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Effect of *Glomus intraradices* and *Rhizobium* on the Growth of Groundnut (*Arachis hypogaea* L.)

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Abstract

Arbuscular mycorrhizal (AM) fungi can increase the capability of the root systems to absorb and translocate phosphorus (P) and minor elements through an extensive network of mycelium. AM fungi are commonly associated with legumes and can increase nutrient uptake of plants growing in high phosphate fixing soils. The present study the efforts of AM fungi *Glomus intraradices* and *Rhizobium* on groundnut *Arachis hypogaea* L. The AM fungi *Glomus intraradices* and *Rhizobium* inoculation plants resulted in production of highest biomass such as plant, shoot length, root length, number of leaves, total number of root nodules, fresh and dry weight of plants and the biochemical content such as chlorophyll 'a' and 'b', total sugar, starch content, proline, carbohydrates and protein contents. The inoculation of *Glomus intraradices* + *Rhizobium* showed an enhanced biomass when compared to other treatments.

1. Introduction

Mycorrhizae are mutually beneficial (symbiotic) relationship between fungi and plant roots. The ancient fungi colonize approximately 90% of the Earths land plant species (Gadkar *et al.,* 2001). AM fungi infect and spread inside the root. They possess special structures known as vesicles and arbuscules. The plant roots transmit substance (some supplied by exudation) to the fungi, and the fungi aid in transmitting, nutrients and water to the plant roots (Auge *et al.,* 2003). The hyphas reach into additional in wetter soil areas and help plants absorb many nutrients, particularly the less available mineral nutrients such as phosphorus, zinc, molybdenum and copper. AM fungi form a kind of sheath ground the roots some times giving it a hairy, cottony appearance. Because they provide a protective cover of plants.

The groundnut (*Arachis hypogaea* L.) is a species in the legume family it is an important oil and protein source and is grown widely in the semi-arid tropics. The groundnut generally cultivated in poor environments, even recently bread cultivars are selected to grow in such a poor environment.

The groundnut plants have a high phosphorus requirement for nodule formation, nitrogen fixation and optimum growth (Turk *et al.,* 2006). Mycorrhizal condition of legume crop found to increase its vegetative growth and seed yield in addition to improve nodulation on it's root system (Mathur and Vyas, 2000). The AM fungi suggested that the plants have universal system for monitoring their microbial affinities that may be either materialistic or antagonistic depending on the form of symbiosis, environmental conditions and individual genetic characters of interacting organisms. These regulatory systems were not only apparently important for evolution of the beneficial microbial interactions that contribute generally to the adaptive potential of terrestrial plants, but also create a more favourable environment for development of ecosystems processes (Azouni *et al.,* 2008).

2. Materials and Methods

This study is to evaluate the effects of *Glomus intraradies* and *Rhizobium* on groundnut plants. Seeds : Seeds of groundnut *Arachis hypogaea* L. variety VIRGIN 7 were obtained from oil seeds research institute Vridhachalam, Cuddalore district, Tamil Nadu.

Glomus intraradices: The AM fungi species of *Glomus intraradices* were collected from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The *Rhizobium* species were collected from Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University.

AM fungus inoculum preparations

Soil (1:1) mixture containing spores and infected root segments of maize, infected with *Glomus intraradies* and grown for 75 days. Served as the mycorrhizal inoculum (approximately 500 spores by placing 3 cm below the seed level and 0.5 ml of rhizobioal culture (10^6 cells).

Experiments

Five seeds of peanut were sown in each earthen pots separately were filled with

5 kg/pot of sandy and loam soil. The experiment was arranged as a randomized black design with five replicates for each treatments. The following replicates were carried out with the following treatments. Control, inoculation with AM fungi, (*G. intraradices*) and inoculation with *Rhizobium* and inoculation with *G. intraradices* + *Rhizobium*.

Sampling

Groundnut plants were collected at 7, 30, 60, 90, 105 days and their shoot and root length was recorded. The roots were dipped in water to remove adhering soil particles and washed with tap water and distilled water the number of root nodules were estimated. Total dry weight of shoots and roots were determined after drying at even 80°C of a constant weight.

The chlorophyll content was assayed according to (Li, 2000) the extraction was made from a 100 mg fresh sample in 25 mL acetone (80%) in the dark at the room temperature and measured at 470, 646 and 663 nm with a UV sepectrophotometers.

The sugar and protein content of plant materials were estimated according to (Nagui 1963) and (Bradford, 1976). Nitrogen (N) content was extracted from sulfuric acid using the semi-microkjeldhal method (Jockson *et al.,* 1973). Phosphorus (P) was extracted by nitric acid and perchloric acid digestion and measured using the vanadoso-molybdophoshoric colorimetric method (Jockson, 1967). Potassium (K) was assayed using a flame spectrophotometer (Allen *et al.,* 1984).

3. Results and Discussion

Shoot, root length: The experiment was carried out to inoculation with *G. intraradices* and *Rhizobium* on root and shoot length were presented in Tables 1 and 2. The data indicated that the dependence of groundnut plant growth on inoculation with *G. intraradices* + *Rhizobium* the increased the root and shoot length on all sampling days. The AM fungi infection is known to enhance plant growth by increasing nutrient uptake. The higher height increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption and greater rates of photosynthesis which obviously could have give to an increase in plant growth (Cooper,1984).

Table 1. Effect of *Glomus intraradices* and *Rhizobium* on shoot length (cm) of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	5.48±0.27	21.64±1.08	29.60±1.48	34.66±1.73	38.00±1.90
AM	9.56±0.47	25.75±1.43	38.60±1.93	45.46±2.27	48.66±2.43
Rhizobium	7.18±0.35	23.08±1.15	34.65±1.73	37.18±1.85	43.45±2.17
AM + *Rhizobium*	13.15±0.65	33.48±1.67	44.00±2.20	50.40±2.52	52.50±2.65

± Standard deviation

Table 2. Effect of *Glomus intraradices* and *Rhizobium* on root length (cm) of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	4.8±0.24	13.94± 0.69	18.48± 0.92	23.18± 1.15	27.15± 1.35
AM	7.14±0.35	18.13± 0.90	23.68± 1.18	28.16± 1.40	34.68± 1.73
Rhizobium	6.70±0.33	15.48± 0.77	20.46± 0.81	25.63± 1.28	30.46± 1.52
AM + *Rhizobium*	9.68±0.481	24.18± 1.20	28.66± 1.43	34.44± 1.72	36.66± 1.83

± Standard deviation

The AM fungi are a group of plant growth promoter reported to improve the overall growth of various crops (Dey *et al.,* 2004; Vikram *et al.,* 2007). The increase in plant growth may be attributed to increased uptake of phosphorus by AM fungi which increase the activity of root nodule bacteria as also reported by Mytton and Livesey (1983) (Table 3). It is evident from the maximum number of arbuscular, vesicles and spores in duel inoculated plants may be due to the fact that both *G. intraradices* and *Rhizobium* are active in not cortical cues (Charitha Devi and Reddy, 2001). That means the presence of one symbiont may be influencing the activity of the other as also suggested by Vejasadova *et al.* (1992) in soybean. It was found that AM fungi symbionts in legume plants has been attributed to high 'P' uptake necessary for nodulation and N_2-fixation and also improve N absorption (Liu *et al.,* 2002).

Table 3. Effect of *Glomus intraradices* and *Rhizobium* on the root nodules of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	2.0±0.10	75.68±3.78	166.45±8.32	275.66±13.75	295.60±147.78
AM	7.15±0.35	115.66±5.75	201.40±10.07	310.14±15.5	345.55±17.27
Rhizobium	5.40±0.27	86.60±4.33	185.80±9.28	290.66±14.53	315.66±15.78
AM + *Rhizobium*	10.18±0.50	164.00±8.20	254.45±12.72	345.10±17.25	370.66±18.53

± Standard deviation

Table 4. Effect of *Glomus intraradices* and *Rhizobium* on the fresh weight (mg/g fr. wt.) of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	8.51±0.42	18.60±0.93	30.65±1.53	38.01±1.90	42.56±2.13
AM	10.15±0.50	21.45±1.07	38.66±1.93	46.68±2.33	50.66±2.53
Rhizobium	9.16±0.45	20.00±1.00	32.45±1.62	40.14±2.00	44.45±2.22
AM + *Rhizobium*	12.15±0.60	25.16±1.25	45.18±2.25	50.44±2.52	56.86±2.74

± Standard deviation

Table 5. Effect of *Glomus intraradices* and *Rhizobium* on the dry weight (mg/g dry wt.) of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	3.13±0.15	9.65±0.48	14.68±0.73	19.56±0.97	24.51±1.22
AM	4.18±0.20	14.63±0.73	17.18±0.85	25.68±1.28	29.60±1.48
Rhizobium	3.04±0.15	11.54±0.57	12.15±0.60	16.01±0.85	19.18±0.95
AM + *Rhizobium*	5.65±0.25	16.18±0.80	20.04±1.00	24.13±1.20	29.60±1.48

± Standard deviation

Table 6. Effect of *Glomus intraradices* and *Rhizobium* on the chlorophyll 'a' of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	0.415±0.020	0.616±0.030	0.918±0.045	1.150±0.057	1.00±0.05
AM	0.565±0.028	1.860±0.093	2.150±0.107	2.863±3.25	2.110±0.105
Rhizobium	0.501±0.025	0.980±0.049	1.140±0.057	1.850±0.092	1.560±0.078
AM + *Rhizobium*	1.860±0.093	2.114±0.105	2.865±0.143	3.150±0.157	2.763±0.138

± Standard deviation

Table 7. Effect of *Glomus intraradices* and *Rhizobium* on the chlorophyll 'b' of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	0.115±0.005	0.246±0.012	0.301±0.015	0.368±0.018	0.315±0.015
AM	0.260±0.013	0.515±0.025	0.718±0.035	0.986±0.049	0.860±0.043
Rhizobium	0.217±0.010	0.463±0.023	0.571±0.028	0.718±0.003	0.700±0.035
AM + *Rhizobium*	0.513±0.025	0.861±0.043	1.650±0.082	1.980±0.009	0.985±0.049

± Standard deviation

Table 8. Effect of *Glomus intraradices* and *Rhizobium* on the total carbohydrates of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	0.651±0.032	0.951±0.047	1.313±0.065	1.763±0.088	1.986±0.099
AM	1.865±0.093	2.631±0.131	2.951±0.147	3.014±0.150	3.654±0.182
Rhizobium	0.947±0.047	1.085±0.054	1.343±0.067	2.850±0.142	3.100±0.155
AM + *Rhizobium*	2.113±0.105	3.154±0.157	4.850±0.242	5.630±0.281	6.850±0.342

± Standard deviation

Table 9. Effect of *Glomus intraradices* and *Rhizobium* on the protein of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	1.840±0.092	2.350±0.117	3.113±0.155	4.103±0.205	4.000±0.200
AM	2.650±0.132	4.630±0.231	6.850±0.342	8.450±0.422	8.150±0.407
Rhizobium	2.003±0.100	3.145±0.157	4.801±0.240	6.153±0.307	7.803±0.390
AM + *Rhizobium*	3.650±0.185	5.810±0.290	8.630±0.431	11.850±0.592	11.001±0.55

± Standard deviation

Table 10. Effect of *Glomus intraradices* and *Rhizobium* on the soluble sugar of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	1.67±0.083	2.15±0.107	3.76±0.188	5.97±0.298	7.81±0.390
AM	2.84±0.142	4.86±0.243	6.15±0.307	8.00±0.400	10.63±0.531
Rhizobium	1.98±0.099	3.13±0.156	4.55±0.227	6.67±0.333	8.13±0.406
AM + *Rhizobium*	3.45±0.172	5.16±0.258	7.67±0.383	9.13±0.456	12.86±0.643

± Standard deviation

Table 11. Effect of *Glomus intraradices* and *Rhizobium* on the starch content of groundnut *Arachis hypogaea* L.

Treatments	7 day	30 day	60 day	90 day	105 day
Control	1.13±0.056	2.45±0.122	3.68±0.184	4.18±0.209	6.18±0.309
AM	2.43±0.121	4.81±0.240	6.16±0.308	7.18±0.359	9.15±0.457
Rhizobium	1.89±0.094	3.18±0.159	4.15±0.207	5.07±0.253	7.23±0.361
AM + *Rhizobium*	3.84±0.192	5.14±0.257	6.89±0.344	8.67±0.433	9.15±0.457

± Standard deviation

Table 12. Effect of *Glomus intraradices* and *Rhizobium* on the Nutrient content of groundnut *Arachis hypogaea* L.

Treatments	105 days		
	N	P	K
Control	1.98±0.099	0.38±0.019	1.75±0.087
AM	2.93±0.146	1.51±0.075	2.60±0.130
Rhizobium	2.21±0.110	1.42±0.071	2.15±0.010
AM + *Rhizobium*	4.31±0.215	2.30±0.115	3.60±0.180

± Standard deviation

The fresh and dry weight of plants affected by due inoculation of *G. intraradices* and *Rhizobium* were shown in Tables 4 and 5. The *G. intraradices* + *Rhizobium* inoculated plants were increased in the fresh, dry weight. This result was in correspond to (Kirshan and Bagyaraj, 1984) who reported that inoculation with the mycorrhizal fungus *Glomus fasciculatum* enhanced peanut growth and increased its dry matter more than 2-fold compared with control. AM fungi inoculated plants have the fungal hypha increase root surface area, resulting in exploring higher volume of soil and overcoming the water and nutrient depletion zones around the roots leading to increased water and nutrient content (Clark and Zeto, 2002).

The pigment content of chlorophyll 'a and b' and carbohydrate significantly increased the *G.*

intraradices + *Rhizobium* inoculation treatment when compared with other treatments (Tables 6 and 7).

Infection with single AM fungi also significantly, increases the chlorophyll 'a' and 'b', higher than those of both *Rhizobium* inoculation and control plants (Hayman, 1982). The AM associations in terrestrial plants have been shown to increase chlorophyll production compared with without fungal associations. The total carbohydrate also significantly increase with inoculation of groundnut plants. The AM fungi increased leaf gas exchange and photosynthetic rate, (Ruiz-Lozano *et al.,* 1996) and enhanced water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity (Dell'Amico *et al.,* 2002). The most effective duel inoculation was observed in the combined treatment with AM fungi + *Rhizobium* which synergistically increased the chlorophyll 'a and b', and carbohydrate content compared with other treatments. The enhancement in chlorophyll and carbohydrate content can be attributed to increase the absorption and translocation of essential metals and ions (Table 8). AM fungi infections which in turn accelerate the metabolic rates related to the synthesis of such constituents and inoculated plants could be as a result of enhanced inorganic nutrient absorption (Copper, 1984). The protein content of *Arachis hypogaea* inoculated with *G. intraradices* + *Rhizobium* after 7th, 30, 60, 90, 105 days of growth studded and the results are given in Table 13. Plants with single inoculation showed significant increase in protein content than control plants. The increase in protein content of groundnut plants was mainly attributed to the beneficial effect of inoculation with the experimental *G. intraradices* + *Rhizobium*. This significant increasing in protein content respectively, comparing to the control. In this experiment there was an increase in the percentage of N and P in plant. It was found that organic acids added to the soils increased the plant uptake of P from a water soluble (Bolan *et al.,* 1991) and also the release of organic acids that both sequester cations and acidity the micro environment near the roots is through to be major mechanisms of P-uptake as well as Mn, Fe and Zn by plants and non AM fungi (Cunningham and Kuiack, 1992).

Total sugar and starch content in groundnut plants inoculated with *G. intraradices* + *Rhizobium* after 7, 30, 60, 90 and 110 days of were studied and the results are given the Tables 10 and 11. The total sugars and starch content were significantly high in AM fungi + *Rhizobium* treatment when compared to control. The AM fungi provide water and nutrition to their host plants and in return host plants transfer their carbohydrate to AM fungi for the energy source (Wu and Xla, 2006).The results suggested that AM symbiosis mainly demanded soluble sugar provided by host plant. A ^{14}C-labelling experiment showed that mycorrhizal roots, respectively, accumulated 66% and 68% of the ^{14}C-labelled photosynthates translocated to the roots of sour orange and Carrizo citrage (Koch and Johnson, 1984). The distribution was independent of the status of phosphorus in levels. Increase in total sugar and starch content of citrus roots colonized by AM fungi however, the transferring processes to carbohydrate from host plant to AM are unclear (Nemee and Guy, 1982; Maronek *et al.,* 1981). AM fungi are capable of converting absorbed soluble sugar into storage compounds that are not ready available to the plant such as glycogen, mannitol or tetreralose.

The growth rate and nutrient uptake were more in AM fungi plants. Any combination containing AM fungi showed significant increase in plant growth and nutrient level.

The nutrient content N, P, K in groundnut plants inoculated with *G. intraradices* + *Rhizobium* after all sampling days were studied and the results are given in the Table 12. The N, P, K content significantly increased in *G. intraradices* + *Rhizobium* treatment when compared to control plants.

In legume plants are the important of AM fungi symbiosis has been attributed to high P requirements on the nodulation and N_2 fixation process which requires enhanced P uptake (Barea and Azcon-Agnilar, 1983) and AM fungi have been shown to improve productivity in soils of low fertility (Jaffries, 1987) and particularly important for increasing the uptake of slowly diffusing ions such as PO_4 (Jacobsen *et al.,* 1992). The mycelium of the AM fungus can access these phosphorus sources and make them available to the plans they colonize (Li *et al.,* 2006).

The mechanism of increased absorption are both physical and chemical. Mycorrhizal mycelia are much smaller in diameter than the smallest root air. For this reason they are able to explore a greater volume of soil and have a much larger surface area for absorption and also the cell membrane chemistry of fungi is different from plants. Mycorrhizae are especially beneficial for the plants partner in nutrient poor soils (Azouni *et al.,* 2008). Krishan and Bagyaraj (1984) reported that the inoculation of peanut plants with *Glomus fasiculatum* increased uptake of phosphorus and micronutrients such as zinc, copper, manganese and iron.

The nitrogen content in AM inoculated plants would be attributed to hyphae uptake the existence of extra-radical hyphal bridges between individual plants permits transfer of nutrient such as N (Marschner and Dell, 1994).

Azouni *et al.* 2008 reported that about 24% of the total nitrogen uptake in AM inoculated plants could be attributed to uptake and delivery by the external hyphae. There is also evidence that nitrogen is taken up by AM fungi hyphae from inorganic sources of ammonium and therefore the higher N inoculation in mycorrhizal plants could be attributed to the hyphae uptake.

4. Conclusion

It could reasonably be said the combined application of *Glomus intraradices* and *Rhizobium* inoculation increased al morphological parameters, biochemical content and nutrient content of groundnut plants.

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