Review Article

The Hidden Powers of Glomalin: Insights into Soil Health and Functionality

Abstract:

Agricultural practices such as the application of inorganic fertilizers and pesticides have profound effects on soil, altering its physical and chemical properties and consequently impacting soil biota composition and diversity. Since plant health is intricately linked to soil health, managing soil in a manner that conserves and enhances soil biota can significantly improve crop yield and quality. The rhizosphere, the zone directly influenced by plant roots, harbours high populations of active microorganisms and plays a vital role in plant-microbe interactions. One of the most crucial symbiotic relationships in the rhizosphere is between plants and arbuscular mycorrhizal fungi (AMF). AMF form mutualistic associations with the roots of various plant species, including major crops and contribute to defence against soil-borne pathogens, nutrient cycling and soil aggregation. Glomalin, a glycoprotein secreted by AMF, plays a crucial role in soil aggregation, stability and carbon sequestration. It enhances soil structure, binds with soil particles, stabilizes aggregates and promotes water infiltration and retention. It also plays a role in sequestering toxic metals, reducing their availability to plants and mitigating their harmful effects on soil biota. Managing glomalin in soil involves practices such as minimizing tillage to preserve the hyphal network, maintaining living roots through cover crops to sustain fungal colonization and optimizing nutrient inputs to support fungal activity without overloading the soil. AMF and glomalin play pivotal roles in agricultural sustainability and soil ecosystem functioning, highlighting the importance of conserving and enhancing these beneficial microbial associations for improved soil health and crop productivity.

Key words: Glomalin, Arbuscular Mycorrhizal Fungi, Soil Aggregation, Nutrient Cycling

Introduction

Agricultural practices such as adding inorganic fertilizers and pesticides can change the physical and chemical nature of the soil environment, there by altering the number of organisms and the ratio of different groups of organisms and the ratio of different groups of organisms. Since plant health is intimately linked to soil health managing the soil in ways that conserve and enhance the soil biota can improve crop yield and quality (Napoli, 2008). The rhizosphere may be defined as the “heart of the soil” as it is the zone under the direct influence of plant roots and with high populations of active micro-

organisms. In the rhizosphere, plant roots influence microbial communities by depositing photosynthates into the rhizosphere and organisms govern plant growth and development (Dhillion and Gardsiord, 2004). The association between plant and arbuscular mycorrhizal fungi (AMF) is one of the most important symbioses on earth, linking the root and the soil system.

AMF are ubiquitous, root-symbiotic fungi and generally mutualistic association with roots of higher plants including major production crop species and pasture plant species. "Mycorrhizae" word is derived from Greek word mycos means fungus, rhiza means root i.e., fungus root. AMF important in defense against soil-borne pathogen and involved in processes such as nutrient cycling and soil aggregation.

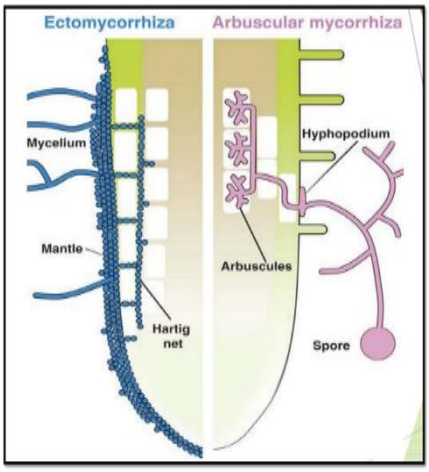

Fig 1. Root-symbiotic fungi

AMF exist in two different phases:

1. Endomycorrhiza (inside root)
2. Ectomycorrhiza (in soil)

- Ectomycorrhiza: A symbiotic relationship between fungi and plants where the fungal hyphae do not penetrate the cortical cell of the plant root.
- Endomycorrhiza: A symbiotic relationship between fungi and plants where the fungal hyphae penetrate the cortical cell of the plant root. Intraradical mycelium consists of hyphae and other fungal structures such as arbuscules and vesicles.

1. Arbuscules: Fungi forms shrub shaped structure inside the root cortical cell by branching several very thin hyphae. This is where the exchange of nutrients and carbon happens between the host plant and the fungus.
2. Vesicles: Blender like structure forms inside the root and site of lipid storage for the fungus.
3. Hyphae: root-like structure that grow outside the root, in long distances to explore the soil for nutrient uptake.

AMF form hyphal networks that can contain over 100m of hyphae per cubic centimeter of soil and are important for nutrient uptake and soil aggregation. Among the different species most dominated species is Glomus and the reason behind it may be the Glomus species can propagate from both spores and broken pieces of hyphae. The tremendous advances in research on mycorrhizal physiology and ecology have led to a greater understanding of the multiple roles of AMF in the ecosystem and the important contribution is glue-like protein to improves soil structure that glue-like structure is "**Glomalin**".

What is Glomalin?

Glomalin is a fungal protein or protein class or group of proteins that is operationally quantified from soil as glomalin related soil protein. It was thought to be exuded by the living fungus found that this glomalin is only released by an AMF into the soil environment during hyphal turnover and after the death of the fungus. The fact that the link between glomalin and these soil protein fractions is not yet clearly established.

How does glomalin work?

Glomalin that gives soil its tilth a subtle texture that enables experienced farmers and gardeners to judge great soil by feeling the smooth granules as they flow through their fingers.

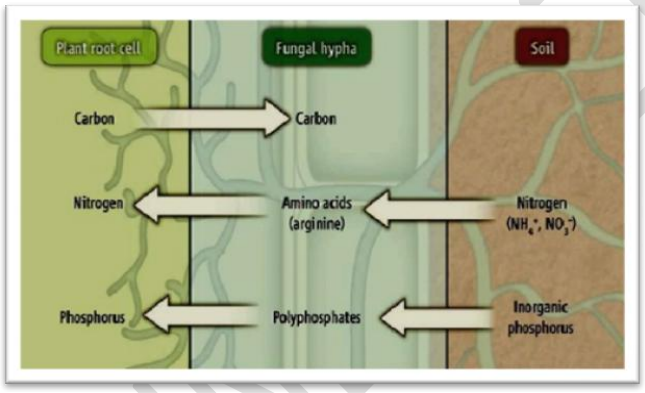

Fig 2. Principle of glomalin

In AMF-plant symbioses, AMF translocate nutrients from soil to plant through the extraradical mycelium and return the plant supplies AMF with carbon in the form of photosynthates about 5-85% of carbon depending on the plant species (Brundrett, 2004). Apart from nutrient uptake the extraradical mycelium also is involved in spore formation and initiation of root colonization. The major benefit for plants when being mycorrhizal is an increase in plant nutrient uptake from the soil(Cappellazzo *et al*. 2008). As glomalin is produced from the hyphae, when hyphae stop transporting nutrient their protective glomalinsloughs off into the soil i.e., it attaches the particles of minerals and organic matter forming a clod.

This type of soil structure is stable enough to resist wind and water erosion but porous enough to letair, water and roots move through it.

History:

This glomalin is discovered in 1996 by united states department of agriculture, Agricultural Research Service soil scientist Sara F. Wright. This soil super glue was mistaken for an unidentifiable constituent of soil organic matter. Rather, it permeates organic matter, binding it to silt, sand and clay particles and its concentration in soil is strongly positively correlated with the water-stability of soil aggregates. In 1998, Wright and her co-worker used different citric acid buffers to extract the glomalin from the soil and based on solubility divided into

i. Easily extractable
ii. Total extractable

Origin of glomalin: The study of glomalin began with the discovery of a monoclonal antibody (Mab32B11) that can be immunologically reactive on the surfaces of spores of *Glomus intraradices.* It is originated from intraradical hyphae in roots and the surface of extraradical hyphae in the rhizosphere and can be released from mycelial surface into soil. It seems that glomalin is believed to be the only protein directly secreted by AMF into soil (Rillig *et al*, 1999).

Table 1: Current terminologies for glomalin and their definitions

Terminology	Description
Glomalin	A yet to be identified putative gene product of arbuscular mycorrhizal fungi
Glomalin-related soil protein (GRSP)	Total soil glomalin fraction, possibly contains other soil protein; fraction of soil glomalin extracted repeatedly using 50 mM sodium citrate solution (pH 8) and autoclaving at 121 °C for 60 min until glomalin extract is straw-colored
Easily extractable glomalin-related soil protein (EE-GRSP)	Fraction of soil glomalin extracted once using 20 mM sodium citrate solution (pH 7) and autoclaving at 121 °C for 30 min Bradford-reactive soil protein (BRSP)
Bradford-reactive soil protein (BRSP)	Glomalin-related soil protein quantified using the Bradford assay; measures all protein in glomalin extract
Easily extractable Bradford-reactive soil protein (EE-BRSP)	Easily extractable glomalin-related soil protein quantified using the Bradford assay; measures all protein in glomalin extract
Immunoreactive soil protein	Glomalin-related soil protein quantified using an indirect enzymelinked immunosorbent assay (ELISA) with monoclonal antibody MAb32B11,

(IRSP)	specific for glomalin, though may cross-react with other soil protein
Easily extractable immunoreactive soil protein (EE-IRSP)	Easily extractable glomalin-related soil protein quantified using an indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody MAb32B11

Evidence that GRSP is (at least partly) of AMF Origin:

There are hence several significant problems in glomalin research; it should be made clear, though, that none of these problems pertain to the operational definition of GRSP per se, but to the link between GRSP and arbuscular mycorrhizal fungi.

There is increasing circumstantial evidence accumulating from decomposition studies that GRSP is of AMF origin. When AMF growth is eliminated, e.g., by incubating soil without host plants, we have observed that GRSP concentrations decline, along with AMF hyphae (Steinberg and Rillig 2003). This design exploited the fact that AMF are obligate biotrophs, whereas most other soil fungi are not. In fact, hyphal lengths of other (saprobic) fungi increased during the course of this study (Steinberg and Rillig 2003), as likely had bacterial biomass. A similar decline in GRSP concentrations was observed after >400 d incubations of soils from three different land use types (forest, afforested area and agricultural land) (Rillig *et al*. 2003). In long-term grassland plots from which AMF were eliminated by a fungicide (hence essentially shutting down glomalin production), GRSP concentrations (BRSP and IRSP) were drastically decreased (Rillig *et al*., 2003). While these data from decomposition studies do not conclusively show that there are no other cross-reactive materials in soil, a link is established between AMF and GRSP. This link is strong, as discussed above, and persists in the presence of the activity of other, non-biotrophic soil biota (which could produce potentially cross-reactive material).

The fact that in detecting IRSP (and EE-IRSP) a monoclonal antibody is used (rather than a polyclonal) also adds to the confidence that these GRSP fractions are of AMF origin, since MAb32B11 would not be expected to cross-react with as many other epitopes as a polyclonal antibody (unless it is against a relatively common structure or there are problems with non-specific binding). Specifically, the antibody has reacted strongly with all tested AMF species to date (Wright and Upadhyaya 1996; Rillig 2004), while exhibiting only negligible cross-reactivity with other soil fungal isolates. Reaction with the antibodyis likely not an artifact of the harsh extraction conditions (e.g., autoclaving), since MAb32B11 can also be used to visualize material on spores and hyphae of AMF in situ (as well as in soil) (Wright and Upadhyaya 1996).

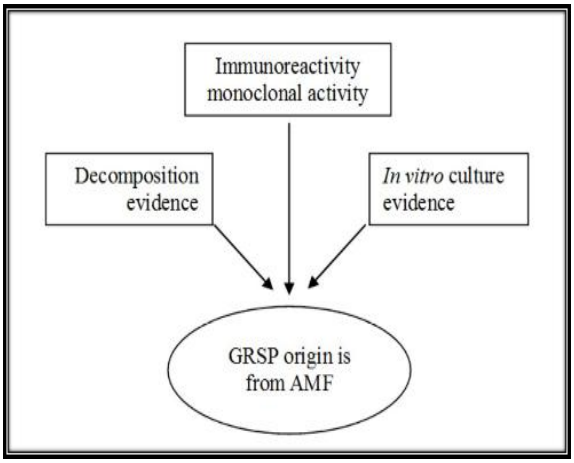

Fig 3. Immunoreactivity activity

Finally, IRSP production was also observed under soilfree, sterile in vitro conditions using transformed root organ cultures (in this case IRSP probably approaches glomalin; (Rillig and Steinberg 2002). In this experiment, IRSP was measured in the hypha-only compartment of split plate cultures, in which roots are pruned back from a barrier that is crossed by AMF hyphae. In subsequent experiments, they have also observed that IRSP accumulates in the liquid culture medium over time (Driver, Holben and Rillig, 2005). While these observations do not exclude the possibility that there are sources of cross-reactivity in soil, these measurements clearly establish that AMF produce IRSP. In particular, these experiments exclude hypha-associated bacteria as likely sources of GRSP.

Structure and composition:

Glomalin is a stable and persistent glycoprotein is released by hyphae and spore AMF in the taxonGlomales. It is resistant to trypsin and chemical hydrolysis. It contains iron appear to have N-linked oligosaccharides and are insoluble and possibly hydrophobic in its native state and resistant to heat degradation because it is a glue like in nature and binds with surface of spores(Wright and Upadhyaya, 1998).

The structure of glomalin still not biochemically defined as it is an N-linked glycoprotein it consists

- N = 3-5%
- C = 36-59%
- H = 4-6%
- O = 33-49%
- P = 0.03-0.1%
- Fe = 0.8-8.8%

Identification:

The glomalin identification is based on solubility characteristics

1. Easily extractable glomalin
2. Total glomalin
3. A “scum” (scum is apparently a sloughed component of glomalin)

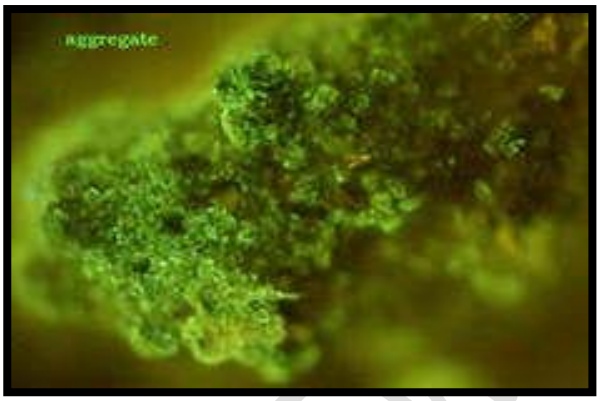

Fig 4 & 5. Identification of glomalin

Soil organic matter, metals (such as iron), clay minerals and other substances may bind to glomalin causing conformational changes and it is in dark red-brown color and soil after extraction losses the brown color and soil after extraction loses the brown color associated with oragnic matter. The unusal extraction remove high quantities of the rich organic material (i.e., glomalin) leaving soil a mineral grey color. Where as a laboratory procedure reveals glomalin on soil aggregates as the green material (Wright and Upadhyaya 1996).

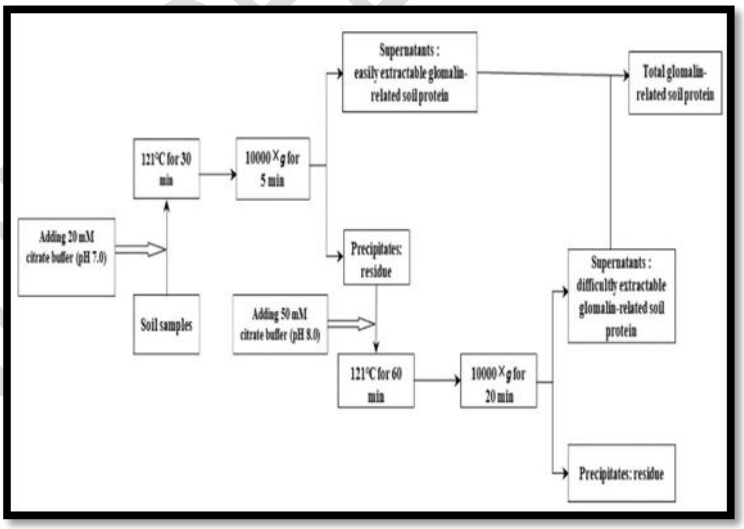

Fig 6:The extraction protocol of different glomalin-related soil protein fractions in soils

The present protocol of glomalin extraction is based on Wright given. For easy understanding Koide and people (2013) divided GRSP into fraction 1(corresponding to easily extractable) and fraction 2 (older glomalin and more difficult to extract). Wu (2014) proposed that fraction 1 as EE-GRSP (easily extractable glomalin related soil protein) and fraction 2 as DE-GRSP (difficultly extractable glomalin related soil protein).

One gram soil sample was mixed with 8mL of 20mmol/L citrate (pH=7.0) for the analysis of EE-GRSP which is incubated at 121°C and 0.11Mpa for 30min and then centrifuged at 10000 Xg for 5min. the supernatant we get that is EE-GRSP. The precipitate at the bottom is residue further used by adding 10mL of 50mmol/L citrate (pH=8.0) which incubated at 121°C for 60min and centrifuged at 10000 Xg for 20min. the supernatant is DE-GRSP. And sum of these EE-GRSP and DE-GRSP is T-GRSP (total glomalin and more difficult to extract) (Fokom *et al*. 2012).

Note: GRSP is extracted from field soil, root, mesh or pot culture media.

Quantification:

The extracted glomalin is used for the quantification with two different methods

1. Bradford protein analysis method
2. ELISA method

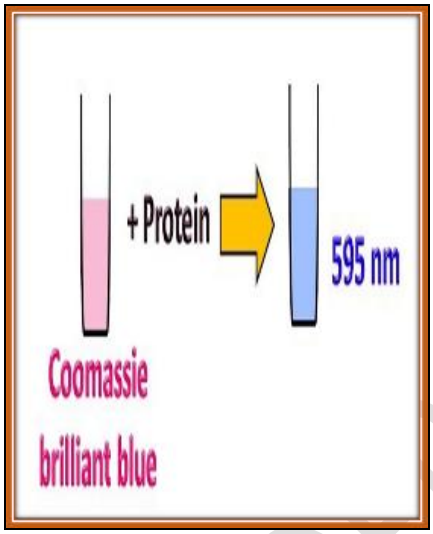

Fig 7. Quantification with two different methods

1) Bradford protein analysis method: Glomalin is defined by the method employed to quantify it (Wright and Upadhyaya 1996; Rillig 2004). Bradford protein analysis is a common method for protein quantification (Bradford, 1976). The Bradford assay is based on the principle that a dye (Coomassie Brilliant Blue G-250) binds with proteins and changes the dye color from red to blue (Bradford 1976; Steinberg and Rillig 2003). The degree of color change, read by a spectrophotometer at a wavelength of 590 nm (A590) as optical density, can be related to protein concentration in a glomalin extract using a standard of known concentration of protein. The standard is prepared in a range of 1.25 to 5 µg bovine serum albumin (BSA) in phosphate-buffered saline (PBS). The equation of the regression line generated by plotting optical density against BSA values is then used to calculate protein concentration in glomalin extracts as Bradford-reactive soil protein (BRSP) for total glomalin (TG) and easily extractable

Bradford-reactive soil protein (EE-BRSP) for the easily extractable glomalin (EEG) fraction (Rillig, 2004).

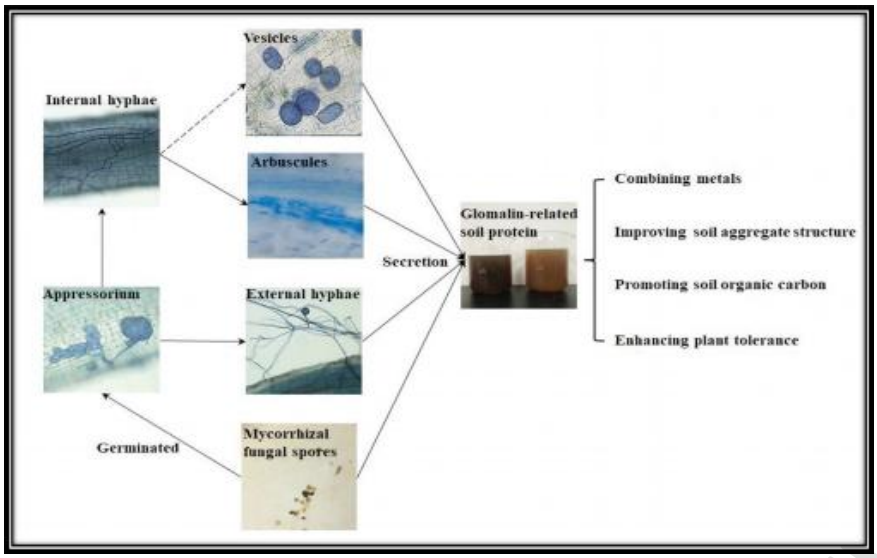

Fig 8. Bradford protein analysis method

2) ELISA (**Enzyme-linked immunosorbent assay**): Also known as enzyme immunoassay. It is a biochemical technique to detect and quantify the specific compound like antigen, antibody and hormone. The amount of compound in the sample to the amount substrate converted to colored product by enzyme-linked antibody. It is a quick and sensitive method to quantifying large number of samples in parallel. Glomalin content in samples was determined by indirect ELISA using the monoclonal antibody Mab32B11 produced against spores of *Glomus intraradices*. TG (total glomalin) quantified using ELISA is regarded as immunoreactive soil protein (IRSP) and the easily extractable fraction is named easily extractable immunoreactive soil protein (EE-IRSP) (Wright and Upadhyaya 1998; Rillig, 2004). The extraction procedure does not eliminate other soil proteins. EE-BRSP and T-IRSP were positively correlated with soil available N and P (Wu *et al*. 2014). Monoclonal antibodies have proved to be highly specific in their ability to differentiate between fungal isolates.

Potential functions of glomalin:

The spores of AMF in the soil germinate, extend and finally form an appressosium to contact with root epidermal cells of host plants. These colonized hyphae continue in depending cortical cells of roots, where arbuscles are formed. The internal hyphae of roots extend outward to form external hyphae on roots which reside on the root surface for absorbing nutrients and water from the soil (Wright *et al*. 1999). Arbuscles, vesicles, external hyphae and spores involved in the secreation of GRSP which performs functions like,

1. Combing with the heavy metals and preventing plants to uptake in toxic level
2. It improves the soil aggregate structure

3. It involved in promoting soil organic carbon
4. It enhancing plant tolerance

1) GRSP functioning of soil organic carbon:

The carbon (C) stored in GRSP is considered highly recalcitrant in nature, lasting for a minimum of 12–22 years (Zou *et al*., 2016), and plays an active role in the development of soil structure (Chi *et al*. 2018). AMF are reported to consume 4–20% of the photosynthates synthesized by host plants. In return, C from mycorrhizas is deposited into the soil via extraradical hyphae as sink for C storage, where mycorrhizal contribution towards organic C accumulated into soil ecosystem involved approximately 54–900 kg hm^{-2} (Zhu and Miller, 2003). Collectively, AMF hyphae and AMF-released GRSP are import components of soil organic carbon (SOC) pools, as evidenced from the positive correlation between GRSP and SOC (Wright and Upadhyaya 1998; Rillig *et al*., 2003). GRSP acts as the most important source of C towards SOC build-up (Kumar *et al*., 2018). The C content of GRSP has been observed to be 2–24 times higher than that of soil humus, accounting for as high as 27% of SOC (Rillig *et al*., 1999). In the oldest soil (oxidized soil, > 4 million years), C in purified T-GRSP accounted for 4% of total C, while the C contribution of T-GRSP was substantially higher than the microbial biomass C. In the peat soil, purified T-GRSP accounted for as high as 52% of the total SOC, and a large amount of GRSP gradually transformed to an active soil C source. Zhang *et al*. (2017) reported that purified T-GRSP extracted from highly saline soils contained relatively high C content (43.41%).

2) GRSP functioning on soil aggregate stability

Aggregation processes in soil are influenced by many factors such as changes in soil organic matter (SOM), moisture content and microbial activity, crop type root development, tillage, and fertilization. The aggregate as a "naturally occurring cluster or group of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent aggregates". The structural stability is dependent on particle size distribution, soil organic matter, vegetation and soil micro-organisms and its stability is influenced by exchangeable cations. One of the most important binding agents for forming stable aggregates is soil organic matter (glomalin). Organic materials are important soil additives to improve soil physical properties. Aggregate formation is a complex process of physical and chemical interactions (Preger *et al*. 2007). Soil aggregates results from a combination of primary mineral particles with organic and inorganic materials. This process, dynamic and complex, is influenced in turn by the interaction of several factors including environmental components, soil management, plant effects but largely by soil properties. Soil structure is often expressed as the degree of stability of aggregates being a major factor which moderates physical, chemical, and biological processes leading the soil dynamics(Harner *et al*. 2004). All the major factors playing a role in aggregate formation and stabilization, the following factors influenced soil aggregation,

(1) soil fauna

(2) soil microorganisms

(3) roots

(4) inorganic binding agent (like glomalin)

(5) environmental variables

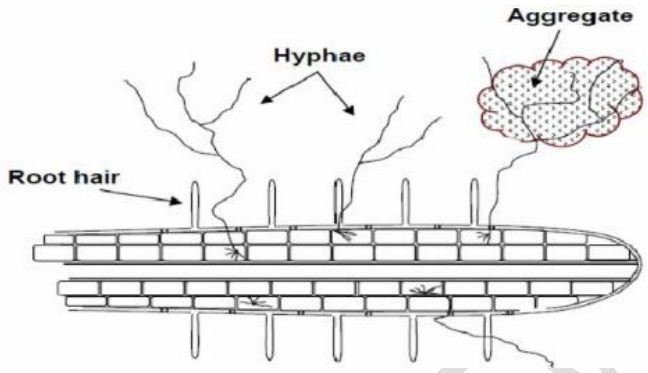

Fig. 9: Hyphae of AMF form a frame for soil particles to collect into aggregates which are coated with glomalin

Soil aggregates are a conglomeration of soil minerals (clay particles, fine sand and silt) small plant or microbial debris, bacteria, organic matter strongly associated with clay coatings Glomalin contributes to the stabilization of aggregates by sloughing off hyphae onto the surrounding organic matter, binding to clays (via cation bridging by iron), and providing a hydrophobic coating (Harner *et al*. 2004). This is demonstrated in several experiments, where total and, especially, immunoreactive concentration of glomalin are positively correlated with percent water-stable soil aggregates in both agricultural and native soils. Hyphae act as a frame upon which soil particles collect while glomalin glues them together and protects them. This is like walls in a house, where boards (i.e., hyphae) are used to frame-up the wall, insulation (i.e., soil particles) fills in spaces between boards, wall board (i.e., microbial glues, like glomalin and fungal and bacterial polysaccharides) help keep everything in place, and finally it is all coated with a protective layer of paint (i.e., glomalin). Sticking soil particles together (i.e., aggregate formation) is just one part of the process and one role for glomalin and other microbial polysaccharides (Preger *et al*. 2007). Glomalin is an important molecule in aggregate stabilization. When aggregates are not stabilized, they break apart with rainfall. Organic matter and nutrients within disrupted aggregates may be lost to rain and wind erosion. The chemistry of glomalin makes it an ideal stabilizing coat.

They increase the contact angle for water penetration, which restricts infiltration and slaking, lowers wet ability, and increases the internal cohesion of aggregates. The Soil aggregates most important strategies proposed for maintaining and improving soil fertility are those which target the physical

properties of the soil. The abundance and stability of the aggregates are critical for several soil functions- Maintaining soil porosity, which provides aeration and water infiltration rates favorable for plant and microbial growth, increasing stability against wind and water erosion, and storing carbon by protecting organic matter from microbial decomposition (Harner *et al*. 2004).

3) GRSP Relationship with Soil Aggregate Water Stability:

It is important to appreciate that the relationship between glomalin related soil aggregation (GRSP) and soil aggregate water stability is a large range of water stabilities. This means that beyond a certain "saturation" GRSP concentration in each soil, additional deposition of GRSP will not result in detectable increases in soil aggregate water stability, at least as measured with the conventional disintegrating forces (Preger *et al*. 2007). The curvilinear pattern of aggregation of soils with high GRSP concentrations saturated with GRSP, perhaps because most pores in these macro-aggregates have already been partially "sealed" by deposition of this substance, slowing down penetration of water into the aggregate. This relationship of GRSP with soil aggregate water stability applies only to hierarchically structured soils, in which organic material is the main binding agent. In a soil in which carbonates are the main binding agent, none of the GRSP fractions are positively correlated with aggregate stability (Harner *et al*. 2004).

4) GRSP functioning on soil toxic elements:

Toxic metal accumulates in the soil which negatively affects plant growth and crop yield and even harms human health through enlarged food chain. AMF reduce the toxicity of toxic metals to plants by converting accumulating and transferring them. GRSP have the buffer capacity on toxic metal release to protect the host plant and also have high binding capacity for certain toxic metals (Cu, Cd and Pd). GRSP can stabilise toxic metals and reduce the availability of toxic metals there by reducing the impact of toxic metals on other soil micro-organisms and plants. Soil organic matter (SOM) can bind potentially toxic elements to alleviate metal toxicity and control metal behaviour in soils. Fulvic acid (FA) and humic acid (HA) make up an important part of soil organic matter, and their binding capacity influences heavy metal behaviour in soil. Besides fulvic and humic acids, humin (HM), an insoluble component of SOM, may also significantly contribute to the metal binding phenomenon seen with SOM. However, less information is available on the contribution of metal-bound GRSP in SOM fractions and the effect of metal-binding on the decomposition of glomalin. Gonzalez-Chavez *et al*. (2004) found that GRSP can stabilize metals such as Pb, Cd, Zn, Cu, Fe and Mn in order to remediate polluted soil.

5) Exogenous GRSP application in crops:

Exogenous GRSP functions in enhancing soil aggregate stability and soil phosphatase activity. Soil tillage heavily disrupts mycorrhizal hyphal network, which had an adverse effect on GRSP production, subsequently weakening the GRSP functioning on aggregate stabilization. Exogenous EE-GRSP had a positive effect on enhancing drought tolerance, due to glomalin behaving as a humic-like substance to stimulate plant growth, as a homologous substance with Hsp60 like a stress moderator, as a hydrophobic layer on fungal hyphae to prevent loss of water, and as a Fe-contained substance to increase plant Fe-SOD activity (Filho *et al*. 2002). In short, exogenous EE-GRSP seems to be used as an effective regulator in plants and soils to affect soil fertility, soil structure, plant growth and plant tolerance. More studies need to be performed to confirm the roles of exogenous EE-GRSP on field crops, besides citrus plants.

Advantages of glomalin:

1. Protect hyphae from nutrient loss
2. Glue soil aggregate and stabilizes it
3. Reduces wind and water erosion
4. Increases water infiltration
5. Increases water retention near roots
6. Improves root penetration by reducing compaction

Management of glomalin in soil:

Soil management to increase aggregation must aim at increasing primary plant production, increasing the amount of C input into the soil, decreasing disturbances and decreasing the rate of C loss by processes such as decomposition and erosion. In this regard, improved management practices include tillage methods, residue management, amendments, soil fertility management and nutrient cycling

1. **Minimum or no-till to reduce disruption of hyphal network**: Use no-till management practices to allow AMF to grow during the cropping season. Tillage disrupts the hyphal network that produces glomalin. Disruption of the hyphal network also decreases the number of spores and hyphae to start the process again on the next crop.
2. **Cover crops to maintain living roots**: Use cover crops to maintain living roots for the fungi to colonize
3. **Reduced inputs, minimum Phosphorus**: Maintain adequate phosphorus level for crops, but does not over-apply P because high levels depress the activity of these fungi.

Conclusion:

AMF play important role in agroecosystem including the involvement of the extraradical mycelium in providing soil aggregation. GRSP has be shown to be correlated with soil aggregate water stability,

although the mechanisms underlying this pattern are not yet understood and it can absorb toxic metals in the soil to decrease the damage of toxic metals on plant. Even though GRSP are beneficial still there is a need to analyse the structure and constituents of glomalin.

Conflict of Interest for Research Paper Submission

- Financial Interests: I don't have any research funding or financial support related to the subject matter of this research.
- Professional Affiliations: I am affiliated with [Name of Organization/Institution] where I hold a position that could be affected by the results or conclusions of this research. This affiliation may influence my perspective or interpretation of the data presented in the paper.
- Personal Relationships: I have personal relationships with individuals involved in this research, such as collaborators, colleagues, or family members, which could potentially introduce bias or influence in the research process.
- I want to assure you that I have made every effort to conduct this research with integrity and objectivity, adhering to ethical standards and guidelines. I have disclosed these potential conflicts of interest to ensure transparency and to allow reviewers and readers to assess the research findings objectively.
- Please let me know if you require any further information or clarification regarding these conflicts of interest. I appreciate your attention to this matter and look forward to your guidance on how to proceed with the submission process.

Data availability Statement

This review article draws from a wide array of current and seminal sources, ensuring comprehensive coverage and up-to-date insights on the subject matter.

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