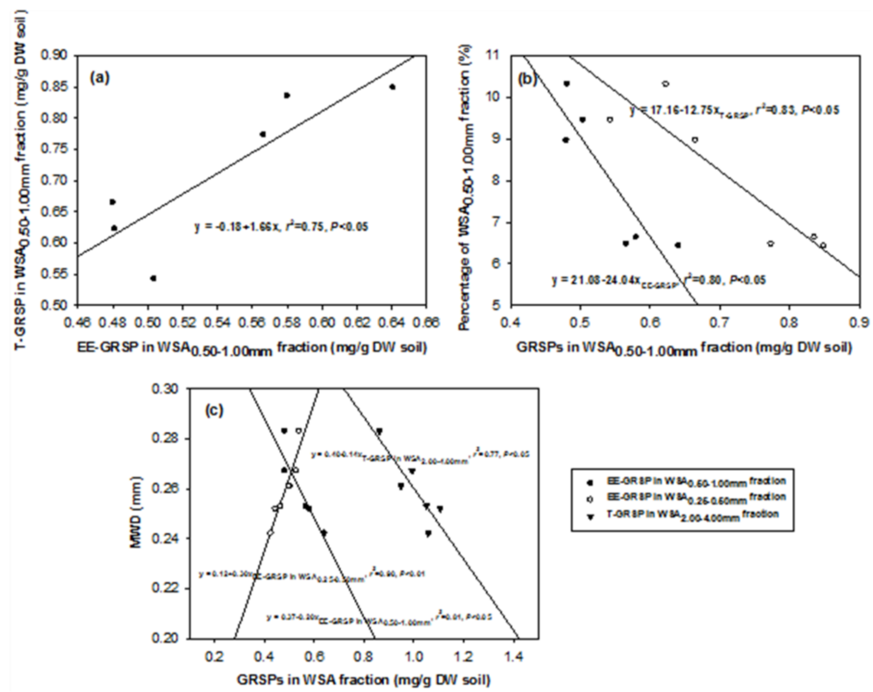

Fig. 3: Relationships between EE-GRSP and T-GRSP in $WSA_{0.50–1.00\ mm}$ fraction (a), between GRSPs and percentage of $WSA_{0.50–1.00\ mm}$ fraction (b), or between MWD and GRSPs in different WSA fractions (c) at 0–30 cm depth of *Citrus unshiu* (n = 6). Abbreviations: EE-GRSP, easily extractable glomalin-related soil protein; MWD, mean weight diameter; T-GRSP, total glomalin-related soil protein; WSA, water-stable aggregate

Acknowledgements

This study was supported by the Key Project of Natural Science Foundation of Hubei Province (2012FFA001), the Key Project of Ministry of Education of China (211107), and the Science-Technology Research Project of Hubei Provincial Department of Education (Q20111301), China.

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(Received 15 October 2012; Accepted 07 February 2013)