

### **Glomalin: A miracle protein for carbon sequestration**

#### **ABSTRACT**

AMF (Arbuscular mycorrhizal fungi)'s hyphae and spore walls releases a special kind of glycoprotein i.e. Glomalin. AMF belongs to the phylum *Glomeromycota* which was previously known as *Zygomycota*. There exists a symbiotic relationship of this fungi with terrestrial plants (~80%), that includes major commercial species viz. wheat, sorghum, corn, and forage species. AMF strongly binds and firmly hold the walls of hyphae and spores. On decomposition of hyphae, glomalin is released in soil. Glomalin depicts recalcitrant behavior and hydrophobic characteristics, and hence prevent the loss of water and nutrients from hyphae (ERM). It can remain as such in soil for years. It's half-life in soil can vary from 6-42 years thus placing it in the category of stable biomolecules. Glomalin Related Soil Protein is quite abundant in wide range of soil The GSRP were found in relative abundance in a wide range of soils (2-15 mg g<sup>-1</sup>), whether it is acid or calcareous or under various crops, such as cereals, vegetables, forage, and agroforestry systems. It plays a significant role in enhancing the soil organic carbon as it acts as an effective carbon sink. It possess strong cementing ability and hence binds the aggregates to enhance structural stability and prevent loss of carbon and nitrogen. GRSP positively correlates with the carbon present in soil.

**Keywords:** Aggregates, AMF, correlation, GRSP, structural stability

#### **INTRODUCTION:**

Arbuscular mycorrhizal fungi (AMF) colonize to the extent of nearly 80% in the plant rhizosphere, it belongs to the phylum *Glomeromycota* (Smith and Read, 2008). Extra-radical mycelium (ERM) network, a key component of AMF, extends far beyond the root zone and acquire labile plant nutrients. It facilitates easy exchange of water as well as nutrients within the mycelium (Zou et al. 2015). The extra-radical fungal hyphae contributes to soil aggregate stability (Rillig et al. 2006; Peng et al. 2013). It indirectly influences the biochemical as well as morphological properties of the host plants (Borie et al. 2008). AMF aids in nutrient and water uptake in exchange of ~20% photosynthetically fixed carbon (Wu et al. 2014). It significantly improves plant-host water relation through different mechanisms viz. direct absorption and indirectly by enhancing the physiological parameters (stomatal regulation, osmotic adjustment, plasma membrane aquaporins, antioxidants and photosynthesis) and nutrient acquiring capacity (Zou et al. 2014). Their relationship with plants is well-known symbiosis on the planet, connecting root and soil, and it has been discovered to be the oldest and most common plant microbe interaction (Smith and Read, 1997). Except for selected groups like Brassicaceae, Amaranthaceae, Chenopodiaceae, and others, most plants in our ecology are inhabited by AM fungus. Hyphae of AM fungi can extend several centimetres beyond the root exploration zone, forming a framework to hold soil particles together by the formation of glomalin, a glycoproteinaceous chemical. Glomalin is only produced by AM

fungus. AM fungus require plant carbon to develop and create glomalin, and the plant receives phosphorus, other nutrients, and water through hyphae, which are tubes (Gomathy et al. 2018). Mycorrhizal hyphae allows effective water flow to the host plants. Under drought-like conditions, up to 4% of water held in the hyphal compartment has been reported to be transported to the root compartment via mycorrhizal hyphae (Khalvati et al. 2005).

Arbuscular mycorrhizal fungi (AMF) are the most important mediators of soil aggregation among the fungi for three reasons. AMF's extraradical hyphae are a significant, and frequently dominant, component of soil microbial biomass (Miller et al. 1995; Rillig et al. 1999). It allows direct tapping of plant carbon resources, and it is independent of carbon reserves in soil which are deliberately limiting (Smith and Read, 1997). Hyphae of AMF, being an indispensable component in path analysis models that allow effect of biotic factors on soil aggregation than other biological influences. AMF (Arbuscular mycorrhizal fungi)'s hyphae and spore walls releases a special kind of glycoprotein i.e. Glomalin (Wright and Upadhyaya, 1996). As extraradical hyphae decays, glomalin is released in the soil and termed as GRSP (Rillig, 2004). It is crucial for maintaining the soil organic carbon levels and hence soil fertility (Wu et al. 2014). Its total carbon and nitrogen contribution (4 to 5%) is higher than that by microbial biomass C in Hawaiian soils (Rillig et al. 2001). It is insoluble, hydrophobic, heat-resistant and recalcitrant in nature, that highly relates to the amount of water stable aggregates. It contains 3 to 5% N, 36 to 59 % C, 4 to 6 % hydrogen, 33 to 49 % oxygen, and 0.03 to 0.1 % P. Vaidya (2011) reported 30- 40% carbon contribution by glomalin which soil clumps. This leads to development of granulated material in soil which mellows the soil and keeps the carbon intact. GRSP enhances aggregate stability, conserve carbon and nitrogen, prevent plant from abiotic stresses and acts as an effective agent for carbon sequestration.

### **Origin of glomalin**

It was first discovered by woman scientist Sara F. Wright in 1996 at the USDA Agricultural Research service (Wright et al. 1996). The name glomalin originates from the order glomales. More the production of glomalin, more will be the carbon sequestration. Glomalin protein can be extracted from the soil by using citrate solution and then centrifugation and decantation. The extracts obtained were reddish coloured due to its iron content (0.8-8.8%) (Wright and Upadhyaya, 1998). Its concentration in soil depends on different agricultural management practices and can be traced in wide range of soils viz. acidic, problematic soils, grassland and cropland (Wright and Upadhyaya, 1998; Wright *et al.*, 1999). Wright et al. (1996) reported ability of glomalin of forming conglomeration with organic matter and root fragments, thus preventing its degradation by soil microbes. Carbon dating was used by Rillig et al. (1999) to trace the time taken for its degradation and observed it to degrade in about 7–42 years. Further studies about glomalin started with monoclonal antibody (mab32b11) cultured against an unknown epitope on crushed spores of the AMF species *Glomus intraradices* (Wright and Upadhyaya, 1996).

### **Procedure for extraction of GRSP content:**

Extracting EE-GRSP and T-GRSP contents from soil samples was quite cumbersome. Both were extracted using procedures as described by Wright and Upadhyaya (1996). Initially, soil

samples were collected and then sieved using 2 mm sieve. Then 1 g of the sieved soil was taken in a centrifuge tube. For EE-GRSP content, extract the sample using 8 ml of 20 mM citrate solution (pH 7.0) by autoclaving it for 30 min (121°C), then centrifuged was carried out at 10,000×g for 5 min to remove the residual soil particles. Similar procedure was adopted for T-GRSP content but the pH of citrate solution was adjusted to pH 8.0 and autoclaving was done for 60 min (121°C), then centrifuged at 10,000×g for 5 min to remove the residual soil particles. This process was repeated again and again and after each cycle, the supernatant was removed, and sodium citrate solution was replenished for the extraction until the supernatant becomes colourless. After decantation, the supernatant was stored at 4°C for analysis. Bradford assay method was used to determine the protein content, by taking bovine serum albumin as standard.

### **Composition of glomalin:**

According to studies on glomalin, Wu et al. (2014) reported division of GRSP into two fractions: fraction 1 and fraction 2. GRSP fraction 1 is easily-extractable GRSP (EE-GRSP), which is a newly produced glomalin and is very labile, while GRSP fraction 2 can be defined as an older glomalin which is quite difficult- to-extract and is recalcitrant in soils, termed as difficultly-extractable GRSP (DE-GRSP). Both EE-GRSP and DE-GRSP combinedly contributes towards T-GRSP i.e. total- GRSP. GRSP is rich in metal ions ( $\text{Fe}^{3+}$ ) contributed by arbuscular mycorrhizal fungi (AMF). GRSP mainly comprises of stretching bonds of O–H, N–H, C–H, C=O, COO–, C–O, and Si–O–Si and bending of C–H and O–H. It consists of seven fluorescent substances of tryptophan-like protein, tyrosine-like protein, fulvic acid-like, humic acid-like, soluble microbial byproduct-like, nitrobenzoxadiazole-like, and calcofluor white-like, with characteristic X-ray diffraction peak at  $2\theta = 19.8^\circ$  and 129.3nm grain size as well as 1.08% low crystallinity (Wang et al. 2014).

### **Effect of disruption of mycelial network:**

There exists an extra-radical mycelium (ERM) that arbuscular mycorrhiza produce, it extends in the soil thus allowing better nutrient and water uptake and also aids in maintaining the aggregate stability. Zou et al. (2015) observed a significant decline in mycorrhizal colonization when the extra-radical mycelium was broken. Root mycorrhizal colonization in the root + hyphal compartment ranged between 32.1 to 49.5%. While, mycorrhizal colonization was found to decrease significantly by 35.2% in case of broken ERM as compared to those with intact ones. Hence, the destruction of ERM network resulted in adverse impact on the magnitude of root colonization. Studies on disruption of ERM network were done and it was concluded that disruption of ERM network (once) decreased only the leaf water status with no considerable effect on plant growth and soil parameters. But, disruption of ERM network (at weekly interval) declined not only the leaf water status but also the plant growth, GRSP production, and Mean weight diameter in both root + hyphae as well as hyphae chambers. More impactful results were obtained with periodic disruption of ERM network at week interval as compared to single time disruption, implying that this network plays crucial role in making the host plant more metabolically active. Out of the GRSP fractions, EE-GRSP showed high response to fluctuating leaf water and mean weight diameter under root + hyphae and hyphae conditions. It suggests that effect of periodical

disruption of ERM network was more impactful than one-time disruption of ERM network with regard to leaf water, plant growth, and aggregate stability responses, thereby, implying ERM network aided in developing the host plant metabolically more active (Zou et al. 2015)

#### **Effect of mycorrhizal colonization on plant growth:**

Colonization with arbuscular mycorrhiza significantly improved the plant growth and yield. Irrespective of AMF species, colonization led to improved stem diameter, leaf number, shoot and root dry weight, and root total length (Wu et al. 2014). Root mycorrhizal colonization with *F. Mosseae* varied from 29.8 to 54.1% in five month-old citrus plants and was observed to be significantly higher in *P. Trifoliata* than the other two citrus genotypes. Inoculation with *F. Mosseae* significantly higher plant growth traits, viz. plant height, stem diameter, shoot and root fresh weight, total root length, surface area, and volume in all three citrus genotypes (Wu et al. 2014). Zou et al. (2015) reported increment in growth (plant height, stem diameter, and leaf stem and root biomass) and physiological characters (leaf relative water content, leaf water potential and transpiration rate) on mycorrhizal inoculation, irrespective of ERM status.

#### **Changes in EE-GRSP and T-GRSP fractions:**

GRSP is release from the soil after the death of fungi, it acts as a binding agent in soil. It is basically insoluble, hydrophobic and heat binding protein that can be classified into two fractions: EE-GRSP and T-GRSP. The classification is based on the different extraction methods (Wright et al. 1998b; Rillig et al. 2001). EE-GRSP and T-GRSP significantly increased in the mycorrhizosphere of root + hyphae and root-free hyphae compartments as compared to non-mycorrhizosphere (Zou et al. 2015). Inoculation with *F. Mosseae* significantly improved the rhizospheric EE-GRSP and T-GRSP concentrations by 26, 23, and 19% and 13, 20, and 15% in *P. Trifoliata*, *C. Tangerina*, and *F. Margarita*, respectively. EE-GRSP and T-GRSP fractions significantly improved under root/hyphae chamber as compared to root-free hyphae chamber, irrespective of AMF species. Difficulty extractable GRSP fraction had no effect on colonization in root and root-free hyphae chamber (Wu et al. 2014). According to a logarithmic function, the concentration of GRSP, including EEG and TG, dropped as soil depth increased. The composition and features of the GRSP are more complicated than previously thought.

#### **SOC Concentration:**

Soil organic carbon concentrations significantly improved in seedlings inoculated with *F. Mosseae* by 39 to 325% as compared to non-AM inoculated controls (Wu et al. 2014). It also accounts for the variation in easily-extractable and total glomalin fractions with the frequency of 71.76 and 70.59%, respectively (Wang et al. 2019). Hodge et al. (2001) observed role of AMF in nitrogen cycling by accelerating the decomposition of organic matter and hence increase SOC levels in soil. Glomalin contributed to total carbon and nitrogen storage by 4-5% in Hawaiian soils. Contribution of glycoprotein to total carbon was much higher than that by microbial biomass carbon (Rillig et al. 2001). This can be attributed to slow turnover rate or the ability of glomalin to accumulate in soil. On suppression of AMF, significant decline in soil C and N content was observed that may be due to decrease in hyphal content and GRSP concentration. Another reason speculated was decline in aggregate stability that has

led to loss of C and N from the bind state. Limited studies have been done on direct influence of glomalin on SOC storage, it was indirectly studied in terms of aggregate stability.

#### **Effect of GRSP on soil parameters and its effect of fungal population:**

It was observed that AM-mediated GRSP production and soil enzyme activities does not depend on Phosphorous concentration provided by chemical means. While, Dai et al. (2013) reported that application of P in long-term experiment significantly enhanced the SOC content, GRSP concentration and AM fungal population size. According to the study by Rillig et al. (2003), Chen et al. (2012) and Wang et al. (2013) and slightly acidic or neutral soils are best for GRSP accumulation, mainly by rhizosphere and various soil fungi. Relation between GRSP concentration and soil bulk density indicated negative correlation thus supporting in improvement of soil structure (Wang et al. 2014, Wu et al. 2011). Thus, both GRSP amount and its composition are crucial for variations in soil bulk density and electrical conductivity GRSP concentration decline with increase in soil depth. The concentration of GRSP was shown to be closely connected to soil pH, bulk density, EC, SOC, and total N, as well as close relationships between soil characteristics and various elements of GRSP composition.

#### **Carbon sequestration and Glomalin:**

Glomalin, itself acts as an important protein in sequestering carbon in soil and hence minimizing the greenhouse gas emission in the atmosphere. It can sequester nearly 27% carbon in soil as an important constituent of soil organic matter. Out of the total carbon storage in soil, glomalin can contribute higher amount of carbon as compared to humic acid (HA) (~ 8% carbon) and weighs 2-24 times more than HA (Nichols et al. 2002). The amount of organic carbon stored in the soil depends on GRSP content in the soil and it varies with soil depth. The qualities, characteristics and composition of glomalin strongly influence the dynamic soil properties and climate change. GRSP, on the other hand, was observed to mitigate nutrient and water loss when it was subjected to abiotic stress (Nichols, 2008). In mycorrhizal biology, the role of GRSP as cementing agent in controlling the soil organic carbon pool under open field settings has received a lot of attention (Zou et al. 2014). GRSP is one of the indicators of active nitrogen along with SOC (Rotter et al. 2017).

#### **Role of glomalin in Water-stable Aggregates (WSA) and aggregate stability:**

Hyphal studies on AM population revealed significantly higher percentage of water stable aggregates of sizes 2.00–4.00, 1.00–2.00, and 0.50–1.00 mm sizes in root + hyphae and root-free hyphae compartments as compared to those obtained under non-mycorrhizosphere, except with WSA at 0.25–0.50 mm size. Glomalin was observed to stabilize the soil macroaggregates by enhancing the release of GRSP and is influenced by the nature and properties of mycorrhizal hyphae. Mycorrhizal hyphae binds the soil particles by entrapping them and hence increases its stability (Zou et al. 2015). Similar results were also reported by Wu et al. (2014), where they observed significant increment in stability of water aggregates under AMF inoculated seedlings than non-AM ones in the rhizosphere of citrus genotype. Mycorrhizal inoculation significantly increased the Mean weight diameter by 89–134 or 78–81% in root/hyphae or rootfree hyphae chamber, respectively (Wu et al. 2014). Drought-

induced aggregate stability is more visible than salinity-induced aggregate stability (Wu et al. 2008; Kohler et al. 2009). Increased mycorrhizal fungi led to higher soil glomalin concentrations, and increased soil aggregate water stability.

### **Correlation analysis:**

Root AM colonization was positively correlated with total root length, hyphal length, EE-GRSP, DE-GRSP and T-GRSP fractions. Hyphal length in both root as well as root-free hyphae chamber positively correlated with all the GRSP fractions. Water stable aggregates of size 2.00–4.00, 0.50–1.00 and 0.25–0.50 mm fractions was found to positively correlate with root colonization, hyphal length, EE-GRSP, DE-GRSP and T-GRSP, except with EE-GRSP for 0.50–1.00 mm and DE-GRSP for 2.00–4.00 mm in both root/hyphae as well as root-free hyphae chamber (Wu et al. 2014). Soil organic carbon, available nitrogen and total nitrogen content (soil fertility-related properties) have positive effects on both GRSP concentration as well as its composition (functional groups, fluorescent substances and elements).

### **Conclusion:**

The nature and content of soil organic matter have a significant impact on the stability of the soil structure. Root and fungal hyphae are among the most essential biotic variables in soil aggregate stabilisation, if not the most critical. AMF play a key role in agroecosystems, including the extraradical mycelium's contribution to soil aggregation. Although the mechanisms underlying this pattern are unknown, GRSP has been demonstrated to be linked with soil organic carbon and aggregate water stability. GRSP pools are vulnerable to management techniques and a range of other impacts at the ecosystem scale, according to phenomenological field research and incipient, more mechanistic analyses. As a result, a better understanding of the factors that control GRSP production such as soil biota, soil physicochemical characteristics, and fungus–host plant species combinations) may eventually inform management strategies aimed at maximising soil aggregation and carbon sequestration.

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