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GLOMALIN AS A POTENTIAL AGENT IN AMELIORATING VARIOUS STRESS CONDITIONS IN MAIZE (*ZEA MAYS*)

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Abstract – Presently, we are facing a major problem of soil contamination by heavy metals through mining activities and pesticides. Another problem of water shortage is also increasing. To address this issue, an important glycoprotein called Glomalin produced by the taxon Glomales plays a significant role. Its main functions are soil aggregation, sequestration of heavy metals, and management of biotic and abiotic stress. Here, we studied the effect of various abiotic stress conditions in maize plants in pot experiments. In drought stress conditions, Glomalin stores water and it is also used to sequester heavy metal in soil. The effect of glomalin was observed in maize cultivated soils pots containing heavy metal $CdCl_2$ at concentrations of 10, 20, 30, 40, and 50 mg/kg soil. Seed germination was observed in maize grown in a 50 mg/kg concentration of heavy metal containing glomalin. Our results also showed that glomalin improved plant growth even under drought stress.

INTRODUCTION

Glomalin was first discovered in 1996 by Wright et al. which is a protein that originates from intraradical hyphae in roots and the surface of extra radical hyphae in the rhizosphere that can be released from the mycelial surface into soils. The protein produced by AMF (Arbuscular Mycorrhizal Fungi) is further confirmed by monoclonal antibody Immuno fluorescence localisation. Glomalin concentration in soil was examined and found to decrease along with the AMF hyphae density that is indicated by earlier studies in 2003 by Dirk. Glomalin, also known as Glomalin Related Soil Protein (GRSP) is a stable and persistent glycoprotein, released by hyphae and spores of AMF. Glomalin is produced by the taxon Glomales, which include fungi of the genera *Acaulospora, Entrophospora, Gigaspora, Glomu*s, and *Scutellospora*. The functions and properties of Glomalin are similar to those of fungal hydrophobins, which are small, self-aggregating, hydrophobic protein. Glomalin is insoluble in water and resistant to heat degradation and has a glue-like nature, so it attaches to horticulture film and soil surfaces. Glomeromycota produces glomalin glycoprotein in significant amounts. Glomalin has not yet been biochemically defined, but studies show that it is an N-linked glycoprotein composed of 3to5% N, 36 to 59% C, 4 to 6% Hydrogen, 33 to 49% Oxygen and 0.03 to 0.1% P. Glomalin also contains Fe, which may be responsible for the reddish color of glomalin. Glomalin was found in different places, such as desert, agricultural, grassland, forest, and non-cultivated soil. Glomalin binds to soil minerals in complexes (Mahendra *et al*., 2018). We need to compare aggregated and non-aggregated soil, in order to test if glomalin production and hyphal growth are responsive to physical growing space (Mtthias *et. al*.,2002). GRSP contributes to the sequestration of heavy metals in the soil. GRSP can bind and sequester some heavy metals such as Cu, Cd, Pd and Zn. GRSP benefits from the environmental pollution of heavy metal in soil (Singh *et al*., 2012). GRSP was extracted from soil in two ways: Easily Extractable GRSP (EE-GRSP) and Total Extractable GRSP (TE-GRSP). Glomalin can be quantified by two methods; Bradford method and the MAb32B11-ELISA assay after extraction (Wei-Qin Gao *et al*., 2019). Glomalin is thermally stable (extraction is carried out by autoclave at 121^{o}C) and resistant to decomposition (Vitezslav and Miroslav, 2019).

Glomalin is produced by AMF, which is one of

the crucial functions of fungi. Glomalin was secreted by hyphae and spores of AMF is defined as a protein that is considered to be an AMF gene product made up of an N-linked glycoprotein. In soil aggregates, by preserving unstable compounds, glomalin significantly reduces soil organic matter degradation. Glomalin has an excellent hyphae protector that helps soil aggregation and is measured as Glomalin-Related Soil Protein (GRSP) from the soil. AMF is not only an element but also a key determinant of soil quality because glomalin is of tremendous prominence in determining numerous aspects of soil quality. It has been reported that the hyphae and spores of AMF excrete this protein at about 80%. AMF produces glomalin not only within the walls but also on the surface of extra-radical mycelium. In the soil, the plethora of AMF and the rate of glomalin production are not always correlated (Aydin and Hulya, 2021).

Cropping systems and land management practices such as tillage, despite their recalcitrant nature, are affected by GRSP content (Wright *et al.*, 1998). GRSP functions as C-sequestration and aggregate stability is beneficial for soil to prevent soil degradation during erosion.

Concentration of glomalin does not depend on the length of the fungus hyphae. During acidification of soil, glomalin increased plant resistance to acidity and toxic levels of heavy metal such as aluminium. Glomalin has shown to increase the plant's resistance to abiotic and biotic stress. Glomalin is homologous to HSP 60 (Heat Shock Protein 60), not only in terms of sequence but also in secondary and tertiary motives. Glomalin observed highly cross-reactivity to HSP 60 (Vitezslav and Miroslav, 2019). Soil Organic Carbon (SOM) was the most important component of the global carbon cycle. The host plant interacts with AMF and produces C into the soil in the form of GRSP, which plays an important role in C cycle and its fixation. Hence plays a beneficial role in the circulation of soil organic carbon.

The structure of Glomalin has not been studied extensively and requires much research as it is only defined as a soil related protein, but its molecular mechanism remains unclear. Hence the future needs studies to analyse chemical constituents and structures. Crop plants must be tested for their response to exogenous GRSP by Treseder and Allen in 2000. The relationship between Glomalin-AMF is still unclear. It is a commonly acknowledged truth that glomalin is a metabolite of arbuscular mycorrhiza. Even so, there is no direct evidence of this statement (Vitezslav and Miroslav, 2019). If it is applied exogenously to the soil, it strongly stimulates root morphology and plant growth. Glomalin can also regulate the phytohormones, especially abscisic acid (ABA), auxin (IAA), and methyl jasmonate, under drought stress (Priscila *et al*., 2021).

MATERIALS AND METHODS

Collection of soil and root samples

Soil and root samples were taken from a depth of 0-25 cm, in polythene bags and brought to the laboratory. The roots were separated from adhering soil by washing gently under tap water and were used for estimation of mycorrhizal colonization. Soil samples were used for the isolation of spores.

Extraction of Glomalin from soil

Total Glomalin extraction

1 g soil was placed in a centrifuge tube with 8 ml of 50 mM sodium citrate. The centrifuge tube was placed in the autoclave for 60-90 min at 121°C. Then it was centrifuged at 4000 rpm for 15 min. Remove the supernatant from the centrifuge tube which contained protein and store it at 4 ºC (Wright and Upadhyaya, 1998).

This supernatant was used for a protein assay to confirm protein. In the supernatant addition of, 1N HCl until pH 2.0 to 2.5 occurred, then solution was placed on ice for 45 min, precipitates formed which was then centrifuged. The pellet was dissolved in 0.1 M NaOH and immediately dialyzed against deionized water in dialysis tubing with an 8,000 to 12,000 Dalton (Da) molecular weight cutoff. Water was changed in the dialysis chamber at least 5 times with an 8-12h incubation period. Dialyzed material was centrifuged, and supernatant collected, and freeze-dried (Nichols and Wright, 2005).

Pot experiments

In the pot experiment, we prepared three pots of maize to compare the effect of varying stress conditions. In each pot were added 30 g of soil and seeds. One pot remained as control, in the second pot 4 ml glomalin was added and in the third pot 0.1 ml of pathogen (*Fusarium oxysporum*) culture was added.

a. Effect of glomalin on maize growth

Glomalin was added in varying amounts (2, 4, 6, 8 and 10 ml) respectively, in each pot having 30 g of soil and seed.

b. Effect of heavy metal

Five pots were taken and addition of heavy metal as $CdCl_2$ in varying concentrations (10, 20, 30, 40 and 50 mg/kg) respectively was done, in pots having 30 gm of soil and seed. For the test, another five pots were taken and addition of 3 ml of glomalin in each pot amended with varying concentration of heavy metal as $CdCl_2$ (10, 20, 30, 40 and 50 mg/kg) respectively was done.

c. Effect of plant pathogenic fungi *Fusarium oxysporum* and glomalin on maize seedling growth Glomalin was added in varying amounts (2, 4, 6, 8 and 10 ml) respectively, in each pot having soil and seed. 0.1 ml of *Fusarium oxysporum* fungi was added to each pot.

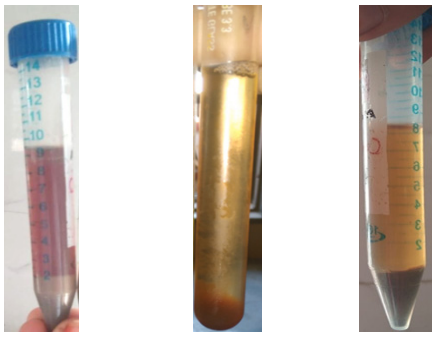
d. Effect of drought stress in maize

One pot was prepared as control, which included only 30 g soil and seeds. In another pot was added 4 ml of glomalin in 30 g soil. To provide abiotic stress, water was added in a 2-day time interval at the minimum level in both pots.

RESULTS AND DISCUSSION

Collection of soil and root sample

Sample 1 was collected from [Latitude-21.267115, longitude - 72.957577]. Sample 2 was collected from [Latitude - 21.269074, longitude - 72.967511]. Sample 3 was collected from [Latitude-21.269811, longitude -72.941647]. All samples were collected in sterile sampling bags.



(A) (B) (C)

Fig. 1. Extraction of glomalin.

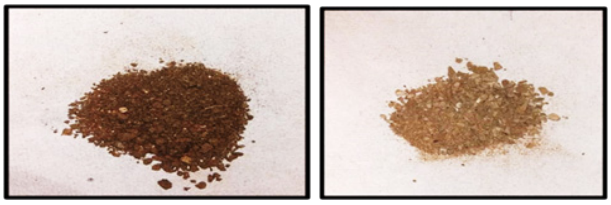
Extraction of Glomalin from soil

Glomalin was extracted from soil using the sodium citrate buffer extraction method. Total glomalin extracted from using 50mM sodium citrate buffer at pH 8.0. Total glomalin was extracted from soil after 3 cycles of autoclave runs until the supernatant color changes to straw color. In this Figure 1[A] centrifuge tube was filled with sodium citrate buffer and soil and then centrifuged. After the centrifugation supernatant contained glomalin, [B] 1N HCl was added in supernatant and glomalin precipitate was observed in the bottom of the tube and [C] After centrifugation precipitated glomalin was collected.

Table 1. Glomalin extraction from different soil sample

Soil sample	pH	Glomalin (mg/g)
Sample 1	8.30	12
Sample 2	8.05	3
Sample 3	7.68	4

Figure 2 shows that the color of soil changes after glomalin extraction. The color of glomalin is brown to slightly red. Table 1 shows that glomalin extracted from sample 1 as the pH of sample 1 is alkaline, which indicates extraction of large amounts of glomalin. Wright and Nichols also suggested that the amount of glomalin varies with the variation of pH of soil (Nichols and Wright., 2005).



(A) (B)

Fig. 2. (a) before and (B) after extraction of glomalin in soil.

Pot experiment

Maize seed germinated within five days after seeds were inoculated in pots. Plant growth parameters were measured in each pot 19 days after seed inoculation. Various plant growth parameters were determined as shown in Table 2 and Figure 3.

Highest shoot length and root length was obtained in maize plants treated with glomalin as compared to untreated plants (S.Cn.). When plants were treated with pathogens, the root and shoot length decreased. Roots were mainly affected by the plant pathogen.

(1) (2) (3)

Fig. 3. (1) S.Cn. (2) S.Cn.G. and (3) S.Cn.P. (S= Soil, Cn= Corn seed, V= VAM fungi and P= plant pathogenic fungus).

Effect of glomalin on germination and seedling growth

The effect of glomalin on various plant growth parameters of maize plants are showcased in Table 3 and Figure 4. Highest shoot length was observed in S.Cn.G. (4ml) and highest root length was observed in S.Cn.G. (10 ml). Larger amount of shoot and root dry weight was detected in S.Cn.G. (4 ml). So that comparison of all plant growth parameters suggested that the useful amount of glomalin is 4 ml. Effect of varying amounts of glomalin were shown to improve plant growth as compared to control maize plant (without glomalin (S.Cn.))

b. Effects of heavy metal

Plant growth was observed in pots containing varying concentrations of heavy metal such as $CdCl_2$ up to (40 mg). Plant growth was absent in S.Cn.H (5^{th} pot) amended with 50 mg of heavy metal.

(1) (2) (3) (4) (5)

Fig. 4. (1) S.Cn.G. (2 ml), (2) S.Cn.G. (4 ml), (3) S.Cn.G. (6 ml), (4) S.Cn.G. (8 ml) and (5) S.Cn.G. (10 ml) (S= Soil, Cn= Corn seed and G= Glomalin).

(1) (2) (3) (4) (5)

Fig. 5. (1) S.Cn.H (10 mg), (2) S.Cn.H (20 mg), (3) S.Cn.H (30 mg), (4) S.Cn.H (40 mg) and (5) S.Cn.H (50 mg) (S= Soil, Cn= Corn seed and H= Heavy metal).

Table 2. Observation of plant growth parameters in soil

No. of pot	Content of soil	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
1	S.Cn.	22.5	16	0.132	0.060
2	S.Cn.G.	25	18	0.143	0.120
3	S.Cn.P.	18	11	0.108	0.056

Table 3. Observation of plant growth parameters in soil containing varying amounts of glomalin

No. of pot	Content of soil	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
1	S.Cn.G. (2 ml)	22	15	0.085	0.056
2	S.Cn.G.(4 ml)	**27.5**	16	**0.150**	**0.153**
3	S.Cn.G.(6 ml)	25.5	17	0.134	0.125
4	S.Cn.G.(8 ml)	24.5	19	0.134	0.083
5	S.Cn.G.(10 m)	25.5	**20**	0.117	0.062

(1) (2) (3) (4) (5)

Fig. 6. (1) S.Cn.H.G. (10 mg), (2) S.Cn.H.G. (20 mg), (3) S.Cn.H.G. (30 mg), (4) S.Cn.H.G. (40 mg) and (5) S.Cn.H.G. (50 mg) (S= Soil, Cn= Corn seed, H= Heavy metal and G= Glomalin).

Germination was absent in S.Cn.H (50 mg) as this concentration of heavy metal proved to be toxic. In this table odd pots numbers indicated the effect of heavy metal in maize plants and even pot numbers indicated the effect of heavy metal when amended with glomalin. When comparing the shoot length, the highest shoot length was observed in S.Cn.H (30 mg). On comparing the root length, the highest root length was observed in S.Cn.H (40 mg). Larger shoot and root dry weight observed in S.Cn.H (30 mg). This showed that 30 mg/kg heavy metal concentration was beneficial for plant growth. 10 and 20 mg/kg concentration was used, but they cannot give significant plant growth. Figure 6 shows maize pots with varying concentrations of heavy metals amended with 3ml glomalin in each pot. Pot number 5, S.Cn.H.G. containing (50mg) of heavy metal showed germination and plant growth. This shows that glomalin can sequester heavy metal and helps plant growth.

All plant growth parameters were observed highest in Pot 4 S.Cn.H.G. (40mg) as seen in figure: 6. 40mg/kg concentration of $CdCl_2$has not shown to be detrimental for plant growth with added glomalin. Glomalin is beneficial because it sequesters toxic concentrations of heavy metal in S.Cn.H.G. (50 mg) that can be observed in Figure 5 and 6. Glomalin shows a positive effect against heavy metal stress in maize crop seedlings. Hussain *et al*, (2013) also studied and reported the effect of Cd heavy metal on maize plants.

c. Effect of glomalin on the biotic stress in plants

Results of the effect of varying concentrations of glomalin that affect plant growth when provided with biotic stress by phytopathogens is shown in Figure 7. Varying amounts of Glomalin were added in pots containing plant pathogenic fungus (0.1 ml). The highest growth parameters were measured in the case of 10 ml of glomalin addition.

Increase in shoot and root dry weight, with increasing amounts of glomalin in the presence of

(1) (2) (3) (4) (5)

Fig. 7. (1) S.Cn.P.G. (2ml), (2) S.Cn.P.G. (4ml), (3) S.Cn.P.G. (6ml), (4) S.Cn.P.G. (8ml) and (5) S.Cn.P.G. (10 ml) (S= Soil, Cn= Corn seed, P= Pathogenic fungus and G= Glomalin).

Table 4. Observations of Plant growth parameters in soil containing varying concentration of heavy metal and heavy metal amendment with glomalin.

No of pot	Content of soil	Shoot length (cm)	Root length (cm)	Shoot dry weight (gm)	Root dry weight (gm)
1	S.Cn.H (10 mg)	22	11	0.085	0.046
2	S.Cn.H.G. (10 mg)	25	11	0.106	0.112
3	S.Cn.H (20 mg)	25.4	11.5	0.120	0.055
4	S.Cn.H.G. (20 mg)	23	11.5	0.116	0.065
5	S.Cn.H (30 mg)	27.3	14	0.151	0.083
6	S.Cn.H.G. (30 mg)	27.5	17	0.135	0.076
7	S.Cn.H (40 mg)	26.7	16	0.121	0.065
8	S.Cn.H.G. (40 mg)	**28.3**	**20**	**0.144**	**0.084**
9	S.Cn.H (50 mg)	–	–	–	–
10	S.Cn.H.G. (50 mg)	22	16	0.099	0.064

plant pathogenic fungus was observed. This may be attributed to Glomalin's formation of a hydrophobic layer surrounding the root that reduces the effect of plant pathogens. Again Glomalin was also shown to reduce the effect of incidence of disease by plant pathogens.

d. Effect of drought stress

To observe the effect of drought stress on plant growth, two pots S.Cn.W and S.Cn.W.G were prepared one without and with glomalin (4ml) and given water stress at interval of two days. Table:6 observed various plant growth parameters in drought stress conditions. Highest shoot and root length was observed in S.Cn.W.G. pot in addition to larger shoot and root dry weight. The result shows that glomalin supports plant growth in drought stress conditions. In many areas, water availability is poor, so added glomalin can store water. Glomalin forms a hydrophobic layer surrounding the root and prevents the loss of water. Huixing reported that VAM fungi enlarge the root area of host plants in drought stress conditions. VAM fungi excrete glomalin in soil and hyphae of VAM protect plants against oxidative damage caused by drought stress (Huixing, 2005).

(a) (b)

Fig. 8. (1) S.Cn.W. and (2) S.Cn.W.G. (S= Soil, Cn= Corn seed, W= Water stress and G= Glomalin).

Figure 9 showed pot (S. Cn) root and shoot lengths were lower as compared to all pots. Maize seeds could not germinate in high heavy metal concentration (50mg), but with the addition of glomalin 4ml which sequesters heavy metal and thereby reduces its concentration, maize seed germination was seen. The chart clearly depicts, plant-pathogen effect is reduced by addition of glomalin. Maize root and shoot length was affected by water stress but the addition of glomalin reduces water stress as it is known to store water.

Figure 10 shows comparisons of shoot and root

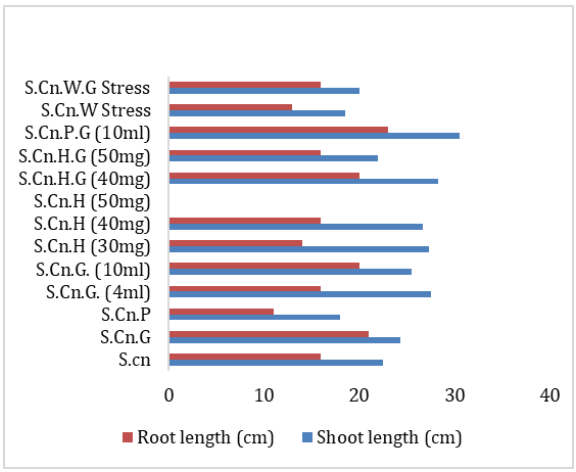


Fig. 9. Comparison of the effect of various stress conditions on root and shoot length in maize plant

Table 5. Plant growth parameters in soil containing plant pathogenic fungus amendment with varying amounts of glomalin.

No. of pot	Content of soil	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
1	S.Cn.P.G. (2 ml)	30	12	0.113	0.055
2	S.Cn.P.G. (4 ml)	29.7	13	0.129	0.080
3	S.Cn.P.G. (6 ml)	28	15	0.140	0.089
4	S.Cn.P.G. (8 ml)	25	11	0.150	0.090
5	S.Cn.P.G. (10 ml)	**30.5**	**23**	**0.163**	**0.098**

Table 6. Observations of plant growth parameters under drought stress condition with glomalin

No. of pot	Content of soil	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
1	S.Cn. W Stress	18.5	13	0.066	0.037
2	S.Cn.W.G. Stress	**20**	**16**	**0.130**	**0.067**

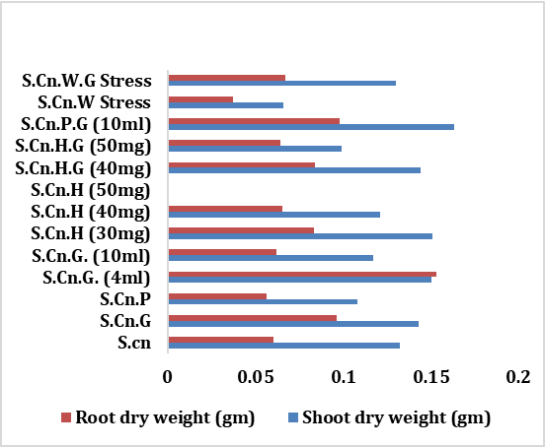


Fig. 10. Comparison of the effect of various stress conditions on root and shoot dry weight of maize plant

dry weight (gm) in different pots. In this chart, the highest root dry weight was detected in pot treated with 4 ml glomalin. Increase in root ultimately increases plant growth because it is the main part of the plant for nutrition and water absorption. Roots of the maize plant are also affected by the phytopathogen. But addition of 10 ml glomalin decreased the effect of plant pathogens.

CONCLUSION

In the twenty-first century, the advancement of industrialization and human activities is polluting the environment. Heavy metal toxicity is increased in soil by mining, industrial waste, and other activities. In this experiment it was observed that the effect of varying concentration of heavy metal $CdCl_2$ (10, 20, 30, 40 and 50) mg/kg in soil containing maize plants. No plant growth was observed in soil containing 50 mg/kg concentration. This implies that 50mg concentration of heavy metal was toxic for plant growth. Addition of 3 ml glomalin in pots containing 50 mg/kg heavy metal plant growth was observed. It suggests that glomalin reduces the toxicity of heavy metal and in turn can also be used in soils that are contaminated with toxic concentration of heavy metal. Plant growth decreased in drought stress conditions but, addition of 3 ml glomalin increased plant growth in drought stress due to soil aggregation and water storage. Glomalin also exhibited antagonistic activity by revealing zones of inhibition against plant pathogenic fungus *F. oxysporum.* In pot experiments, glomalin had a positive effect and increased plant growth even in biotic stress given by the fungus.. Looking to the rewards offered by Glomalin, it can be used in bioremediation of soil contaminated with petroleum, polycyclic aromatic hydrocarbon and pesticides. In this experiment it can be observed that glomalin work as potential agents in ameliorating heavy metal stress, drought stress and biotic stress.

Conflict of Interest- None

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