ПРОБЛЕМИ ГЕОБОТАНІКИ ТА КУЛЬТУРБІОГЕОЦЕНОЛОГІЇ

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ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* UNDER VARIED DOZES OF NPK ON GREEN GRAM

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Pot culture experiments were carried out to assess the influence of variable levels of nitrogen and phosphorus, a consortium of arbuscular mycorrhizal fungi alone and the arbuscular mycorrhizal fungi along with *Rhizobium* on green gram, keeping potassium at full recommended level. Crop with full recommended doses of nitrogen, phosphorus and potassium, without the microbes was the control. *Glomus mosseae* and *Glomus microcarpum* were the arbuscular mycorrhizal fungi applied as a consortium in these experiments. *Rhizobium* isolated from a natural source was used as the bacterial strain along with the arbuscular mycorrhizal fungi. Dry biomass, total leaf area, total chlorophyll, percentage colonization, nodulation, tissue nitrogen, phosphorus and potassium, seed yield, and the post harvest spore count were the significant parameters of growth and yield of the crop examined in these experiments. The plants were grown in six replicated samples, in plastic pots, in a completely randomized design. Samplings were done on the 30th and the 60th day of growth after inoculation and the harvest data was taken on 68th day after inoculation. Overall assessment was that the application of arbuscular mycorrhizal fungal consortium and *Rhizobium* to green gram can significantly enhance its growth parameters and yield, at lesser levels than the full recommended doses of chemical fertilizers.

Key words: AMF consortium, Rhizobium, NPK, green gram.

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ВПЛИВ ДЕРЕВОПОДІБНОГО МІКОРИЗНОГО ГРИБА ТА БАКТЕРІЇ *RHIZOBIUM* НА КВАСОЛЮ ЗОЛОТИСТУ ПРИ ВНЕСЕННІ РІЗНИХ ДОЗАХ АЗОТУ, ФОСФОРУ ТА КАЛІЮ

Експериментальним шляхом оцінювався вплив різних рівнів азоту та фосфору, консорції деревоподібного мікоризного гриба окремо та разом з бактерією *Rhizobium* на квасолю золотисту, підтримуючи калій на необхідному рівні. Рослина з внесенням рекомендованих доз азоту, фосфору та калію, без мікроорганізмів, була контрольною. Для експерименту були узяті *Glomus mosseae* та *Glomus microcarpum* як консорція деревоподібного мікоризного гриба. *Rhizobium,* відокремлений від природного джерела, був використаний як бактеріальна раса разом з деревоподібним мікоризним грибом. Суха біомаса, загальна листова площа, загальний уміст хлорофілу, процент колонізації, утворення бульб, тканинний азот, фосфор та калій, урожай насіння, підрахунок спорового збору були значущими показниками росту та врожаю досліджуваної культури. Рослина вирощувалася у шістьох екземплярах у пластикових горщиках з повністю рандомізованим планом. Відбір зразків здійснювався на 30 та 60-й день розвитку після інокуляції, а збір клітин, які виросли в культурі, виконаний на 68-й день після інокуляції. Було доведено, що застосування консорції деревоподібного мікоризного гриба та бактерії *Rhizobium* суттєво збільшує показники росту та врожай квасолі золотистої у меншій мірі, ніж використання мінеральних добрив у рекомендованих дозах.

Ключові слова: консорція деревоподібного мікоризного гриба, бактерія Rhizobium, азот, фосфор, калій, квасоля золотиста.

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ВОЗДЕЙСТВИЕ ДРЕВОВИДНОГО МИКОРИЗНОГО ГРИБА И БАКТЕРИИ *RHIZOBIUM* НА ФАСОЛЬ ЗОЛОТИСТУЮ ПРИ ВНЕСЕНИИ РАЗЛИЧНЫХ ДОЗ АЗОТА, ФОСФОРА И КАЛИЯ

Опытным путем оценивалось влияние различных уровней азота и фосфора, консорции древовидного микоризного гриба отдельно и вместе с бактерией *Rhizobium* на фасоль золотистую, поддерживая калий на необходимом уровне. Растение с внесением рекомендованных доз азота, фосфора и калия, без микроорганизмов, было контрольным. Для эксперимента были взяты *Glomus mosseae* и *Glomus microcarpum* в качестве консорции древовидного микоризного гриба. *Rhizobium,* отделенный от естественного источника, был использован как бактериальная раса вместе с древовидным микоризным грибом. Сухая биомасса, общая лиственная площадь, общее содержание хлорофилла, процент колонизации, образование клубней, тканевый азот, фосфор и калий, урожай семян, подсчет спорового сбора были значимыми показателями роста и урожая исследуемой культуры. Растения выращивались в шести экземплярах в пластиковых горшках с полностью рандомизированным планом. Взятие образцов осуществлялось на 30 и 60-й день развития после инокуляции, а сбор клеток, выросших в культуре, проведен на 68-й день после инокуляции. Было доказано, что применение консорции древовидного микоризного гриба и бактерии *Rhizobium* существенно увеличивает показатели роста и урожай фасоли золотистой в меньшей степени, чем использование минеральных удобрений в рекомендуемых дозах.

Ключевые слова: консорция древовидного микоризного гриба, бактерия Rhizobium, NPK, фасоль золотистая.

Arbuscular mycorrhiza (AM) is accepted as a synergist for pulse crops in general (Powell and Bagyaraj, 1984). *Glomus* isolates are effective in increasing growth and seed yield in pulse crops such as soybean (Carling and Brown, 1980; Vejsadova et al., 1992). The effect of interaction between AM fungi (AMF) and *Rhizobium* in soybean and other leguminous plants is also well known (Bagyaraj et al., 1979; Manjunath et al., 1984; Kumar and Potty, 1998; Devi and Reddy, 2001). Among pulse crops, green gram (*Phaseolus aureus* Roxb.) is unique with its better nutritional and medicinal properties (Sabnis and Daniel, 1990). Increased plant growth and seed yield are recorded in green gram consequent to inoculation with AM fungi (Rao and Rao, 1996). In green gram, *Rhizobium* and AM are known to have compatible and synergistic interrelationship and a common better antagonism to the nematode than their individual effects (Ray and Dalei, 1998). Synergistic effect of *Glomus* and *Rhizobium* in nodulation, nitrogen fixation, growth, dry matter production and seed yield in green gram is also known (Thakur and Panwar, 1997: Hazarika et al., 2000).

Agricultural systems aiming at high productivity need the use of microbial synergists and chemical fertilizers such as Nitrogen (N), Phosphorus (P) and Potassium (K); P (Murdoch et al., 1967; Mosse, 1973; Bolan et al., 1984; Chandrashekara et al., 1995), N (Lanowska, 1966; Hayman, 1970) and NPK (Lanowska, 1962; Alexandrova, 1968). Two species of *Glomus* (*G. mosseae* and *G. microcarpum)* and *Gigaspora margarita,* and a species of *Scutellospora* are the common synergistic fungi found associated with the roots of this crop in its traditionally cultivated natural fields in South India (Valsalakumar et al., 2007). Experimental applications of those individual species of AM fungi, *Piriformospora indica*, an axenically culturable fungus that mimics phytopromoting properties of AMF, and *Rhizobium* in green gram revealed that the impacts of certain of them on growth parameters of the crop such as total biomass, tissue nitrogen, tissue phosphorous, total leaf area, total chlorophyll, percentage colonization and total number of root nodules in the crop are positively and significantly different over the different microbial combinations used (Ray and Valsalakumar, 2009).

Use of AM and other microbial substitutes are essential in developing sustainable farming procedures of crops in general, in order to reduce costly chemical fertilizers which are harmful to environment as well. However, it is an established fact that microbial associates can improve plant growth only if nutrients are available in the soil at a specific concentration (Tsai et al., 1993). Moreover, small additions of nutrients are necessary to improve plant growth and AMF infection in nutrient poor soil (Mosse, 1973a).

Present experiments were to test the quantitative requirement of NPK on the synergistic influence of AM fungal consortium rather than individual species of AMF, and *Rhizobium* on green gram. The consortium of two species of AM fungi used in the present experiment were *Glomus mosseae* (*G. mosseae*) and *Glomus microcarpum* (*G. microcarpum*) which were effective in promoting productivity in green gram, when applied individually (Ray and Valsalakumar, 2009) and the *Rhizobium* used was an experimentally derived good isolate. There were two sets of experiments; first set included a consortium of AM fungi alone and variable amounts of N and P, and the second set included consortium of AM fungi along with *Rhizobium* and variable amounts of N and P; both sets received full recommended dose of K.

MATERIALS AND METHODS

The variety of green gram used in the experiment was 'Co6' supplied by the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The plants were grown in plastic pots (Allen et al., 1976) of 23cm diameter in completely randomized design (CRD). The pot mixture consisted of thoroughly mixed soil, fine sand and powdered and dried cow dung in the ratio 3:1:1. The soil used in potting mixture was the top layers of a Each pot was filled with 5 kg potting mixture. The pots were filled to ¾ of their capacity. Gravelly Clay Loam on Laterites with mechanical composition of about 40.1% coarse sand, 28.5% fine sand, 8.8% silt and 22.6% clay; the available nitrogen, phosphorus and potassium of the pot mixture were at amounts 21.2mg kg^{-1}, 4.48mg kg^{-1} and 60.07mg kg^{-1} respectively, and the pH was 6.33. According to Muhr et al., 1965, N and P at this amount in a soil are considered low and K is medium. Therefore, addition of N and P along with the microbes was considered in the experiment. The pots and pot mixture were sterilized by formaldehyde treatment (Rajithkumar and Potty, 1998). Pure cultures of *G. mosseae* and *G. microcarpum* used were obtained from the AM fungal collections of Microbiology Division of Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India. The inoculums of the different AM fungi were multiplied in double autoclaved soil (N- 18mg/kg; P- 4.81mg/kg; K- 59mg/kg), employing a gramineaceous plant (Kabberathumma et al., 1986), Italian millet (*Setaria italica* Beauv.), as host in pot culture for eight weeks. The soil along with spores, hyphae and root segments were used for inoculating the test plants. The spore load of soils after multiplication were; *G. mosseae* - 200/100g soil, and *G. microcarpum* - 734/100g soil. The total spore load of the inoculum was adjusted to 400-spores/100g soil, with approximately equal number of spores of each AM fungus. Spore count of the soil was derived as per Smith and Skipper, 1979.

The AMF inoculum was added and mixed with the topsoil in each pot. The pots included 19 sets with six replications each, which consisted of control, nine combinations of AMF consortium and NPK, and nine combinations of AMF consortium plus *Rhizobium* and NPK. The treatment combinations were as given in the Table 1.

The *Rhizobium* culture used in the experiment was an isolate from *Phaseolus multiflorus*, which was evaluated for its effect on green gram and was found to be more productive among six isolates tested. The *Rhizobium* inoculum was prepared in Yeast Extract-Manitol (YEM) medium as per Dye, 1979. The *Rhizobium* cultures were incubated at 35^0C for 48 h and used for inoculating the seedlings. The *Rhizobium* inoculation was made on the next day after sowing the seeds. 10ml of 48 h old bacterial culture (approximately 10^9 cells/ ml) was added to the soil surrounding the seed using a pipette. 10 ml sterile bacterial culture medium was added to those pots which were not given rhizobial inoculation.

Urea was used as the source of N, while Rock phosphate (Raj Phos) was used as the source of P to supplement the nutrient poor soil. Experiments included application of both these fertilizers at different combinations of 50 and 100% of their recommended dose (RD) along with the AM fungal consortium and *Rhizobium*. Control for both the set of experiments was crops with full recommended doses of NPK without the microbes. The nutrient K used was muriate of potash (MOP) and the dose of which was kept constant (100% of the RD) to all the treatment combinations. The full recommended doses of N, P and K for green gram were; N – 20kg ha^{-1}; P2O5 – 30kgha^{-1}; K_2O – 30kg ha^{-1}, derived as per methods of Kerala Agriculture University (2002).

Table 1

Treatment combinations of AM fungal consortium, *Rhizobium*, N and P

Sl. No.	First set of Treatment combinations (AM fungal consortium alone with variable doses of N and P)	Sl. No.	Second set of Treatment combinations of AM fungal consortium and *Rhizobium* with variable doses of N and P)
1	M0 R0 P2N2 **Control**: (Full RD of NPK **without microbes**)		
2	M1 R0 P0N0	11	M1 R1 P0N0
3	M1 R0 P0N1	12	M1 R1 P0N1
4	M1 R0 P0N2	13	M1 R1 P0N2
5	M1 R0 P1N0	14	M1 R1 P1N0
6	M1 R0 P1N1	15	M1 R1 P1N1
7	M1 R0 P1N2	16	M1 R1 P1N2
8	M1 R0 P2N0	17	M1 R1 P2N0
9	M1 R0 P2N1	18	M1 R1 P2N1
10	M1 R0 P2N2	19	M1 R1 P2N2

M0- without mycorrhiza
M1 – with AM fungal consortium
N0- without additional N
N1 – with 50% RD of N
N2 – with 100% RD of N
P0- without additional P
P1 – with 50% RD of P
P2 – with 100% RD of P
R0 – without *Rhizobium*
R1 – with *Rhizobium*

The pots were carefully arranged to avoid contamination between the treatments. Two surface sterilized and pre-soaked seeds of green gram were placed in each pot with a distance of 15cm in between. The fertilizer application was done on the 3rd day after sowing. The plants were watered once in a day up to 30 days and then twice in a day until harvest so as to avoid any chance of water stress (Borse et al., 2002). Pure freshwater (pH 6.8) from a well free of fungal spores was used for watering all the test plants throughout the experimental period. Samplings were done on the 30th and the 60th day of growth after inoculation (DAI) for studying growth parameters. Two pots from each treatment were selected during each sampling date and the remaining pots were kept for final harvest. The plants were uprooted, without damaging the root system. The root system was cleaned with water and all the fine fragments of roots were recovered. Total dry biomass, total leaf area, total chlorophyll, total number and fresh weight of nodules per plant, percentage of root colonization (Philips and Hayman, 1970), tissue NPK and total seed yield per plant were analyzed. For recording dry weight, the root and shoot were dried in an oven at 80^{0}C to constant values (Daft and Hogarth, 1983). Total leaf area was calculated using image processing with the help of MATLAB software. Fully expanded third leaf from the tip of the plant was taken for estimating total chlorophyll and tissue NPK (Thakur and Panwar, 1997). Chlorophyll content of the leaves was estimated as per Arnon, 1949. Sample preparation for analyzing tissue NPK was done as per (AOAC 1978). The estimation of nitrogen was carried out by micro-Kjeldahl method (AOAC 1978) using Kjeltec 1026 distilling unit. Tissue phosphorus (P) and potassium (K) were measured after triple acid digestion; P was estimated by Vanado-molybdate yellow- colour method and Flame photometric method – Instrument Model 128 Systronics - was used for the estimation of K (Jackson 1973). The harvest data (total seed weight per plant) was taken on 68th day after sowing the seeds. Statistical analysis such as critical difference (CD) and correlation were done with GENSTAT-6 software.

RESULTS

Details of various measurements of the treatments are given in Table 2. From among the first set of treatments involving AMF consortium alone and various doses of N and P, the highest mean total dry biomass per plant was obtained from M1R0P2N1 (15.16g). Dry mass at this treatment level showed an increase of 41% over that of the control. When *Rhizobium* was also incorporated in the treatment (second set), the highest dry biomass observed was in treatment of M1R1P0N1 (16.3g), which was 51.6% higher than that of the control. Among the treatments involving AMF consortium and different doses of N and P, the highest mean leaf area (LA) was observed in M1R0P0N1 (1316.12 cm^2). In treatments involving dual inoculation of AMF consortium and *Rhizobium* with different levels of N and P, highest mean LA was observed in M1R1P2N2 (1053.1 cm^2). In all treatments the average total chlorophyll content was significantly higher than that of the control. Among all the treatments, the highest amount of chlorophyll was noticed in M1R0P1N1 (0.906 mg/g FW). It represented an increase by 58% over that of the control. It became clear that AMF consortium significantly increased total chlorophyll in green gram at 50%RD of both N and P together. The highest recorded mean total chlorophyll among treatments involving AMF consortium, *Rhizobium* and fertilizer was 0.804 mg/g fresh weight (FW) in M1R1P2N1. It was 40% above than that in the control.

Table 2

Different treatments and the measures of various growth and yield parameters

Treatment		Total Dry biomass / plant (g)	Total LA/plant (cm^2)	Total Chloro-phyll (mg/g FW)	Leaf tissue			Nodules/ plant		% root colonization	Total weight of seed/ plant (g)
					N (%)	P (%)	K (%)	No.	FW (g)		
M0R0P2N2 (Control)		10.75	1135.44	0.573	3.95	0.355	1.31			0	5.24
M1R0	P0N0	8.95	736.63	0.807	3.86	0.345	1.38			46.3	5.06
	P0N1	11.96	1396.00	0.627	3.69	0.355	1.23			68.2	3.44
	P0N2	14.24	1281.58	0.738	4.24	0.36	1.19			60.6	5.23
	P1N0	3.81	657.53	0.738	4.6	0.44	2.16			46.4	4.24
	P1N1	6.5	784.32	0.906	4.64	0.405	1.97			41.8	4.84
	P1N2	10.29	1021.53	0.646	4.01	0.32	1.72			34.9	6.70
	P2N0	8.74	755.93	0.868	4.22	0.37	1.28			70.8	5.96
	P2N1	15.16	1195.2	0.790	4.0	0.335	1.43			58.6	5.26
	P2N2	12.06	1128.8	0.857	4.12	0.385	1.3			65.7	5.77
CD at 0.05 level		**4.29**	**243.5**	**0.0788**	**0.150**	**0.02**	**0.11**			**3.35**	**0.80**
M1R1	P0N0	11.44	983.14	0.666	3.15	0.325	0.87	85	0.87	72.2	4.57
	P0N1	16.3	902.21	0.634	3.25	0.32	1.25	147	2.27	49.7	6.16
	P0N2	7.73	620.01	0.632	3.65	0.36	0.92	45	0.78	63.9	4.43
	P1N0	9.63	674.17	0.636	4.21	0.39	1.74	129	1.14	82.2	3.72
	P1N1	10.22	989.36	0.747	3.78	0.385	1.51	103	1.48	61.8	5.92
	P1N2	9.21	752.81	0.723	3.85	0.405	1.28	51	0.53	55.2	4.29
	P2N0	10.61	922.34	0.743	3.9	0.465	1.695	87	0.74	60.2	5.99
	P2N1	9.42	678.74	0.804	3.98	0.385	1.64	166	1.63	55.0	6.02
	P2N2	12.28	1053.1	0.707	3.76	0.345	1.535	134	1.18	46.4	5.37
CD at 0.05 level		**4.954**	**344.4**	**0.1115**	**0.122**	**0.02**	**0.086**	**9.8**	**0.086**	**2.733**	**0.652**

In the absence of *Rhizobium*, the highest mean percentage of root colonization was observed in M1R0P2N0 (70.8). But along with *Rhizobium* the highest mean colonization (82.2) was observed in the treatment M1R1P1N0. There was 16% increase in percentage colonization in dual inoculation compared with AMF consortium alone treatment. In both cases, highest colonization was obtained with zero addition of N. In dual inoculation the P requirement for maximum colonization was recorded as 50% of RD. *Rhizobium* inoculation was found synergistic in AM fungal colonization. The highest number of nodules was observed in M1R1P2N1 (166), but the highest total weight of nodules was recorded in M1R1P0N1 (2.27g).

Among all the treatments, the total seed weight per plant (6.7g) was highest in M1R0P1N2. There was 28% increase in seed weight in this treatment over that of the non inoculated control supplemented with full RD of NPK. Thus it became clear that the AM association, even without *Rhizobium* could enhance the seed yield to its maximum in green gram at 50% reduced supply of P. When AM fungal consortium, *Rhizobium* and fertilizers at different levels were given, the highest total seed weight per plant (6.16g) was recorded in the treatment M1R1P0N1. The increase in seed yield in this case was 17.6% over that of the control. Result of the dual inoculation of AMF consortium and *Rhizobium* revealed that an increase of seed yield in green gram is possible with zero addition of P and only 50% of the RD of N. It was also noted that in the presence of AMF consortium and *Rhizobium,* significantly higher seed yield is possible with 50% RD of both N and P, compared to that in the control.

Among treatments involving AM and fertilizers, the highest percentage of tissue N was observed in M1R0P1N1 (4.64%), which was 17.5% higher than that of the control (3.95%). The mean leaf tissue P was the highest in M1R0P1N0 (0.44%). The P concentration in M1R0P1N0 represented an increase by 24% over that of control. The highest K concentration in leaf tissues among all the treatments was noticed in M1R0P1N0 (2.16%) which represented an increase by 65% over that of the control, suggesting a positive role of AMF in K nutrition in green gram.

In treatments involving AM, *Rhizobium* and fertilizers, the highest amount of leaf tissue N (4.21%) was observed in M1R1P1N0, which was 6.6% higher than that in the control. When the highest leaf tissue N in AMF consortium and fertilizer treatments was compared with that in dual treatments of AMF and *Rhizobium* together with fertilizers, there was 11% reduction in leaf tissue N in the latter than that in the former. The highest mean tissue P was noted in M1R1P2N0 (0.465%). The highest amount of P in leaf tissues (P use efficiency of the plants) observed in this dual inoculated plants was 31% higher than that of the control with full RD of N and P. It was evident that AMF consortium acted synergistically with *Rhizobium* with respect to P nutrition in green gram. In presence of NPK, the general behaviour of K content in the leaf tissue in dual inoculated plant resembled that of AMF consortium alone inoculated plants mentioned earlier. The highest mean tissue K (1.74%) was observed in M1R1P1N0 which was of 33% over that in the control plants. However, the highest amount of K noticed in leaf tissues among dual inoculation was about 32% lesser than that in single inoculation with AMF consortium alone and fertilizers.

Post harvest spore count of two species of AMF

Analysis of spore count in post harvest soil indicated that the two species of *Glomus* behaved differentially to treatment conditions (Fig. 1). It was observed that there was a general tendency for *G. microcarpum* to reduce sporulation in presence of the *Rhizobium*. There was no correlation (r = 0.048) between the spore count in *G. mossae* and *G. microcarpum*. The percentage root colonization and spore count were also not correlated. The average spore count of *Glomus mosseae* and *Glomus microcarpum* was 1110 and 957 per 100g soil respectively in the treatments involving AM fungal consortium, while it was reduced to 766 and 447 per 100g soil respectively in treatment involving AMF consortium and *Rhizobium*.

DISCUSSION

In production-intensive, integrated and sustainable farming procedures, analyses of microbial influence in presence of chemical fertilizers are important. Present experiments enabled a detailed examination of the combined effect of two species of *Glomus* together with *Rhizobium* to assess the optimum N and P fertilizer requirement under individual or dual treatments of AM and *Rhizobium* on an important pulse crop, green gram. These kinds of studies provide the base for field trials towards development of eco-friendly and sustainable increase in productivity of pulse crops in general.

It became evident from results of the present experiments that application of AMF species such as *Glomus* enables a significant increase in total biomass of the crop over that of the control at 50% reduced levels of N along with P and K at 100% of the RD**.** Even in the absence of N and P fertilizer AMF consortium along with K at the recommended full dose could ensure an average biomass of green gram comparable to that of the control. This is in agreement with the reports of Chandrashekara et al., 1995 that there is improvement of biomass in plants under AM along with different doses of fertilizers. When *Rhizobium* also was added along with AMF consortium, a significant increase in dry weight was noticed in the crop at zero addition of P fertilizer and N fertilizer at 50%RD. These observations agree with the findings of Mosse, 1973 that AM along with *Rhizobium* produced extra advantage on growth parameters when fertilizers are available at a certain critical level. The critical dose observed in the present experiments were N at 50% of RD and K at full RD. The conclusion was that application of the AMF consortium and *Rhizobium* to green gram can enhance the growth and biomass of the crop over that of cultivation using synthetic chemical fertilizers alone and that too at a lesser cost of production (less amount of N and no P fertilizer). Lesser use of chemical fertilizers with AMF consortium-*Rhizobium* mixture can reduce environmental damage which is prevalent in chemical intensive green revolution agriculture.

Results of the experiments of AMF consortium and different doses of N and P, keeping K at constant level, were compared with that of the control. It was revealed that keeping K at full RD, no additional P is required, if the two species of *Glomus* were applied to the crop for a significant increase in total leaf area of the crop over that of the control, provided N is given at 50% RD. Moreover, it was observed that in presence of these microbes and if K is maintained at the full of RD, further increase in the amount of N (to 100% RD) or P (to 50% or 100% RD) has no significant positive influence on average total leaf area of the plant over that of the control. It was also evident that a significant reduction in the average total leaf area of the crop happened when AM alone were applied without N and P, even though K was at full RD. Therefore, Dixon's, 1990 view of higher hormonal activity in AM fungal associated plants may be further examined, in connection with the role of extra nitrogen supply as a factor contributing to the enhancement of the leaf area in green gram. Compared to the control there was no significant difference in the average total leaf area of the crop in treatments involving *Rhizobium* and AMF consortium if extra addition of P and N fertilizers were not made. However, a significant reduction in the total leaf area was noticed when certain levels of P or N were added to the dual treated plants whereas at certain other combinations of P and N there was no significant change in leaf area from that of the control. Thakur and Panwar, 1997 reported increase in leaf area in green gram treated with AM-*Rhizobium* combination. But the present observation showed the need of additional P and N along with dual inoculation for a significant increase in leaf area. Therefore, it may be concluded that the relationship of P, N, AMF consortium and *Rhizobium* in enhancing the leaf area of the crop is a specific and complex process. Application of organic fertilizer may be more suitable in such situations (Alloush et al., 2000). Kabeerathumma et al., 1986 reported the advantage of using farm yard manure along with mycorrhiza as a source of nutrients to enhance yield in sweet potato.

The average total chlorophyll content in AMF consortium inoculated plants without N and P was significantly higher than that in the control. Similar significant increase was also observed when AMF consortium together with certain combinations of P and N were used. But at the same time certain other combinations of AMF consortium and N and P could not yield a significant change in total chlorophyll from that of the control. Therefore, it may be concluded that the relationship of P, N and AMF consortium in enhancing the total chlorophyll in leaf tissues of the crop is a complex process involving certain critical nutritional or environmental requirements. No significant difference in total chlorophyll from that of the control in all dual treatments of *Rhizobium* and the AMF consortium together with N alone. However, there was a significant enhancement in total chlorophyll from that of the control when *Rhizobium* and the AMF consortium together with P alone were applied at the full recommended dose. Thus it became evident that in presence of both AMF consortium and *Rhizobium*, P and N at 50% RD or P at 100% RD has a positive influence on the total chlorophyll of the plant.

Influence of AM in enhancing the growth and yield of a crop depends on the percent colonization of AM fungi on roots of the crop (Zhu et al., 2003). Hence, a comparison of percentage colonization of the fungi on roots of the crop in presence of different doses of P and N in the absence and presence of *Rhizobium* was also important to be analyzed. Present experiments showed that N either in half or full dose with out P has significant positive influence on percent colonization of AMF consortium on roots in green gram. This observation agrees with that of Mosse, 1973 that neither mycorrhizal nor non mycorrhizal plants responded to phosphate unless a minimum amount of N was given. In the present experiments P had a positive effect only when applied at its full dose in the absence or presence of N. A significant negative effect on percentage root colonization of AM was observed when N was combined with half dose of P. Bolan et al., 1984 observed that P has a positive influence on AM colonization of plant roots at low doses whereas at higher doses P addition is not beneficial. However, higher doses (full RD) of P showed enhancement in percentage root colonization in green gram, but the utility of which in productivity depends on the overall impact of this aspect on growth parameters and final yield. Since soil used in the experiment was nutrient poor (Muhr et al. 1965) and the AMF consortium showed a positive reaction to external supply of N, nitrogen requirement may be considered critical. Overall, it appeared that there is an optimum interrelationship in the amount of N and P required for efficient association of AMF with the roots of a crop.

Percentage root colonization of AMF consortium in green gram in presence of *Rhizobium* was found significantly positive at the 50% RD of P without N application. At 50% RD of P with any dose of N, and either absence or application of P at full RD always caused a significant negative influence on root colonization in green gram. Therefore, the influence of *Rhizobium* on percentage colonization of AMF consortium in green gram in presence of N and P is a delicate and complex process to be further analyzed. Vanlauwe et al., 2000 observed a positive relationship between P availability and root colonization of AM in *Mucuna* and Lablab. It is an established fact that the hyphae of AM fungi may extend beyond the plant rhizosphere and take up N and transport it into the rooting zone (Ames et al., 1983). The present findings showed that in presence of *Rhizobium*, the N application at higher doses has a negative influence on percentage colonization of AMF consortium in roots of green gram. Overall finding was that for successful colonization of AM on crops, fertilizer doses significantly lower than that of the full RD are beneficial and the full RD is wasteful.

The total number of nodules and total weight of nodules per plant are very good indicators of effective *Rhizobium* symbiosis (Hazarika et al., 2000). There was significant increase in the number and total weight of nodules per plant when P or N alone or both were given at 50% RD. At full doses of P, both the 50% and 100% RD of N were significantly effective in increasing nodulation characteristics. However, when N was given at its full dose, there was a significant reduction in nodulation with no addition of P or 50% RD of P. These findings pointed out the fact that when AMF consortium and *Rhizobium* are applied together, only small doses of N and P are needed for significant improvement of root nodulation characteristics in green gram.

Tissue level of N, P and K is a very significant parameter to assess the rate at which nutrient assimilation takes place. A significant increase in tissue nitrogen was noticed when either N at full dose or P at 50 or 100% or both P and N in equal proportions were given to the crop along with AM. Another important observation was that the application of AMF consortium without N and P caused no significant change in tissue nitrogen content from that of the control. However, when *Rhizobium* also was given along with the AMF consortium, a significant increase in tissue N was observed in treatments involving 50% RD of P without N. In the absence of P, N supply at all levels caused a significant reduction in tissue N from that of the control. These observations gave the idea that when AMF consortium and *Rhizobium* are applied together P nutrition is critical to leaf tissue N content of the crop.

In the case of P in leaf tissues, application of either P alone or N and P at equal proportions could yield a tissue P level significantly higher from that of the control. Another finding was that when P and N were given at non-proportionate doses, the tissue P significantly got reduced from that of the control in green gram. As in the case of tissue N, the application of AMF consortium without N and P did not cause a significant change in tissue P content from that of the control. When *Rhizobium* also was added to these treatments, a significant increase in tissue P was noticed only when P was externally applied in the soil, irrespective of its doses. All treatments without P showed a significant reduction in tissue P from that of the control. Therefore, it appeared that to enhance the tissue P in tripartite applications of AMF consortium, *Rhizobium* and green gram, 50% RD of P is essential.

In all the treatments and the control K was kept constant (at 100% of RD). However, it was noted that addition of P irrespective of its dose was essential for a significant enhancement of K from that of the control in the leaf tissues if AM alone or AM together with *Rhizobium* was applied to the crop. Similarly, when treatments involving AM alone or AM and *Rhizobium* without P was applied, there was a significant reduction from that of the control in tissue K except when N was applied at 50% RD. In this treatment, the tissue K content was not significantly different from that of the control. These findings suggested the need of an external source of P for significant enhancement of K in leaf tissues in green gram when the crop is cultivated with the support of AMF consortium and *Rhizobium*. Bethlenfalvay et al., 1989 also observed a similar mycorrhizal interaction with P in tissue K concentration.

In general, the present examinations of NPK in leaf tissue in relation to AM and *Rhizobium* agree with the observations of Ames et al., 1983, and Krishna and Bagyaraj, 1984 that the interaction between N, P, AM and *Rhizobium* is significant in N and P nutrition in legumes.

Ultimately, yield is the best parameter to represent the utility of microbial or chemical fertilizer applications in all crops. Total weight of seeds per plant is the most important characteristic of yield in legume crops (Hazarika et al., 2000). In the treatments of AMF consortium without N and P additions and most of the treatments with N and P additions, yield characteristic was not significantly different from that of the control. However, in the treatment of AMF consortium along with 50% RD of P and 100% RD of N there was a significant increase in the seed yield from that of the control. A significant reduction was noticed in cases where AMF consortium was applied together with 50 % RD of N and 0% P or 50% RD of P and 0% N. When *Rhizobium* also was added to the AMF consortium, there was significant increase in the total seed weight per plant, from that of the control in certain treatments.

Therefore, it may be noted that application of *G. mosseae* and *G. microcarpum* in a consortium and *Rhizobium* to green gram could significantly enhance its yield at reduced levels (50%) of chemical fertilizers when compared to the purely chemicalized agriculture methods. The present observations of enhancement of yield in green gram in presence of these AMF consortium involving two species of *Glomus* agrees with the observations Chandrashekara et al., 1995 that inoculation of AM can reduce P application for enhancement of yield in crops.

The spore count in the soil after harvest also explains the successful interaction of the crop with AM fungal associations. Sporulation in AM fungi may be influenced by various factors such as soil organisms (Ross, 1980), N fertilization (Land et al., 1993) and the physiological state of the host (Smith et al., 1994). In the present study the rhizobial interaction resulted in reduced sporulation in both AM fungi but the sporulation in the two AM fungi (*G. mosseae* and *G. microcarpum*) showed no correlation with each other. The proportion of *Glomus mosseae* spores was higher than that of *Glomus microcarpum*, indicating competitiveness of the former over the latter. Talukdar and Germida, 1994 made a similar observation between two species of *Glomus* on lentil and wheat. As sporulation demands more resources from the host (Daft and Hogarth, 1983) and AM fungi in the consortium sporulate independently, increasing the number of AM fungal component in the consortium may lead to increased stress on the host, especially during sporulation of the

AM fungal partner. However, in annuals like green gram the expenditure of resources on fungal sporulation may occur during senescence so that it would not affect the harvestable yield (Smith et al., 1994).

CONCLUSION

Overall analysis revealed that the AMF consortium and *Rhizobium* treatments could ensure an average biomass comparable to that of the control even without the application of N and P if K was given at the full recommended dose. It became also evident that AMF consortium together with *Rhizobium* can enhance the growth and biomass of the crop significantly higher than that of the control if small amount of N and full recommended dose of K are given. In other growth parameters such as leaf area, leaf chlorophyll, tissue NPK and yield of the crop the AM or AM and *Rhizobium* along with certain amount of NPK additions was found positive and useful to green gram. It was quite evident that application of AMF consortium and *Rhizobium* to green gram can significantly enhance its yield at reduced levels of chemical fertilizers when compared to the purely chemicalized agriculture methods.

The present pot culture experiments therefore strongly put forward the hypothesis that AM-*Rhizobium* combination can significantly reduce use of chemical fertilizers in pulse crops, which may be further investigated using intensive field trials. Reduced use of chemical fertilizers along with suitable AMF consortium-*Rhizobium* mixture can definitely ensure reduced cost of production and sustainable growth in productivity of pulse crops without further environmental damage. Since increased cost of production and environment damage are inevitable in chemical intensive green revolution agriculture, increase in productivity using AM and *Rhizobium* ensures a lesser cost of production and lesser environment damage, which is visualized in a production intensive sustainable farming process.

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