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Carbon Sequestration by Glomeral Fungi in Soil is Influenced by Phosphorus and Nitrogen Fertilization

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Abstract*— Glomalin is the most abundant gylcoprotein in soil which produces by glomeral fungi in symbiosis with plant roots. It improves soil physical, chemical and biological properties. Assimilated plant C which is allocated to the mycorrhizal fungus, appears as a recalcitrant glycoprotein (glomalin) in cell walls of hyphae and spores. Considering global warming due to increasing greenhouse gases, this phenomenon could be important in carbon sequestration and reducing CO_2 in atmosphere. Chemical fertilizers could affect symbiotic relations of these fungi, which in turn affect glomalin production. In a pot culture experiment, the sterile soil was treated with 0, 100, 200 mg N kg^{-1} soil as urea or 0, 20, 40 mg P kg^{-1} soil as triple superphosphate, in two separate factorial experiment based on completely randomized design with three replications. Corn plant (*Zea mays* L.) was inoculated with *Rhizophagus clarus* (formerly, *Glomus clarum*) or *Rhizophagus intraradices* (formerly, *Glomus intraradices*) in each set of experiment. Easley extractable glomalin (EEG) and total glomalin (TG) in soil were determined by Bradford method at the end of experiment. Root colonization by both fungi increased EEG and TG compared to the non-mycorrhizal control ($p<0.05$). Nitrogen levels of 100, 200 increased EEG by 75 and 112% and TG by 59 and 76%, respectively, compared to the no nitrogen treatment. P levels of 20, 40 caused 27% increase and 6% decrease in EEG, and 24% increase and 13% decrease in TG, respectively, compared to the zero addition of phosphorus. Regarding glomalin production in this condition, *R. clarus* was more efficient than *R. intraradices.* Application of 20 mg P kg^{-1} increased root colonization, dry weights of shoot and root, chlorophyll index, leaf area, amount of shoot and root nitrogen and potassium compared to the 40 mgP. kg^{-1} and control. Thus, application of 100 mg N kg^{-1} increased root colonization, dry weights of shoot and root, chlorophyll index, leaf area, amount of shoot and root phosphorus and potassium compared to the 200 mg N kg^{-1} and control.

***Keywords*— Glomalin; Mycorrhizal fungi; Corn; Chemical fertilizers; Soil carbon**

I. INTRODUCTION

Over the past 150 years, the amount of carbon in the atmosphere has increased by 30%. Most scientists believe there is a direct relationship between increased levels of carbon dioxide in the atmosphere and rising global temperatures. One proposed method to reduce atmospheric carbon dioxide is to increase the global storage of carbon in soils. Soil carbon storage is an important function of terrestrial ecosystems.

It is recently coming to light that mycorrhizal fungi may play an important role in maintaining this pool in soil [1],[2],[18]. Measurements of plant carbon allocation to mycorrhizal fungi have been estimated to be 5-20% of total plant carbon uptake [1] and in some ecosystems the biomass of mycorrhizal fungi can be comparable to the biomass of fine roots. Recent research has shown that mycorrhizal fungi hold 50 to 70 percent of the total carbon stored in leaf litter and soils of forest lands [8],[13]. Based on the magnitude of mycorrhizal fungal inputs to the soil carbon pool, some have suggested that variation in the recalcitrance of mycorrhizal biomass may be important for predicting soil carbon storage, as it would affect the rate at which the contribution of mycorrhizal fungi to soil carbon is returned to the atmosphere [3],[5],[8].

The glomalin, a glycoprotein produced only by arbuscular mycorrhizal fungi, has been found to accumulate in some soils, and may be a substantial fraction of the soil carbon pool in these ecosystems [6],[16]. It may be hiding place for up to %30 of soil organic carbon. Glomalin as a recalcitrant glycoprotein with half life of about 50 years, persist in soil for a long time. By this way, the key role of glomalin in carbon sequestration in terrestrial ecosystems is well obvious [4], [17].

Glomalin is synthesized by the fungi and accumulates in cell wall of spore and hyphae [17]. There are evidences that glomalin protect fungi from environmental stresses (temperature, moisture, salinity, heavy metals, etc). It also protects hyphae during transport of nutrients from the plant to the hyphal tip and from soil to the plant. Amino acid

sequencing of glomalin indicates that it has similarity with heat shock proteins (HSPs) [7]. It releases to the soil after fungal dead, therefore, it accumulates in soils in considerable amounts annually. The growth of arbuscular mycorrhizal fungi completely depends on its host plant status. Any change in host plant growth conditions, will reflect on fungal development and consequently on glomalin production [12]. Wu et al. [24] pointed out that the application of nitrogen and phosphorus fertilizers at low level could induce arbuscular mycorrhizal development and consequently enhance glomalin production. Others reported no significant effect of nitrogen on glomalin production [22]. Unlike to the nitrogen, the higher level of phosphorus may inhibits glomalin production by reducing AM fungal development in host roots.

This study was aimed to evaluate the effects of N and P fertilizers on AMF development and glomalin production. We hypothesized that supplying nitrogen would enhance but phosphorus (specially at higher levels) would diminish glomalin production by the fungi.

II. Materials and Methods

A. Fungal Species

Rhizophagus clarus (formerly, *Glomus clarum*) and *Rhizophagus intraradices* (formerly, *Glomus intraradices*) were obtained from Biology Department of Lund University, Sweden. The fungal species were propagated with maize plants in 4-L pots containing sterile sandy loam soil. Rorison's nutrient solution prepared with deionized water with 1/2 strength of phosphorus was added to the pots twice a week to bring the soil moisture to field capacity [15]. Pots were kept in greenhouse with 28/20 °C day/night and 16/8 h light/dark period. After 4 months, top plants were cut off and pot materials containing soil, mycorrhizal roots, hyphae and spores were thoroughly mixed and used as fungal inoculum.

B. Plant Material and Fungal Inoculation

Seeds of corn plant (*Zea mays* L. Single Cross 704) were surface sterilized with %1 sodium hypochlorite and sown in pots containing 2 kg sterile soil. A loamy sand soil with pH 7.8, ECe 1.4 dSm^{-1}, organic carbon 0.22%, $K_{ava.}$ 182.6 $mgkg^{-1}$ and $P_{ava.}$ 4.4 $mgkg^{-1}$ was used in this study. The sterile soil was treated with 0, 100, 200 mg N kg^{-1} soil as urea or 0, 20, 40 mg P kg^{-1} soil as triple superphosphate, in two separate factorial experiment based on completely randomized design with three replications. All pots received K fertilizer as K_2SO_4 at a rate of 80 mg K/ kg soil.

Fungal inoculants at a rate of 80g/ pot was spread at depth of 5cm below the soil surface as a thin layer, in each set of experiment.

C. Chlorophyll Index, Plant N, P and Dry Weight

Eighty days after sowing, the chlorophyll index was measured using Spad Hanstech Cl-01, then plants were harvested and shoot and root dry weights were determined after drying at 60 °C for 48h. Nitrogen and phosphorus concentrations in shoot and root were measured according to the standard methods.

D. Root Colonization

Fine roots were cleaned in %10 KOH followed by staining with lacto-glycerol trypan blue [23]. Root colonization percentage was determined as per described by Giovannetti and Mosse [25].

E. Glomalin Determination

Easley extractable glomalin (EEG) and total glomalin (TG) in soil were extracted with 20 and 50 mM citrate buffer, respectively [14]. Briefly, one gram of soil (<2 mm) was mixed with 8 mL of 20 mM citrate buffer , pH 7 for EEG or 50 mM citrate buffer, pH 8 for TG in a test tube and autoclaved at 121 °C for 30 min. It was then centrifuged at 5000 RPM for 15 min. The supernatant was assayed for glomalin concentration using Bradford method [26]. Bovine serum albumin (BSA) was used for preparation calibration standard curve.

III. Results and Discussion

A. Plant Dry Weights and N, P Concentrations

Root and shoot dry matter were significantly increased by N fertilization up to 100 mg/kg but the higher level of nitrogen (N200) had adverse effect on both criteria. In each level of nitrogen, mycorrhizal plants had higher shoot and root biomass than non-mycorrhizal ones, although the Rc was efficient than Ri fungus. The highest shoot and root dry weights were achieved in Rc inoculated plants with N100 level. Nitrogen concentration in shoot and root was enhanced by rising of N level to N200.

TABLE I
EFFECTS OF N FERTILIZATION AND FUNGAL INOCULATION ON PLANT BIOMASS AND NUTRIENTS CONCENTRATIONS.

Treatments		Shoot DW g pot^{-1}	Root DW g pot^{-1}	CI	Shoot		Root	
					%N	%P	%N	%P
N0	NM	4.34f	1.95h	1.59g	0.30h	0.30c	0.35g	0.36b
	Rc	6.10e	3.21g	2.21f	0.34g	0.33b	0.44e	0.43a
	Ri	6.44e	4.16f	2.41e	0.34g	0.33b	0.40f	0.44a
N100	NM	14.22c	4.93e	6.54d	0.80f	0.18g	0.65d	0.38d
	Rc	19.94a	10.94a	7.29c	0.95d	0.20e	0.67d	0.31c
	Ri	17.61b	9.27b	7.13c	0.88e	0.19f	0.67d	0.30c
N200	NM	10.11d	3.65fg	11.07a	1.53a	0.20e	1.48a	0.24e
	Rc	17.33b	8.07c	11.06a	1.38b	0.21d	1.20b	0.27d
	Ri	14.76c	6.85d	10.43b	1.28c	0.20e	1.14c	0.28d

Means in each column followed by same letter are not significantly different at $p<0.05$.

DW: dry weight, CI: chlorophyll index, NM: non-mycorrhizal, Rc: *Rhizophagus clarus* , Ri:*Rhizophagus intraradices.*

Higher N uptake by plant in N200 level did not cause any increase in shoot and root biomass but prevented them to a marked extent. The positive effect of both fungi on N uptake was seen in N0 and N100 levels. Phosphorus concentration of shoot and root was negatively affected by increasing of n level, likely due to dilution effect. In each level of N fertilizer, mycorrhizal inoculation lead to marked increase in shoot and root P compared to the non-mycorrizal plants. Increment of chlorophyll index was obtained by both N fertilization and fungal inoculation, although the positive effect of fungi was distinct in N0 and N100 levels (Table.1).

The effects of phosphorus fertilization on shoot and root growth and N and P uptake had same trend as nitrogen, but the phosphorus level of P40 had more preventing effect on shoot and root biomass production.

Symbiotic efficiency of both fungi on shoot and root growth and P allocation to the plant was considerable at P0 level and to the some extent at P20 level, but they were not efficient in P40 level (Table.2). There are evidences that higher availability of P in soil inhibits the development of mycorrhizal symbiosis which lead to decrease in beneficial effect of fungi [9], [11]. Chlorophyll index was significantly increased in mycorrhizal plants compared to the non-mycorrhizal ones at P0 and P20 levels. There was no positive effect of fungal inoculation on chlorophyll index at P40 level (Table 2). It seems that at higher P level, the fungi could not exert beneficial effect on chlorophyll synthesis.

B. SoilEEG

Soil EEG was significantly increased by increasing nitrogen level in both fungal inoculations. The enhancement of EEG in the presence of Rc fungus was more than Ri ($p<0.05$). The increase was also seen in non-mycorrhizal treatment (Fig.1). Glomalin synthesis by the fungi is positively affected by nitrogen availability in soil. Nitrogen is the main constituent of this glycoprotein.

The results indicate that glomalin as a recalcitrant carbon source can accumulate in soil by N fertilization. Plant photosynthates are translocated to the fungal organs via roots and mainly utilize for glomalin synthesis in hyphal and spore cell walls. During this process, nitrogen plays an important role as constituent of the glycoprotein [22]. The Bradford method [26] was used for glomalin determination in this study. The method is not specific for glomalin and therefore measures other glomalin related proteins and glycoproteins as well [17]. By this way, other proteins which increase by N fertilization, come to account in Bradford assessment. This might be a reason for increase of EEG by rising N level in non-mycorrhizal treatment.

TABLE II
EFFECTS OF P FERTILIZATION AND FUNGAL INOCULATION ON PLANT BIOMASS AND NUTRIENTS CONCENTRATIONS.

Treatments		Shoot DW g pot^{-1}	Root DW g pot^{-1}	CI	Shoot		Root	
					%N	%P	%N	%P
P0	NM	3.77g	2.39e	6.68d	0.99a	0.18e	0.96a	0.23e
	Rc	9.11e	5.47bc	8.65a	0.85c	0.21d	0.81b	0.26c
	Ri	7.85f	4.66d	8.28b	0.89b	0.21d	0.75c	0.24d
P20	NM	14.80b	5.89b	5.40g	0.67f	0.22c	0.54f	0.32a
	Rc	19.48a	8.99a	6.43e	0.84c	0.24b	0.63de	0.32a
	Ri	18.60a	8.32a	6.17f	0.79d	0.23bc	0.57ef	0.30b
P40	NM	12.53c	5.71b	7.39c	0.72ef	0.27a	0.55f	0.29b
	Rc	12.62c	5.15bcd	7.47c	0.77de	0.28a	0.58def	0.30b
	Ri	11.49d	4.74cd	7.50c	0.74e	0.27a	0.64d	0.30b

Means in each column followed by same letter are not significantly different at $p<0.05$.
DW: dry weight, CI: chlorophyll index, NM: non-mycorrhizal, Rc: *Rhizophagus clarus* , Ri:*Rhizophagus intraradices.*

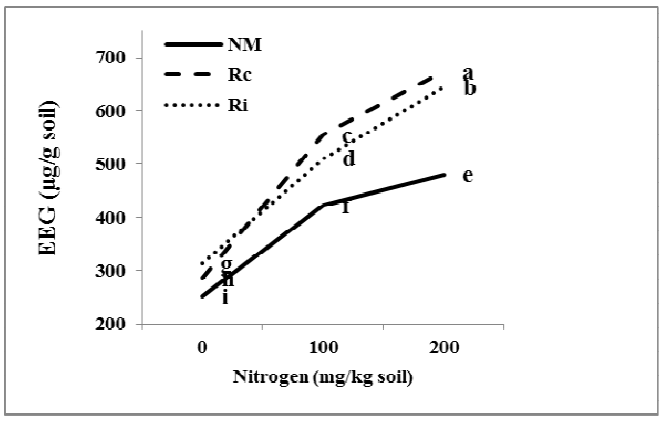

Fig. 1 Effects of nitrogen levels on soil EEG content in mycorrhizal and non-mycorrhizal treatments.

The effect of P fertilization on EEG production was different from that of N fertilization. Increasing level of phosphorus fertilizer from P0 to P20 caused a marked enhancement of EEG in both fungi, although the increase was more in Rc than Ri fungus (Fig.2). It seems that P20 level supplies plant with sufficient phosphorus, which in turn carbon allocation to the fungi is enhanced. Under P-limiting conditions the plant will invest in mycorrhizas and/or more roots, simply because it needs to acquire more P [10],[20],[21]. Unlike to the N fertilizer, increasing of P to the highest level (40 mg/kg) severely diminished EEG synthesis.

Carbon allocation to the plant may be influenced by phosphorus availability in soil [1]. Higher available P in soil inhibits mycorrhizal fungal development in root and soil, therefore the root pathway for P uptake dominates mycorrhizal pathway, thus the carbon allocation to the fungus is decreased [11]. Schnepf et al. [19] pointed out that the apparent deactivation of the direct pathway in AM plants might be caused by down regulation of the plant phosphate transporters in root epidermis plus root hairs (directly via the presence of AM fungi or indirectly via increased plant P status) and/or by competition for P between roots and hyphae in the depletion cylinder around the root.

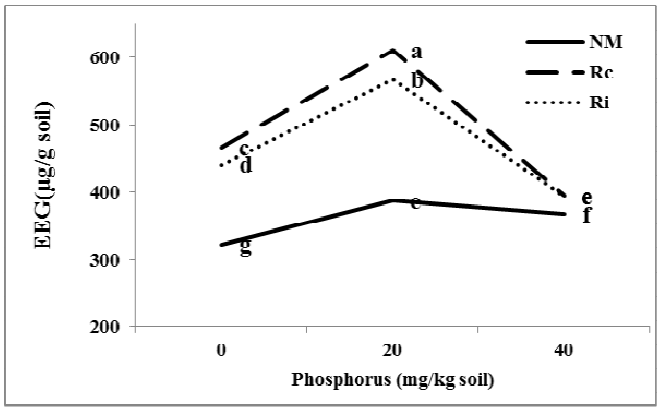

Fig. 2 Effects of phosphorus levels on soil EEG content in mycorrhizal and non-mycorrhizal treatments.

A little increase was seen in non-mycorrhizal treatments as a result of P fertilization(Fig.2). The likely reason is that higher soluble phosphate in soil at P20 and P40 levels may enhances glomalin extraction with citrate buffer.

C. SoilTG

Total glomalin (TG) showed same trend as EEG in response to the N and P fertilizations. As described for EEG, the Rc was also more efficient than Ri fungus in TG production (Figs.3&4). TG is consisted of aged plus newly synthesized glomalin by the fungi.

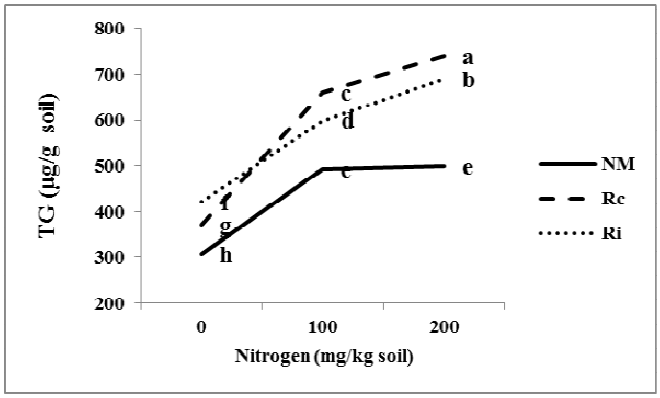

Fig. 3 Effects of nitrogen levels on soil TG content in mycorrhizal and non-mycorrhizal treatments.

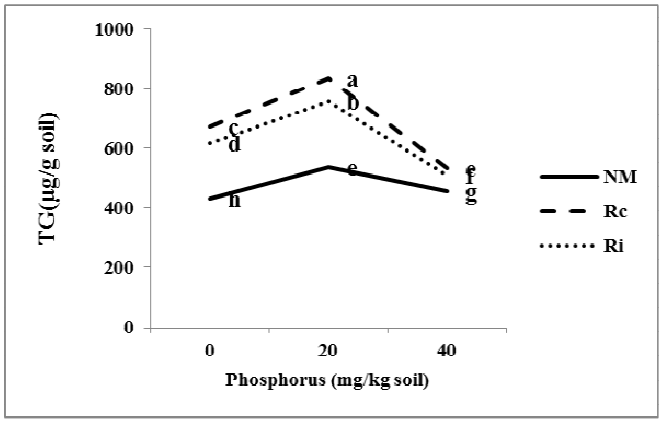

Fig. 4 Effects of phosphorus levels on soil TG content in mycorrhizal and non-mycorrhizal treatments.

D. Glomalin and Root colonization correlations

Soil EEG and TG were positively correlated with mycorrhizal root colonization (Figs 5&6). By increasing root colonization the fungal organs in soil are also proliferated around the root. All AM fungal propagules possess glomalin on their cell walls [16] therefore a marked increase in both glomalin contents was recorded at higher root colonization levels.

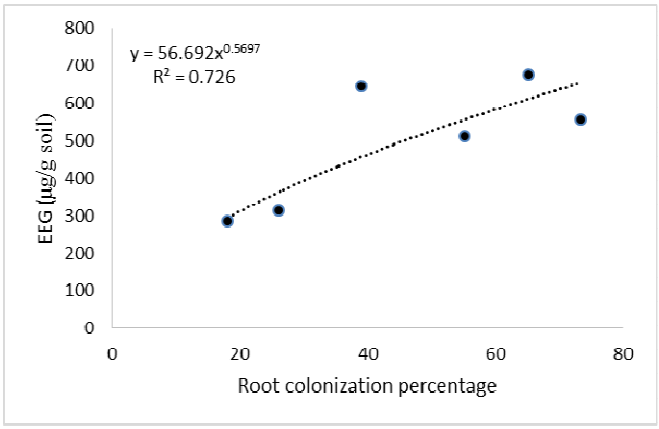

Fig.5 Regression between soil EEG content and mycorrhizal root colonization.

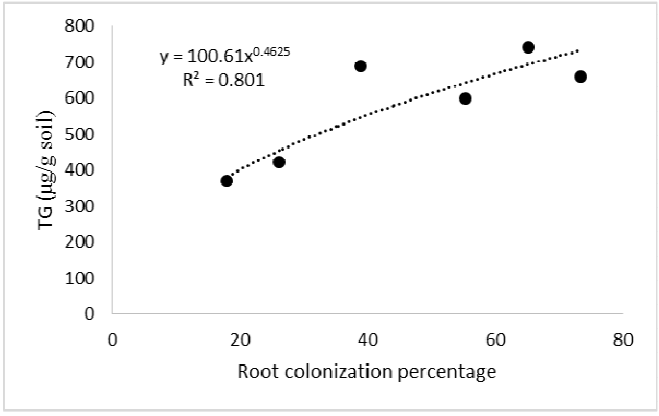

Fig.6 Regression between soil TG content and mycorrhizal root colonization.

IV. Conclusions

Carbon sequestration via glomalin synthesis by AM fungi is an important pathway for capturing CO_2 from atmosphere. Field managements which help AM development in soil ecosystems, would lead to enhancement of glomalin production [12],[14]. Based on our results, nitrogen fertilizers could positively affect the production of this glycoprotein. Once plant assimilates are translocated to the fungi, they could transform to the nitrogenous compounds if sufficient nitrogen sources are available. By this way, a considerable amount of fixed carbon is assimilated in fungal organs and soil particles as well. Phosphorus application showed some different trend in this respect. By increasing of P to medium level (20 mg kg^{-1}) a marked increase in glomalin production was seen because of improvement of plant growth. But the enhancement of P to the higher level (40 mg kg^{-1}) had adverse effect on mycorrhizal root

colonization and consequently on glomalin production. There are many evidences that sufficient P supply could diminish carbon allocation to the fungi [1],[11]. It could be concluded that carbon sequestration by arbuscular mycorrhizal symbiosis in terrestrial ecosystems can be improved by N fertilization at optimum level but P fertilization at restricted conditions.

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