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Glomalin – an interesting protein part of the soil organic matter

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Abstract: The negative effects of the current agricultural practices include erosion, acidification, loss of soil organic matter (dehumification), loss of soil structure, soil contamination by risky elements, reduction of biological diversity and land use for non-agricultural purposes. All these effects are a huge risk to the further development of soil quality from an agronomic point of view and its resilience to projected climate change. Organic matter has a crucial role in it. Relatively significant correlations with the quality or the health of soil parameters and the soil organic matter or some fraction of the soil organic matter have been found. In particular, C_{tot}, C_{ox}, humic and fulvic acids, the C/N ratio, and glomalin. Our work was focused on glomalin, a glycoprotein produced by the hyphae and spores of arbuscular mycorrhizal fungi (AMF), which we classify as Glomeromycota. Arbuscular mycorrhiza, and its molecular pathways, is not a well understood phenomenon. It appears that many proteins are involved in the arbuscular mycorrhiza from which glomalin is probably one of the most significant. This protein is also responsible for the unique chemical and physical properties of soils and has an ecological and economical relevance in this sense and it is a real product of the mycorrhiza. Glomalin is very resistant to destruction (recalcitrant) and difficult to dissolve in water. Its extraction requires specific conditions: high temperature (121°C) and a citrate buffer with a neutral or alkaline pH. Due to these properties, glomalin (or its fractions) are very stable compounds that protect the soil aggregate surface. In this review, the actual literature has been researched and the importance of glomalin is discussed.

Keywords: arbuscular mycorrhiza; fungus; heat-shock protein; soil; water retention

Arbuscular mycorrhizal fungi (AMF) what is it?

Arbuscular mycorrhiza (AM) is currently considered to be of key importance for the functioning of the majority of the current terrestrial ecosystems (e.g., moor lands or boreal forests), (Read 1991). It can now be found in 80% (Smith & Read 2008), respectively 70–90% (Błaszczowski 2012) of tracheary plants (Tracheophyta species). A typical feature of arbuscular mycorrhiza is its absolutely unique structures inside the roots as well as outside it (the so-called arbuscula and vesicle). This highly specialised symbiosis was

known earlier as vesicular-arbuscular mycorrhiza (i.e., VAM). This type of a so-called endomycorrhizas means that the fungus penetrates inside the root cells of tracheary plants where it forms a tree-like structure (arbusculus) outside the root and then it forms storage organs in the shape of a bag (so-called vesicles) inside the root cells. Within the symbiosis, the AMF “invests” less available elements from the soil to the plant (prevailing phosphorus) and, vice versa, the plant provides organic substances from its own photosynthesis (carbon). The hyphae of the arbusculi colonise or intergrow through the root of

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the host plant and, according to modern studies, they can increase the surface of the root hairs by up to 80% (Millner & Wright 2002). Thanks to this, the plant reaches more distant sources of nutrition and water and it can, thus, use elements hard to obtain in soil (the previously mentioned phosphorus). Harris and Paul (1987) estimate that 40–50% of the carbon captured by photosynthesis is transferred to the AMF; a conservative estimate states “only” the values of 10–20% (Jakobsen et al. 2002). The higher the nutritional demand of the plant, the higher the carbon amount must be delivered to the plants by the AMF. For example, Treseder and Allen (2000) state that up to 85% of the carbon may be transferred to the AMF, where a large part of this carbon is then used to form glomalin (Treseder & Turner 2007). Wright et al. (1999) compared the glomalin production on permanent growths, pastures and plough lands. A lower glomalin concentration was measured in soils which have been cultivated and agriculturally used, which is probably highly related with the soil aeration by agricultural technologies and the organic matter mineralisation. Another parameter which is relatively interestingly affects the glomalin concentration in the agricultural systems, with respect to the AMF representation, is the cropping patterns (Wright & Anderson 2000). The individual field crops significantly differ according to their closeness depending on the AMF. According to Plenchette (1983), the following field crops currently grown that are strongly dependent on the AMF are: corn (*Zea mays*) and flax (*Linum usitatissimum*); legumes (Leguminosae), beans (Fabaceae) and potato (*Solanum tuberosum*) have benefits from mycorrhiza. Wheat (*Triticum* spp.), oats (*Avena sativa*) and barley (*Hordeum* spp.) have a benefit from the mycorrhiza, but are not dependent on it. Polygonaceae, Amaranthaceae and Brassicaceae as some of the few that do not form a symbiosis with the AMF (Harley & Smith 1983). Rape (*Brassica napus*), mustard (*Brassica juncea*), sugar beet (*Beta vulgaris*), buckwheat (*Fagopyrum esculentum*) or spinach (*Spinacea oleracea*). would be from our better-known crops that don't have this symbiosis. The host specificity of the AMF is very low (Smith & Read 2008). The plants are very often colonised by mixtures of AMF within the same root (Helgason et al. 1999). But, there are favourable and less favourable known combinations of plant-fungus symbionts.

In general, this is not a new symbiosis; some fossil records of Glomeromycota arbusculas show it played an important role in terrestrial ecosystems even

250 to 400 million years ago (Harper et al. 2013). These records, thus, indicate that Glomeromycotas were already present at the earliest stadiums of terrestrial ecosystem colonisation, which confirms the hypothesis that they could support this process (Blackwell 2000).

AMF can, therefore, provide a differential advantage within an ecosystem (van der Heijden et al. 1998). In addition to making nutrition available, the soil-plant system also draws upon some other benefits. The symbiosis is interesting for plants which are situated in an environment containing heavy metals. The hyphae of fungi are able to accumulate these toxic elements (Cu, Pb, Zn etc.) in their bodies and, thereby, protect the root of the host plant (Chern et al. 2007; Vodnik et al. 2008). Heavy metals are bound by carboxyl groups of pectic compounds (hemicelluloses) that fill the space between the fungus and the plant cell (Gałązka & Gawryjolek 2015). According to Cornejo et al. (2008) and Chern et al. (2007) 1 g of glomalin is able to bind 4.8 mg of Cu and 188 mg of Pb. In addition to heavy metals, the accumulation was also observed in organic pollutants, e.g., phenanthrene (Gao et al. 2017). Thanks to the AMF, some other soil properties may even be improved:

- The content of water-stable aggregates (WSA) is growing: the correlation with the glomalin content – according to the fraction of the extracted glomalin, the soil and the manner of the management, there is a correlation at the range of $r = 0.03\text{--}0.92$ (Wright & Anderson 2000; Harner et al. 2004), but the relationship between the glomalin and the content of WSA is curved (Wright & Upadhyaya 1998). It means, above a specific saturation level of glomalin, additional deposition of glomalin will not cause an increase in the WSA. For low levels of glomalin (and WSA), the relationship seems to be linear. This relationship (glomalin and WSA) refers only to the soils where the organic material is the main binder. In soils where carbonates are the major binder (e.g., Calcisol), none of the glomalin fractions positively correlates with the WSA (Rillig et al. 2003),
- the growth of the micro-organisms is stimulated (by the root exudate) which are antagonistic to the present pathogens (Phytophthora, Pythium, Rhizoctonia or nematode) (Borowicz 2001; Gałązka & Gawryjolek 2015),
- during the acidification of the forest ecosystems, the increased plant resistance to acidity and phytotoxic levels of aluminium (Seguel et al. 2013),

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- it is a reservoir of soil carbon and nitrogen (Nichols & Wright 2004),
- increases the plant resistance to abiotic and biotic stress (Augé 2004; Whipps 2004) including drought,
- influence on the osmotic potential: we can use recycled/salted water for irrigation, (Gómez-Bellot et al. 2015).

On the other hand, people can affect the AMF activity:

- By balanced crop rotation which supports the high species diversity of the AMF (compared to monocultures). The higher content of the AMF was also observed in organic (ecological) farming systems compared to monocultures (Gałązka et al. 2017).
- Or by the site preparation: a strong positive correlation has been found, for example, between the glomalin (in many fractions) and direct sowing or reduced/conservation tillage. Even though the conventional tillage method stimulates microbial activity, this stimulation is the result of the disintegration of the soil aggregates and respiration in the decomposition of the organic matter (Gałązka et al. 2017, 2018).

Smith et al. (2011) showed that the effects of AMF are less pronounced in soil with high or excessive phosphorus content (most acidic soils, and soils containing free calcium carbonate – i.e., chernozems, or some coluvic soils and regosols). The current phosphorus fertilisation recommendations are very inaccurate. Most phosphorus-tests only test for the acceptable P in the soil mineral fraction and ignore the phosphorus that is potentially available from the organic soil component and the soil mycorrhizal potential.

Relationship of the AMF and glomalin

There are several unclarities regarding the glomalin–AMF relationship. It is a generally recognised fact that glomalin is a metabolite of arbuscular mycorrhiza, however, there is no direct evidence of this statement. It is not possible to completely exclude the possibility that there are other cross-reacting substances or bindings affecting its behaviour in the relatively complex medium which the soil definitely is (Rillig 2004). The hypothesis of the AMF and glomalin relationship is more or less based on experiments with samples of the root colonised by the AM and samples without the colonisation by fungi. Glomalin was only detected on roots infected by the fungi. When the growth of the AMF is eliminated (e.g., by

soil incubation without the host plants), the glomalin concentration decreases together with the concentration of the AMF hyphae (Steinberg & Rillig 2003). There are further unclarities concerning the impulse which would initiate the production of glomalin by the AM hyphae (Rillig et al. 2001). The glomalin physiological function is not well known although Gadkar and Rillig (2006) found evidence that glomalin may relate to a heat shock protein (HSP).

Another unknown factor is also the route through which glomalin is deposited in the soil. There are two theories:

- (1) The glomalin forms a permanent part of the AM hypha and it only gets to soil after the fungus dies. In this case, the glomalin is an important substance for the functioning of the AM and its effect on the soil is negligible compared to it (Driver et al. 2005). The author states that 80% of the glomalin was contained in the hyphae.
- (2) The release of glomalin as metabolite or secretion by an AM hypha. In this case, we can expect a certain mobility of this protein in the soil, on the other hand, it can be more easily degraded by the soil micro flora (St-Arnaud et al. 1996).

Despite the fact that glomalin is highly probable to be a product of AMF, Lutgen et al. (2003) proved that its concentration is not proportionate to the length of the fungus hyphae. Steinberg and Rillig (2003) performed a laboratory incubation for the period of 150 days, and the glomalin content increased by 25%, and in the case of the AM, the length of the hyphae increased by 60%, i.e., it was significantly higher. The authors stated in the same paper that seasonal fluctuations of the glomalin concentrations are negligible. Several papers also focused on the AMF colonisations and glomalin concentrations in relation to the photosynthesis of the host plant. The majority of scientists agrees that the plants respond to the increasing concentrations of CO₂ by a higher biomass production, but the effect of the edaphons is still unclear. Kasurinen et al. (1999) state that the higher concentrations of CO₂ in the environment also cause the growth of the AMF activity and the glomalin concentration. However, e.g., the results of Jones et al. (1998) indicate that the higher atmospheric concentrations of CO₂ will have a relatively high effect on the entire soil nutritional chain.

Glomalin or another name?

A compound named as glomalin was described for the first time by Sara Wright during the study of AMF

in 1996. It was a glycoprotein produced abundantly on the hyphae and spores of an AMF. One of the reasons for its relatively late discovery were the quite specific characteristics of glomalin: it is hydrophobic, thermally stable (the extraction is carried out in an autoclave at 121 °C) and recalcitrant (resistant to decomposition). Some papers (e.g., Gadkar & Rillig 2006) indicate that it could be an HSP60 homologue (heat shock protein 60).

Rillig et al. (2001) estimated (based on the analysis of C_{14}) an average glomalin turnover in the environment to be between 6 and 42 years. One of possible explanations for such a high difference may be that the AMF contains two functionally completely different locations: the roots and the soil. This may also be one of the reasons why it is difficult to assess the AMF flows or its persistence in the environment, as recognised by Miller and Kling (2000) or other authors (Steinberg & Rillig 2003). A similar turnover time as the one shown by Rillig et al. (2001) can be found in the combination of organic carbon with a clay fraction (an organo-mineral complex), which may indicate that the glomalin in the soil is protected from degradation by the bond to the clay minerals, although the concentration with clay is relatively low. Glomalin can contain: 28–45% of carbon, 0.9–7.3% of nitrogen, 0.03–0.1% of phosphorus and it may also contain ions of metals in some soils (Huang et al. 2011). It represents almost one third of the soil carbon and 1–9% of the bound iron (Wright & Nichols 2002). Glomalin is also marked as the highest stock of soil nitrogen in the organic mass of extractable soil (Nichols & Wright 2004).

The glomalin concentration highly depends on the vegetation cover and the manner of soil management (Mirás-Avalos et al. 2011). An increased occurrence of glomalin is usually observed after the application of an organic material, especially stable manure, cattle slurry or compost (Curaqueo et al. 2011). Garcia-Orenes et al. (2012) also observed an increase in the glomalin concentration in the case when combining a mineral fertiliser and straw. Reduced concentrations are usually measured in soils where the soil structure is physically disrupted, by ploughing or by a dry climate (Wright & Anderson 2000). Important substances, with regard to the effect on the availability, concentration or possibility to determine the glomalin, are the secondary metabolites of the plants, especially tannins. These phenolic substances can enter numerous biochemical processes in the soil (Hättenschwiler & Vitousek 2000; Fierer et al. 2001).

The common contents of glomalin in the various environments:

- Agricultural land 0.3–0.7 mg/g (Wright & Anderson 2000; Wuest et al. 2005)
- Boreal forest 1.1 mg/g (Treseder et al. 2004)
- Desert 0.003–0.13 mg/g (Rillig et al. 2003; Treseder & Turner 2007)
- Temperate forest 0.60–5.8 mg/g (Steinberg & Rillig 2003; Nichols & Wright 2005; Treseder & Turner 2007)
- Temperate grassland 0.23–2.5 mg/g (Lutgen et al. 2003; Batten et al. 2005; Nichols & Wright 2005)
- Tropical rainforest 2.6–13.5 mg/g (Lovelock et al. 2004; Treseder & Turner 2007)
- Antarctic region 0.007–0.15 mg/g (Pohanka & Vlček 2018)

The highest concentrations were measured in the samples of Hawaiian soils (> 100 mg/g) and in general, it applies that arid regions have permanently lower glomalin concentrations (< 1 mg/g), (Rillig et al. 2001). As far as the glomalin profile distribution, it is especially deposited in the top layers of the soil and its concentration decreases with the depth; a limit depth of approximately 140 cm is stated as this is highly probably related to the depth of the organo-mineral horizons and the depth of the root binding. A certain exception was probably the colluvial minerals where the glomalin could be found even in deeper depths. In addition to the soil, glomalin has also been detected in rivers (Harner et al. 2004) probably as a result of soil erosion.

Glomalin taxonomy

According to Rillig (2004) we can only apply the term glomalin to the protein synthesised by the putative AMF gene and it should be named as a glomalin related soil protein (GRSP), because the specific protein glomalin has not been isolated yet. A GRSP determined by monoclonal, for a glomalin-specific antibody (MAb32B11) is immunoreactive soil protein (IRSP). The formal terminology for glomalin is outlined in Table 1 (according to Rillig 2004). In the following text, we are going to simplify terminology (albeit, not quite correctly) and equate the terms for the individual proteins and the term glomalin.

The biochemistry of glomalin

Glomalin is a glycosylated protein exerting high stability under non-natural physical conditions. Though it

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can appear that the glomalin is only a fungal protein and that it has no resemblance in the other organism, the contrary is true. Glomalin has its counterpart in the other organisms which is the heat shock protein 60 (HSP60), a protein present in the mitochondria including the mitochondria from human cells. It is believed that glomalin is a homolog of HSP60, not only in sequence, but also in the secondary and tertiary motives and even the antibodies against glomalin exert a high cross-reactivity to the HSP60 (Gadkar & Rillig 2006).

The exact structure of glomalin has not been fully studied yet, hence the molecular mechanisms related to glomalin remain unclear (Singh et al. 2013). The term glomalin-related soil protein is frequently used in order to emphasise the uncertainty about the number of protein types. The glomalin content in the environment strongly correlates with the aggregate water stability (Driver et al. 2005) and immobilisation of the metals like aluminium is also proven (Seguel et al. 2016). However, highly toxic heavy metals like lead and cadmium can also interact with glomalin (Malekzadeh et al. 2016).

The findings advert to the systematic environmental function. The persistence of the protein is another feature providing unique characteristics to glomalin (Gillespie et al. 2011).

As aforementioned, the structure of glomalin is not well understood because the molecular structure was not crystallographically studied yet. The mentioned homology in the primary sequence of HSP60 and glomalin has the presumption of a structural resemblance. The HSP60 is a protein weighing around 60 kDa giving rise to the oligomeric rings (Kagawa et al. 1995). HSP60 is an evolutionary conserved protein which can be found in both prokaryote and eukaryote organisms (Gammazza et al. 2012). If not only the primary, but also the final structure of glomalin, is close to the HSP60, similar properties like the adhering of other molecules and the ability of self-polymerisation can be expected. The fact that HSP60 can be expressed under stress conditions is also an interesting similarity because glomalin can compensate for the exogenous stressogenic conditions. A typical stressogenic condition for HSP60 is heat (Cheng et al. 2016), but other types of cell

Table 1. Formal terminology and proposed names for glomalin according to Rillig (2004)

Current usage	Identity	Proposed name/usage	Justification
Total glomalin (TG)	Bradford-reactive soil protein (after autoclave and citrate extraction)	Bradford-reactive soil protein (BRSP)	the Bradford assay is non-specific for the particular protein
Easily extractable glomalin (EEG)	Bradford-reactive soil protein (easily extracted; autoclave and citrate extraction)	Easily extracted BRSP (EE-BSRP)	the Bradford assay is non-specific for the particular protein
Immunoreactive glomalin (IRTG)	immunoreactive (MAb32B11) soil protein (after autoclave and citrate extraction)	immunoreactive soil protein MAb32B11 (IRSP)	there is the possibility of the cross-protein reactivity in the soil
Immunoreactive easily extractable glomalin (IREEG)	immunoreactive (MAb32B11) soil protein (easily extracted; autoclave and citrate extraction)	easily extracted immunoreactive (MAb32B11) soil protein (EE-IRSP)	there is the possibility of the cross-protein reactivity in the soil
Glomalin	currently used loosely to describe all of the above pools – and putative gene product	glomalin-related soil protein (GRSP)	“glomalin” in the currently used sense refers to the very different entities; hence it is confusing
Glomalin as a putative gene product	currently unknown identity; hypothesised to be substantially similar to soil glomalin pools (in particular immunoreactive pools)	glomalin(s)	historically, the goal was to identify an arbuscular mycorrhizal fungi protein glomalin; hence this name should be reserved for this gene product (or group of gene products) only

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Table 2. Comparison of heat shock protein 60 (HSP60) and glomalin

	Glomalin/glomalin related soil protein	HSP60
Why the protein is expressed?	probably the stabilisation of the environment, protection against multiple factors including heavy metals	A typical chaperone, protection against multiple factors including heat, pH, heavy metals
Site of action	in as well as out of the organism	mitochondrial
Produced by	mycorrhizal fungi	prokaryotic and eukaryotic cells
Detailed structure	unknown	60 kDa subunit, able to be associated and forming oligomeric rings

threatening conditions like pH, osmotic stress, the presence of heavy metals lead to an expression of HSP60 (Shi et al. 2016). A comparison of HSP60 and glomalin is provided in Table 2.

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