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# Short Communication
# Glycoprotein Associated with *Funneliformis coronatum*, *Gigaspora margarita* and *Acaulospora scrobiculata* Suppress the Plant Pathogens *In vitro*

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## Abstract

**Background and Objective:** Arbuscular mycorrhizal fungi (AMF) form symbiotic association with most plants and are known to play significant role in higher nutrient uptake and soil aggregation as well as, carbon sequestration through production of specific glycoprotein characterized as glomalin or glomalin related soil protein (GRSP) contained in their hyphae and spores. The objective of this study was to find out the fungi static effects of this glycoprotein in suppression of growth of two soil borne plant pathogens *in vitro*. **Materials and Methods:** Sorghum was used as the test plant, *Funneliformis coronatum* (*F. coronatum*), *Acaulospora scrobiculata* (*A. scrobiculata*) and *Gigaspora margarita* (*G. margarita*) were used as AMF inoculums, *Rhizoctonia solani* (*R. solani*) and *Colletotrichum falcatum* (*C. falcatum*) were used as test pathogens. The GRSP was extracted from the soil of sorghum pots after harvesting, two different concentrations of GRSP was supplied to the agar medium and test pathogens were inoculated, control plates received extractant from non-AMF inoculated soil. The experiment was performed in completely randomized design with 4 replicates each. The data were analyzed by one-way ANOVA. **Results:** The level of GRSP production varied with different AMF species. *In vitro* testing of suppression of *Rhizoctonia solani* and *Colletotrichum falcatum* by GRSP extracted from *Funneliformis coronatum* inoculated soil was higher followed by *Gigaspora margarita* and *Acaulospora scrobiculata* inoculated soil. **Conclusion:** Production of GRSP differs with AMF species and was found to suppress the growth of pathogens *in vitro*.

Key words: *Colletotrichum falcatum*, *Rhizoctonia solani*, glomalin related soil protein, *Funneliformis coronatum*, *Acaulospora scrobiculata*, *Gigaspora margarita*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Arbuscular mycorrhizal fungi directly participate in the process to accumulate soil aggregate stability by physically forming a network around soil particles and indirectly by the hyphal exudation of an iron-containing, heat stable glycoprotein (extracted at 121°C) named glomalin as an aggregate binding agent[1,2]. Glomalin has been operationally defined as glomalin-related soil protein (GRSP) by extraction and detection conditions from soil and it is detected in large amounts in different ecosystems[1]. The sticky GRSP acts as biological glue, helping to bind soil tiny particles into small aggregates of different sizes[3]. Well-aggregated soil is stable enough to stand against calamities such as heavy winds and water erosion and has better air and water infiltration rates which work in favor of plants and microbial growth[4,5]. Additionally, GRSP is recalcitrant enough to have a long residence time in soils (7-42 years)[6,7] and plays a pivotal role in long-term carbon/nitrogen storage and heavy metal sequestration[2,8]. Therefore, the release and accumulation of GRSP in soils can be a very important mechanism for ecological restoration of soils.

Improved plant nutrition, competition for colonization sites and activation of plant defense mechanism are some of the mechanisms proposed in controlling diseases[9]. Though, role of AM fungi in plant tolerance to disease has already been proven[10]. The role of GRSP in suppression of *Rhizoctonia solani* and *Colletotrichum falcatum* has been worked out probably for the first time in this study.

## MATERIALS AND METHODS

The study was carried at Rhizosphere Biology Lab, Department of Biological Sciences, G.B Pant University of Agriculture & Technology, Pantnagar (U.K., India) between January-July, 2017.

**GRSP production:** The seeds of sorghum were sterilized with 0.1% clorox solution (water, sodium hypochlorite, sodium chloride, sodium carbonate, sodium chlorate, sodium hydroxide and sodium polyacrylate) followed by four rinsing with sterilized water. The infective propagules were estimated from separately maintained AM cultures[11]. Two hundred infective propagules each of *Funneliformis coronatum*, *Acaulospora scrobiculata* and *Gigaspora margarita* were used to inoculate sorghum in 500 mL pots containing steam sterilized soil: sand (1:1) mixture. AMF inoculum was provided in holes and one germinated sorghum seed was kept on to the inoculum and covered with the same pot soil. Plants were watered 3 times a week with autoclaved distilled water and Hoagland's solution was added weekly having ¼ dose of phosphorus. AMF inoculum was produced separately for two cycles of 60 days each. Plants without inoculation of any AMF served as control, however they did receive microbial wash from 1 g inoculum of AMF which was obtained by filtering 1g inoculum through Whatman filter paper No. 1. The experiment was conducted in greenhouse with 18 h of light (600 µmol photons $m^{-2}$ $sec^{-1}$) and 27±2°C temperature. Each treatment (inoculated and uninoculated) was replicated 4 times. After every harvest, the shoot was harvested from both AMF and control treatments. Shoot was dried in oven at 65°C for 48 h and weighed.

**Extraction, precipitation and dialysis of protein:** After harvesting the plants, soil from each replicate was mixed thoroughly for extraction of GRSP, 0.25 g samples of dry-sieved 1–2 mm aggregates extracted with 2 mL of extractant (20 mM citrate, pH 7.0 at 121°C for 30 min), following the method of Wright and Upadhyaya[12]. Extraction process was repeated twice from the same sample. After extraction, precipitation was carried out in 1 N HCl and reconstituted in 100 mM sodium borate (pH 8.0). Dialysis was done with 10 mM sodium borate at pH 8.0. GRSP samples were lyophilized and again reconstituted in distilled water. Reconstituted protein was stored at -20°C.

**Estimation of GRSP:** GRSP was estimated by modified Bradford dye binding assay[13] with BSA as the standard and concentration was extrapolated to mg $g^{-1}$ soil.

***In-vitro* evaluation and estimation of inhibition of *Rhizoctonia solani* and *Colletotrichum falcatum*:** Poisoned food technique was used to see the impact of GRSP on two pathogens i.e., *Rhizoctonia solani* and *Colletotrichum falcatum*. In control, extractant was taken from control pot grown in absence of AMF. Two concentrations; 0.4 and 0.8 mg/petri plate of GRSP, were used from three species of *F. coronatum*, *A. scrobiculata* and *G. margarita*, separately. Discs of 5 mm diameter was taken from full grown petri plate of either *R. solani* or *C. falcatum* and was placed over GRSP containing petri plate as well as control petri plates. Each treatment was replicated 4 times. Growth inhibition was measured as reduction in the radial growth of pathogen with GRSP over control. Percent inhibition was calculated using formula[14]:

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where, C and T is radial growth of pathogen in control and treatment, respectively.

**Statistical analysis:** The data were analyzed by one-way ANOVA. All treatment means were tested for significant differences at $p<0.05$.

## RESULTS

**Plant growth and production of GRSP:** Significant growth enhancement of sorghum was observed in either of the AMF inoculated plants. Sorghum plants were inoculated with *F. coronatum* showed significantly ($p<0.05$) higher shoot dry weight followed by *A. scrobiculata* and *G. margarita*.

Color of GRSP from pot soil varied from dark brown to yellow brown with different AMF species, however, no color was detected in control. Significantly higher ($p<0.05$) amount of GRSP was recorded in case of *F. coronatum* followed by *G. margarita* and *A. scrobiculata* (Table 1).

**Percent inhibition of *R. solani* and *C. falcatum*:** Percent inhibition of *R. solani* and *C. falcatum* was highest by GRSP extracted from pot inoculated with *F. coronatum* followed by *A. scrobiculata* and *G. margarita* (Table 2). The percent inhibition of pathogen was higher at higher concentration of GRSP in petri plates (Table 2). All the data were significantly different at $p<0.05$.

## DISCUSSION

In the current study, results support the possible role of AM in suppression of soil borne plant pathogens. Research on plant mycorrhizal interactions has mostly focused on the physiology of nutrient/carbon exchange and plant signal-transduction mechanisms controlling the bidirectional interaction. Comparatively less is reported about the mechanisms conferring non-nutritional contributions by mycorrhiza, such as suppression of soil-borne diseases and enhancing plant resistance to pests and diseases[15]. There are evidences obtained from the decomposition studies that accumulation of GRSP in soil is of AMF origin[7]. It has been reported that AMF can reduce disease incidence and propagule number of several soil borne pathogens including *Aphanomyces*, *Fusarium*, *Phytophthora*, *Pythium* and *Verticillium* species in the plant and mycorrhizosphere[10,16,17]. Although the mechanisms implicated are still not well characterized, direct and indirect interactions between AMF and pathogens have been put forward as a plausible hypothesis to explain the role of AMF in biological control of root diseases[3]. Present finding is additional information proving the role of GRSP in suppression of soil borne pathogens.

In this experiment, shoot and root growth of sorghum plants varied with different mycorrhizal species: *F. coronatum*, *A. scrobiculata* and *G. margarita*. It indicates that different species of AMF shows different effects on same variety of plant. GRSP production was found significantly higher in soils used to grow plants with *F. coronatum*, followed by *G. margarita* and *A. scrobiculata*. This could be the result of the performance of an AMF species to produce extra radical hyphae which is ultimately responsible for the release of GRSP in soil. Significant plant growth enhancement by *F. coronatum* reflects more functionality of extra radical hyphae and therefore, there is a possibility that the amount of extra radical hyphae is more in this case and so, the amount of GRSP. Absence of GRSP in the control pots assures the production of this glycoprotein from AMF only. This describes implode of GRSP from different species of AMF is variable. *In vitro* studies showed that at different concentrations of GRSP percent, inhibition of *R. solani* and *C. falcatum* was significantly higher

Table 1: Concentration of GRSP from different AMF pure culture

| AMF species | GRSP (mg g$^{-1}$ soil) |
|---|---|
| *F.coronatum* | 12.8 (21.02) |
| *A. scrobiculata* | 8.06 (16.72) |
| *G. margarita* | 9.12 (17.35) |
| Control | 0.0 (0.0) |
| LSD at 5% | 3.12 |

Angularly transformed values are given in parenthesis

Table 2: Percent inhibition of *Rhizoctonia solani* and *Colletotrichum falcatum* at different concentration of GRSP

| | Percent Inhibition of *R. solani* | | Percent Inhibition of *C. falcatum* | |
|---|---|---|---|---|
| AMF species | % inhibition at 0.8 mg GRSP/petri plate | % inhibition at 0.4 mg GRSP/petri plate | % inhibition at 0.8 mg GRSP/petri plate | % inhibition at 0.4 mg GRSP/petri plate |
| *F. coronatum* | 66.11 (54.4) | 23.30 (28.85) | 63.3 (52.69) | 18.44 (25.4) |
| *A. scrobiculata* | 23.96 (29.28) | 11.74 (20.02) | 31.55 (34.16) | 9.94 (18.35) |
| *G. margarita* | 17.36 (24.59) | 7.61 (15.93) | 24.27 (29.49) | 8.75 (17.16) |
| Control | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| LSD at 5% | 2.53 | 1.97 | 2.00 | 2.62 |

Angularly transformed values are given in parenthesis

at higher concentration of GRSP showing antifungal activity. The result indicated a direct antibiosis of GRSP in the suppression of pathogen under in vitro condition.

A new putative focusing result was found, which provide a new possible and promising explanation to the involvement of AM fungi in plant protection against soil borne pathogens and point to their use as biological control agents.

## CONCLUSION

The current study suggests that the amount of GRSP production varies greatly with different AM species. *F. coronatum* showed the highest level of GRSP production in the soil. The study reveals alternative roles of GRSP other than soil particle aggregation.

## SIGNIFICANCE STATEMENTS

This study discovers the plausible explanation of involvement of AMF in contributing towards plant protection against soil borne plant pathogens that can be beneficial for understanding its mechanism of mycorrhizae induced resistance. This study will help the researchers to uncover the critical areas of mycorrhizae induced resistance related transcriptomics. Thus, a new theory on glomalin related soil protein (GRSP), acting as 'shield glycoprotein' against soil borne plant pathogen is being presented.

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