



Review Article

Glomalin: A Potential Soil Conditioner for Perennial Fruits

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Abstract

Glomalin, a special glycoprotein secreted by hyphae and spore walls of arbuscular mycorrhizal fungi (AMF), possesses some diverse characteristics, of them its hydrophobic nature and stability to heat are most profound. Glomalin sloughs off cementing material following the hyphal degradation that coat soil organic matter and soil particles, thereby, forming a protective surface layer against loss of water and nutrients from soil aggregates. These properties of glomalin would influence plant and/or soil water relations. Assessed through Bradford assay, glomalin contains proteins of non-AMF and AMF origin, in addition to large amount of soil-related heat-stable proteins, collectively defined as glomalin-related soil protein (GRSP) in soils. In perennial fruits, GRSP concentration has been observed up to 1 mg/g DW soil, which is strongly affected by physiography, soil management practices, and season. GRSP represents a type of glue agent that stabilizes soil aggregates, as one of the fractions of soil organic matter in perennial fruit orchards. There is an increasing demand to discern whether the functionings of exogenous GRSP application is similar to those of endogenously synthesized GRSP. These accrued results provide strong support in favour of glomalin as a potential soil conditioner that holds promise in perennial fruits. This review also makes an attempt to address the future lines of research on glomalin-related issues. © 2016 Friends Science Publishers

Keywords: Aggregate stability; Fruit crops; Glomalin-related soil protein; Mycorrhiza; Soil fertility

Introduction

Arbuscular mycorrhizas (AMs) are a mutualistic symbiosis between arbuscular mycorrhizal fungi (AMF, a member of Glomeromycota) and roots of ~80% land plants (Fig. 1), where AMF receive plant photosynthates and in return, provide the host plant the required water and mineral nutrients through the extraradical mycelium (ERM, Fig. 2) (Gutjahr and Parniske, 2013). During the formation of AMs, an exchange of signaling molecules between AMF and the host plant takes place for so many physiological and biochemical functions. It is estimated that AM is one of the most widespread symbionts so effective in terrestrial ecosystems.

Many functionings of AMs are still unknown. For example, glomalin, a newly discovered glycoprotein, is produced by AMF. The glomalin has been found in a series of soil environments, such as acid, calcareous, grassland, and cropland soils. Moreover, glomalin stocks could represent diversity of both AMF and the host plants, and appears to be linked to soil C pools (Treseder and Turner, 2007). Therefore, glomalin could predict global C change in ecosystem feedbacks.

At present, glomalin-based research is considered as

one of the most important multidisciplinary subject interfacing fungal physiology and soil biochemistry. In this review, we simply highlight the origin of glomalin and important advances made in utility of glomalin as a soil conditioner in perennial fruit orchards.

Discovery and Origin of Glomalin

In 1996, Sara E. Wright, a soil scientist at the Agricultural Research Service, United States Department of Agriculture (USDA), firstly identified an unique fungal glycoprotein secreted only by spores and ERM of AMF in the taxon Glomales, including fungi of the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, and *Scutellospora* (Wright *et al.*, 1996). The gooey protein was named as glomalin, because these secreted fungi belong to Glomales. The monoclonal antibody, MAb32B11, produced against fresh spores of *Glomus intraradices* FL208, was used to detect the presence of glomalin. Immunofluorescence, using MAb32B11 detected the glomalin on extraradical hyphae with an uneven sheath and on the surface of small and large water-stable aggregates (Wright, 2000). In *in-vitro* cultures of *G. intraradices*, glomalin was tightly bound in hyphal and spore walls, and ~80% of glomalin was released by

hyphae and spores of the AMF (Driver *et al.*, 2005). As a result, glomalin appears in soil, primarily through release of hyphae. Application of proteomics showed that glomalin is a thioredoxin-containing chaperone.

The glomalin either coats on the surface of ERM or remains as a component of spore and hyphal walls. Once these mycorrhizal hyphae degrade, the glomalin would enter into the soils, called as glomalin-related soil protein (GRSP), as a consortium of proteins of AMF and non-AMF origin, including soil-related heat-stable proteins (Rillig, 2004; Gillespie *et al.*, 2011). After extracting with heated citrate buffer, GRSP showed a dark reddish-orange color, due to abundance of 0.8~8.8% iron in the molecule (Wright and Upadhyaya, 1998).

As earlier described by Wright and Upadhyaya (1996), total glomalin is extracted with 0.25 g soil in 2 mL of 50 mM citrate buffer (pH 8.0) for 90 min at 121°C and 0.11 Mpa. While, easily-extractable glomalin is extracted with 20 mM citrate (pH 7.0) for 30 min at 121°C and 0.11 Mpa. Later, Koide and Peoples (2013) and Wu *et al.* (2014a) divided GRSP into two fractions, easily-extractable glomalin-related soil protein (EE-GRSP) and difficultly-extractable glomalin-related soil protein (DE-GRSP). Meanwhile, EE-GRSP is considered as a newly synthesized glomalin and relatively more labile, while DE-GRSP is comparatively an older glomalin originated from EE-GRSP turnover and more difficult to extract and recalcitrant in soils. Total glomalin-related soil protein (T-GRSP) is the sum of EE-GRSP and DE-GRSP.

GRSP Changes

EE-GRSP concentration has been observed in the range of 0.3–0.6 mg/g DW soil in the rhizosphere of *Citrus unshiu* grafted on *Poncirus trifoliata* (Wu *et al.*, 2012). And, total GRSP concentration has been reported to range from 0.5 to 0.8 mg/g DW soil in citrus orchard. Dynamics of GRSP showed maximum concentration of T-GRSP level during July, maximum EE-GRSP was observed during September or March with minimum EE-GRSP and T-GRSP levels during May and November (Wu *et al.*, 2014c). Our further studies (Wu *et al.*, 2013) revealed that both EE-GRSP and T-GRSP significantly decreased with reduction in water-stable aggregate (WSA) size (0.25, 0.50, 1.00, 2.00 and 4.00 mm) and soil depth (0–15 and 15–30 cm).

Mycorrhization has been observed to induce 19–26% higher EE-GRSP and 13–20% higher T-GRSP in the mycorrhizosphere than in non-mycorrhizosphere following the inoculation with AMF (Wu *et al.*, 2014b), implying that AMF inoculation could help in production of endogenous GRSP fractions for further utilization. Significantly higher EE-GRSP, DE-GRSP and T-GRSP induced by mycorrhization was absolutely dependent on AMF genotype since hyphal diameter, hyphal wall thickness, and its branching pattern collectively influenced the production of GRSP (Wang *et al.*, 2014). In addition, soil management



Fig. 1: Root colonization of trifoliate orange [*P. trifoliata* (L.) Raf.] by *Funneliformis mosseae* (Wu QS, unpublished data). Blue zones mean mycorrhizal infection in the roots



Fig. 2: Extraradical mycorrhizal mycelium from the roots of trifoliate orange [*P. trifoliata* (L.) Raf.] colonized by *F. mosseae* (Wu QS, unpublished data). These mycelium into soils are considered as soil hyphae

strongly affected GRSP levels in fruit orchards. In 13–15-year-old Newhall navel orange trees (grafted on trifoliate orange) located at Three Gorges Region (China), the highest T-GRSP content was detected in no-tillage and sod culture soil and the lowest in clean-tillage soil management (Wang *et al.*, 2011). On the other hand, infection with *Funneliformis mosseae* showed an increase in production of EE-GRSP, DE-GRSP and T-GRSP in trifoliate orange seedlings, irrespective of substrate P level (Wu *et al.*, 2015a).

Field observation in citrus orchards also revealed that T-GRSP content decreased with the increasing altitude (200 to 800 m) in most part of the year except summer (Wang *et al.*, 2015a). A significantly positive correlation between T-GRSP and soil hyphal length or spore density indicated that better AM levels in roots or soils is favored in maintaining higher GRSP contents in citrus orchards.

GRSP and SOC Pool

GRSP fractions are reported to contribute positively in enriching the soil organic carbon (SOC) pool (Rillig, 2004; He *et al.*, 2010), since glomalin contains ~37% C and accounts for 4–5% of soil total C (Rillig *et al.*, 2001; Lovelock *et al.*, 2004). In C stock, GRSP contained high aromatic (42–49%) and carboxyl (24–30%) C contents and low aliphatic (4–11%) and carbohydrate-type (4–16%) C contents (Schindler *et al.*, 2007). Haddad and Sarker (2003) considered GRSP as a newly discovered component of soil organic matter. However in citrus orchards, EE-GRSP and T-GRSP showed no significant relation with SOC in soil WSA in the size of 0.25–4 mm, but root glomalin correlated positively with SOC (Wu *et al.*, 2014c). Since GRSP turnover has a long settling time, to the tune of 6–42 years (Rillig *et al.*, 2001), which could facilitate in blocking the functioning of GRSP with regard to changes in SOC pool. However, other studies revealed, EE-GRSP and T-GRSP were positively correlated with SOC in three citrus genotypes, namely, *Citrus tangerina*, *Fortunella margarita*, and *P. trifoliata* (Wu *et al.*, 2014b) and in the potted trifoliolate orange colonized by *F. mosseae*, *G. intraradices*, *G. versiforme* and *Paraglomus occultum* (Wang *et al.*, 2014). These observations advocated that under less disturbed conditions, GRSP has more positive contribution towards enriching the SOC pool in perennial fruits.

GRSP and Soil Aggregation

Many studies have shown that GRSP strongly influenced the soil aggregate stability in a variety of soils, but the GRSP effect was absolutely dependent on GRSP concentrations in WSAs in different sizes (Rillig, 2004). Linear correlation of GRSP with soil WSA stability was further observed within a specified concentration of GRSP. And beyond this critical concentration of GRSP, such linearity turned into curvilinear relationship between GRSP and WSA stability (Rillig, 2004). Rillig (2004) reported that under high concentration of GRSP, these GRSP fractions could seal and deposit in pores of these macro-aggregates, resulting in slowing down of water penetration into the WSAs, finally decreasing WSA stability.

In citrus orchard, we found no significant correlation of GRSP or root glomalin with WSA stability (based on the determination of mean weight diameter), revealing that GRSP is not the main binding agent of soil WSA formation and its subsequent stabilization (Wu *et al.*, 2014c). However, under potted condition, EE-GRSP and T-GRSP in mycorrhizosphere of citrus and peach plants (Fig. 3) were significantly and positively correlated with WSA stability (Wu *et al.*, 2014b; Zhang *et al.*, 2014), indicating that under less disturbed conditions, GRSP plays a key role in stabilizing WSAs. The glue functioning of GRSP is also beneficial to alleviate the toxicity of heavy metals either on AMF as inoculants and host crops as different fruit crops,

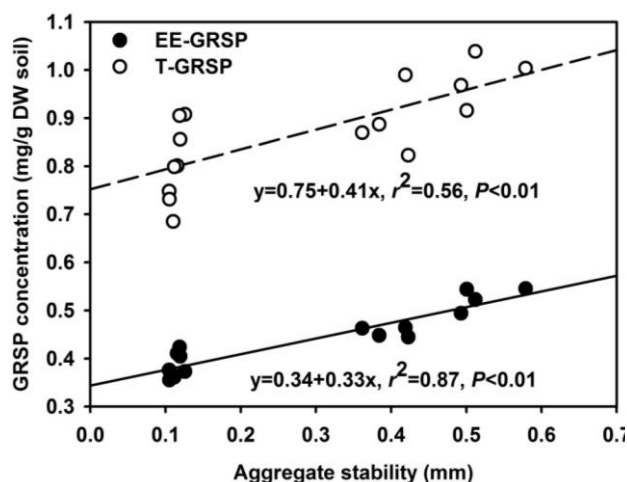


Fig. 3: Linear correlation between soil aggregate stability (in terms of determination of mean weight diameter) and EE-GRSP or T-GRSP concentration in rhizosphere of *Prunus persica* seedlings inoculated with *Funnelformis mosseae* and grown in non-replanted and replanted soil ($n=16$) (Wu QS, unpublished data)

displaying the strong buffering capacity of glomalin against heavy metal stress (Wang *et al.*, 2010).

In a non-sterilized soil of peach orchard, inoculation with *F. mosseae* increased EE-GRSP content by 17.8 and 11.1% and T-GRSP by 10.1 and 17.6%, irrespective of non-replanted soil and replanted soil (Zhang *et al.*, 2014). Higher GRSP contents in mycorrhizosphere could cement harmful substances and plant growth inhibitors released by peach roots, coupled with higher soil aggregate stability, due to mycorrhizal-induced GRSP changes in peach mycorrhizosphere.

GRSP and Soil-plant Water Relations

Researches revealed that GRSP-induced aggregate stability is more conspicuous under drought (Wu *et al.*, 2008) than under salinity (Kohler *et al.*, 2009). Nichols (2008) proposed that GRSP aided in conserving the loss of water and nutrients from the soil exposed to abiotic stress, due to formation of hydrophobic layer on the surface of aggregate by GRSP. Studies further indicated that EE-GRSP and T-GRSP were, to some extent, increased by drought stress within rhizosphere of trifoliolate orange seedlings (Wu *et al.*, 2008; Zou *et al.*, 2014), because drought-stress-induced death/senescence of mycorrhizal hyphae released more GRSP into soils (Driver *et al.*, 2005). Correlation studies showed that T-GRSP but not EE-GRSP had a significantly negative correlation with soil and leaf water potential in the mycorrhizosphere of potted trifoliolate orange (Zou *et al.*, 2014), suggesting that T-GRSP was more active under drought stress than EE-GRSP. As revealed by earlier studies (Augé, 2001; Wu *et al.*, 2008; Zou *et al.*, 2014), mycorrhizal

inoculation improved soil WSA distribution and WSA stability in terms of GRSP and mycorrhizal hyphae, beneficial for the host plants to drive soil water. In addition, soils with higher GRSP contents are less susceptible to soil erosion (Haddad and Sarker, 2003).

Functioning of Exogenous GRSP

GRSP functionings on soil aggregation, SOC pools, and water relation are delineated through regression analysis and path coefficient analysis (Wright and Upadhyaya, 1998; Fokom *et al.*, 2012; Wu *et al.*, 2012, 2014a). However, these studies considered only GRSP-induced changes in soil properties through the evaluation of endogenous GRSP levels. In fact, many factors, such as soil microorganisms, roots, SOC, and experimental conditions influenced the evaluation of GRSP functionings. Based on varied functionings of GRSP, some researchers considered the application of exogenous GRSP into the rhizosphere of fruit crops, in order to confirm the hypothesis, whether exogenous GRSP as an analogue of humic substance has any roles on plant growth and soil aggregation. Recently, Wu and his group for the first time extracted EE-GRSP solutions from citrus rhizosphere and applied in different strengths of EE-GRSP solutions into the potted trifoliate orange seedlings and the field-planted Satsuma mandarin (Wu *et al.*, 2015b; Wang *et al.*, 2015b). The results showed that exogenous EE-GRSP solutions induced an increase of WSA stability, SOC concentration, and soil phosphatase activities, absolutely dependent on exogenous EE-GRSP strengths. Moreover, in controlled experimental conditions, the magnitude of WSA stability and plant biomass production was curvilinearly and positively correlated with strength of exogenous EE-GRSP application (Wang *et al.*, 2015b). These studies further confirmed the hypothesis of Rillig (2004) about the role of endogenous GRSP. Therefore, exogenous EE-GRSP can be considered as a soil conditioner/regulator with regard to favourable transformations in soil fertility and soil structure in fruit crops, as well as a plant growth regulator.

Future Perspectives

The number of researches have been conducted on AMF-induced secretion of glomalin and soil GRSP since its discovery in 1996. Some expected advances in the glomalin-related research have been made. A global consensus is that glomalin is a kind of potential soil conditioner that holds huge potential in perennial fruit crops. While, there are still many unknown properties of about this unique soil protein. Following further studies are suggested:

- (1) Clarify the role of GRSP in soil aggregation at different WSA sizes in soils of diverse mineralogy.
- (2) Define the mechanisms that operate toward the contribution of GRSP in WSA stability.
- (3) Explore GRSP functionings in different perennial

fruit orchards, possessing variation in rhizosphere properties.

(4) Understand the mechanisms involved in efflux of glomalin from roots or fungal hyphal walls to the rhizosphere.

(5) Define different components of glomalin through proteomics and metabolomics.

(6) Evaluate the physiological functionings of different exogenous GRSP fractions on plant growth and soil properties in perennial fruit crops.

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