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Response of Groundnut ('JL-24') Cultivar to Mycorrhiza Inoculation and Phosphorous Application

Khirood DOLEY*, Paramjit Kaur JITE

*University of Pune, Department of Botany, Pune, 411007, Maharashtra, India; khirood_doleys@yahoo.com (*corresponding author)*

Abstract

A pot experiment was conducted on peanut *Arachis hypogaea* L. during 2007 growing season to determine their growth characteristics due to mycorrhizal inoculation and two different levels of soluble phosphorous application. Due to inoculation by AM fungi the growth parameters such as leaf number, shoot length, root length, fresh weight, dry weight, pod number and nodule number were significantly increased but two different level of phosphate also showed growth. However, growth parameters showed variable results when two different level of phosphate was applied along with AM fungi. Without phosphorous the mycorrhizal groundnut showed significant growth but when first low level of phosphorous was applied it showed more significant growth, however most significant result was observed with second high level of phosphorous application to the groundnut plant. Total chlorophyll content and acid and alkaline phosphatase activity was also significantly higher but most significant were observed when first level of phosphorous was applied followed by second level of phosphorous. The percent root colonization by mycorrhizal fungus *Glomus fasciculatum* was higher due to application of phosphorous but mycorrhizal dependency went on decreasing due to increase in the level of phosphorous. The different level of phosphorous had significant effect on growth and physiological parameters of mycorrhizal and non-mycorrhizal *Arachis* plants after 30, 60 and 90 days of growth period. However, the obtained results proved the improvement in plant growth with application of phosphorous. Thus, for increase in production of groundnut in the state of Maharashtra seems to be feasible option for increasing the overall production and yield.

Keywords: *Arachis hypogaea* L., dependency, *Glomus fasciculatum*, plant growth, root colonization

Introduction

Groundnut (*Arachis hypogaea* L.) is considered to be one of the most important oilseed crops worldwide. India has been producing groundnut since long time and currently ranks among top three producers in world with 5.9 million tons and annual groundnut oil production of 1.5 million tons. In the state of Maharashtra (India), the groundnut varieties that are under cultivation are of Spanish Bunch Varieties which are confined mainly in northern region of Khandesh, Vidharbha area and certain parts of rest of the Maharashtra. Since 1993-1994 the area under groundnut is showing continuous instability or decrease in average productivity in Maharashtra. In 1993-1994 the productivity of the crop was 1004 Kg/ha which came down to 744 Kg/ha in 2006/2007 (Patil, 2009).

Arbuscular mycorrhizal association is considered to be widely symbiotic association between specific soil fungi and a plant root (Schüßler *et al.*, 2001) which is becoming important constitute in our modern day agriculture systems. These Glomeromycotan fungi bank on their plant host for carbon in return for which fungus improves nutrition especially phosphate nutrition (Smith and Read, 1997). Phosphorus is an essential plant macronutrient which is required to build important molecules such as nucleic acids and phospholipids, and plays central role during energy transfer in processes like NADPH, ATP and regulation of enzymatic and metabolic reactions (Bieleski and Ferguson, 1975; Theodorou and Plaxton, 1993). Phosphorous moves by diffusion in soil and is taken up by plants through root interception. The free phosphate levels available to plant around the soil are used to be very low and may range from less than 1 to 10 μM (Marschner, 1995). The presence of phosphate varies in their forms in the soil, with an organic and a mineral pool (Holford, 1997). As phosphorous typically constitutes around 30% or 65% or 80% of total P in the soil as organic P (Fageria, 2009) but still the availability of 80-99% phosphorous for uptake in plant is scarce because of different factors like adsorption, precipitation or conversion into organic forms (Smith and Read, 1997). As a result, the role of mycorrhizal association is of significant importance for the P supply since the fungal hyphae extends into the soil and allows roots to explore a larger soil volume (Smith and Read, 1997). The extraradical hyphae of AMF act as root extensions and draw P from soil to supply it to plants (Ramos-Zapata *et al.*, 2009). The form of P most readily accessed by plants is Pi (in-organic Phosphate) (Bieleski, 1973). If the root is mycorrhized, it is primarily captured from the soil by external hyphae and transferred to the plant root cortex (Sanders and Tinker, 1971; Smith and Gianinazzi-Pearson, 1988).

Despite numerous studies, the mechanisms underlying this P translocation in the hyphae are largely unknown (Smith *et al.*, 1994). Many researchers have reported the

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increase of P concentration in mycorrhizal plants (Pasqualini *et al.*, 2007; Smith and Read, 1997; Yao *et al.*, 2008). The transfer of P to plants by AM fungi is influenced by P addition in soil, as it decreases the mycorrhizal association (Vierheiling, 2004). Moreover, plants take up Pi as orthophosphate ions from soil (Holford, 1997) due to which there may be decrease of P in soil around the roots but extraradical hyphae of AMF grow beyond this depletion zone and provide positive effects on plants (Smith and Read, 1997).

The conceivable role of AM fungi in terms of their ability in phosphate nutrition has been gaining much importance in recent years (Karandashov, 2005). And since external supply of nutrient in terms of costly fertilizers to crops by farmers of low economy may lead to low yield and supply to common society in general. Hence, the study was undertaken to increase yield altogether with low dependency on chemical fertilizers with high yield.

Materials and methods

Soil characteristics

Study of initial chemical characteristics of used soil was carried out before commencement of the experiment.

Chemical characteristics of soil used in the experiments (Source: Zuari Laboratory)	
pH	7.80
Electrical conductivity	10 Siemens/meter
Organic carbon	0.52 %
P_2O_5	12.00 kg/ acre
K_2O	80 kg / acre
Zinc	0.98 pm
Copper	3.70 ppm
Iron	11.00 ppm
Manganese	31.00 ppm

Isolation, identification and inoculum preparation of AM

Isolation of AM fungal spores was carried out by wet sieving and decanting methods described by Gerdemann and Nicolson (1963). One-hundred grams of rhizosphere soil was suspended in 1000 ml of tap water. The mixture was stirred for 1-2 minutes and coarse particles were allowed to settle down for 10-15 minutes. The supernatant was decanted through a series of sieves arranged in descending order of mesh size (100-60 μ). These steps were repeated twice more to ensure maximum spore recovery from the soil sample. Sievates from each sieve were collected separately in beakers. The supernatant from each beaker was then separately filtered through Whatman filter paper. The filter papers were placed in the Petri-plate, care was taken to ensure that they remain moist. The contents of the filter papers were examined for spores and sporocarps under the stereomicroscope. The identification of arbuscular mycorrhiza was carried out by complying keys recommended by Trappe (1982) and Schenck and Prez (1987). Determination of colour, shape and dimensions of arbuscular mycorrhizal (AM) fungal spores were determined according to Kornerup and Wanscher (1983). The estimation of arbuscular mycorrhizal (AM) fungal spore number was carried out by Gaur and Adholeya (1994). The AM fungi *G. fasciculatum* (Thaxter Sensu Gerd.) Gerd. and Trappe was mass multiplied and maintained in pot cultures with hosts *Sorghum vulgare* and *Panicum maximum* (Jacq.) roots grown on 30 cm earthen pots containing 10-15 kg of sterilized soil and sand in a proportion of 1:1. After three months mycorrhizal inoculations were made to each groundnut plant with 20 g of AM fungi inoculum of *G. fasciculatum* mixture containing spores, colonized root pieces and extrametrical mycelium in rhizospheric soil (obtained from the pot culture). The mycorrhizal inoculum was placed at about 3-5 cm below each groundnut seeds under the soil surface before sowing.

Plant material used

Seeds of groundnut local cultivar 'Phule Pragati' ('JL-24') kindly obtained from Naik seeds, Maharashtra, Pune, India, were used in this study. For rising the seeds in greenhouse, the seeds were surface sterilized with 0.02% $HgCl_2$ for 5 mins and then washed three times with sterile distilled water.

Greenhouse trials

Pot culture experiments in which seeds of groundnut with healthy appearance, surface sterilized were sown in plastic pots, was adopted. Four seeds were sown per pot (18 × 15 cm) in diameter filled with 9 kg of autoclaved soil (121°C for 1 hour), with 3-4 openings to drain excess water. Plants were irrigated regularly for 80% moisture in pots. The pots were inoculated with soil based 0.2% inoculum of AM fungus (*G. fasciculatum)* as mentioned above. After three weeks, phosphorous was applied using two different concentrations (50 mg and 100 mg/ kg of soil) in deionized water weekly with three replications and after three weeks of mycorrhizal inoculation. The experiments were arranged in Completely Randomized Block Design (CRBD), with six treatments consisting of non-mycorrhizal control (C, C+1P, C+2P) and mycorrhizal (Gf, Gf+1P, Gf+2P) groundnut plants. The control (C) was without any inoculations or a treatment at any particular interval of time and mycorrhizal (Gf) was treated with *G. fasciculatum* inoculum only. All other treatments were applied with two levels (1P or 2P level) of phosphorous *viz*., 50 or 100 mg phosphorous kg^{-1} respectively. Except control ones and mycorrhizal ones were treated with *G. fasciculatum* inoculum (20 g) below seeds (3-5 cm) before sowing.

Efficay of AM and P application on plant growth and yield

Twelve plants from three pots of each treatments were carefully harvested after growth period of 30, 60 and 90 days of AM fungus *G. fasciculatum* inoculation, washed under running tap water and were sampled for various morphological parameters: shoot and root length (cm), number of leaves, number of nodules, number of pods, fresh and dry weight of all plant (g). Dry weights were recorded after drying the samples at 70°C for 48 hours.

Study of AM colonization in peanuts roots

Randomly selected root samples were cleared in 10% KOH at 90°C for 1 hour and stained in 0.01% trypan blue (Phillips and Hayman, 1970) for 10 minutes. The fungal structures were visualized under a compound microscope and the measurement of root colonization by *G. fasciculatum* were determined by Grid-line intersect method (Giovannetti and Mosse, 1980).

Mycorrhiza dependency in colonized peanut roots

Dependency of AM was determined by measuring the dry weights of the plants after drying the tissue to constant weight at 70°C for 48 hours according the method described by Plenchette *et al.* (1983):

Percent Mycorrhizal Dependency = [(dry mass mycorrhizal plant – dry non mass mycorrhizal plant) / dry mass mycorrhizal plant] × 100%

Efficacy of AM and P application on some physiological activities

Total chlorophyll was estimated according the method described by Arnon (1949). Acid and alkaline phosphatase was analyzed as the method described by Lowry *et al.* (1951).

Statistical analysis

The data were subjected to statistical scrutiny following one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± standard deviation (SD). Duncan's multiple range test was applied as post hoc test at $p = 0.05$. All the calculations were made by using a Statistical Package for Social Sciences (SPSS) for windows version 9.0 and Microsoft Excel 2007 to analyze the data.

Results

Growth response of peanut plants to AM inoculation and P application

In 2007, inoculation of *G. fasciculatum* and phosphorous application led to significant results in the groundnut cultivar ('JL-24') in sterilized soil. There was significant increase in various developmental parameters such as number of shoot and root length, fresh and dry weight of plant, leaf, pod and nodule numbers due to mycorrhizal inoculation with *G. fasciculatum* and phosphorous application with low (1P) and high (2P) dosage after the growth pe-

Tab. 1. Shoot length and root length in *Arachis hypogaea* L. after 30, 60 and 90 days of AM inoculation

	Shoot length (cm)			Root length (cm)		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	17.33±0.94d	20.33±1.25d	34.67±2.49d	22.33±1.70d	26.33±2.62d	35.00±2.45c
C+1P	19.50±0.41cd	24.00±2.16cd	49.67±1.70c	25.17±1.43cd	28.33±2.49d	42.50±1.08b
C+2P	19.17±0.24c	26.67±2.05bc	52.00±1.63bc	28.67±2.62bc	30.00±1.63cd	43.00±2.45b
Gf	25.67±0.94b	27.67±2.05bc	56.67±2.05c	31.00±2.16ab	33.67±2.49bc	54.67±2.05a
Gf+1P	29.00±0.82a	30.00±2.27ab	64.33±4.19b	32.33±1.25ab	36.67±2.62ab	57.67±1.70a
Gf+2P	30.00±1.63a	33.67±1.25a	65.33±1.70a	35.33±2.49a	39.00±2.45a	58.33±3.30a

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different ($p<0.05$) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

Tab. 2. Fresh weight and dry weight in *Arachis hypogaea* L. after 30, 60 and 90 days of AM inoculation

	Fresh weight (gm)			Dry weight (gm)		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	2.62±0.56c	5.46±0.61c	12.95±1.20d	0.98±0.15d	1.24±0.22d	1.54±0.06d
C+1P	3.27±0.09bc	5.94±1.08c	14.84±2.03cd	1.33±0.20cd	1.71±0.49cd	1.96±0.20cd
C+2P	3.44±0.20b	6.75±1.12bc	16.70±1.10bc	1.80±0.10bc	2.00±0.14bc	2.20±0.22bc
Gf	5.46±0.18a	8.52±1.13b	18.26±0.65b	1.82±0.41bc	2.32±0.10ab	2.65±0.37ab
Gf+1P	5.51±0.35a	8.81±0.93b	22.11±1.50a	2.24±0.08ab	2.49±0.14ab	2.73±0.21a
Gf+2P	6.03±0.16a	11.60±1.01a	24.13±0.76a	2.31±0.17a	2.84±0.11a	2.94±0.06a

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+ 50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different ($p<0.05$) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

Tab. 3. Leaf number and nodule number in *Arachis hypogaea* L. after 30, 60 and 90 days of AM inoculation

	Leaf number (no.)			Nodule number (no.)		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	22.00±2.16b	27.00±3.56c	59.00±2.94d	9.33±2.05c	12.33±3.09c	35.00±2.45c
C+1P	22.33±1.70b	35.67±5.25c	66.00±1.63cd	10.00±4.97c	18.00±3.27c	42.50±1.08b
C+2P	24.67±1.25b	36.00±3.27c	68.67±2.87c	17.33±1.70c	23.33±8.73c	43.00±2.45b
Gf	35.67±2.87a	48.33±3.40b	92.67±4.11b	29.33±1.25b	41.67±7.59b	54.67±2.05a
Gf+1P	39.33±1.89a	53.33±8.50b	101.67±3.68b	30.67±6.02b	43.00±7.26b	57.67±1.70a
Gf+2P	40.00±4.32a	57.00±6.16b	108.00±7.12a	44.00±8.52a	48.00±5.89b	58.33±3.30a

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+ 50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different ($p<0.05$) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

Tab. 4. Pod number and Mycorrhizal dependency in *Arachis hypogaea* L. after 30, 60 and 90 days of AM inoculation

	Pods number (no.)			Mycorrhizal dependency (%)		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.67±0.47b	1.67±0.47c	2.00±0.82c	-	-	-
C+1P	1.00±0.00ab	2.33±0.47c	2.33±0.47c	-	-	-
C+2P	1.00±0.00ab	2.67±1.25c	3.33±0.47c	-	-	-
Gf	1.00±0.00ab	5.00±0.82b	5.33±1.25b	43.3±14a	46.1±10.5a	40.4±9.3a
Gf+1P	1.33±0.47ab	5.33±0.94b	7.00±0.82ab	40.7±7a	31±21.6a	28.4±1.9a
Gf+2P	1.67±0.47ab	5.67±1.25b	7.67±1.25a	22.2±3.3a	29.7±3.86a	25.4±6.7a

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+ 50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different ($p<0.05$) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

riod of 30, 60 and 90 days as compared to control non-mycorrhizal with both phosphorous levels. The various morphological parameters were significantly higher in mycorrhizal (Gf, Gf+1P, Gf+2P) plants as compared to control ones (C, C+1P, C+2P), the overall morphological parameters went on increasing with low level followed by high level application of phosphorous and highest morphological parameters were recorded with high phosphorous level. (Tab. 1-4).

Dependency of AM in colonized peanut roots

The percent mycorrhizal dependency (MD) of mycorrhizal groundnut plants was higher as compared to low or high level of phosphorous or controls ones. The data showed lower mycorrhizal dependency due to low level phosphorous dosage and high level phosphorous dosage demonstrated lowest mycorrhizal dependency on the basis of dry mass as compared to any other treatments (Tab. 4).

Efficacy of AM and P application on some physiological activities

Plant chlorophyll content

The total chlorophyll content as referred in Fig. 1, showed significant rise in their content at both low and high level of phosphorous application. But total chlorophyll content were significantly risen in mycorrhizal treated groundnut plants and marked increase were observed with increase in phosphorous dosage of low and high levels after growing period of 30, 60 and 90 days.

Acid and alkaline phosphatase activity

As illustrated in Tab. 5, the acid phosphatase activity was appreciably higher in mycorrhizal (Gf) groundnut plants at both low and high levels of phosphorous (Gf+1P and Gf+2P) after 30, 60 and 90 days when compared to non-mycorrhizal control groundnut plants. The acid phosphatase activity was higher due to mycorrhizal inoculation but low and high phosphorous dosage led to significant increase at latter period of growth. Highest activity was observed with high levels of phosphorous followed low level of application as compared to control groundnut plants.

The alkaline phosphatase activity showed rise in its activity Tab. 5, after 30, 60 and 90 days of growth at both low and high level of phosphorous as compared to uninoculated non-mycorrhizal control groundnut plants. The alkaline phosphatase activities were higher in mycorrhizal groundnut plants at low and high levels of phosphorous as compared to non-mycorrhizal control ones. However, highest activity was observed at high level of phosphorous in mycorrhizal groundnut plants than any other treatments.

Root colonization of AM

The groundnut seeds inoculated with spores of *G. fasciculatum* were harvested after 30, 60 and 90 days to determine percent root colonization. The AM colonization was found to be in increasing manner at both levels of phosphate or without it (Fig. 2). Arbuscules, intraradical vesicles and colonization were seen because of which it was assumed that the mycorrhizal symbiosis was established. However, colonization was nil in non-mycorrhizal groundnut plants.

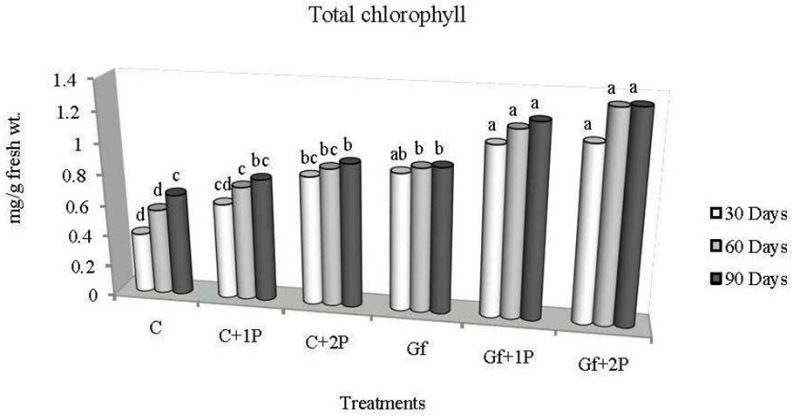

Fig. 1. Total chlorophyll content in peanut plant

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different (p<0.05) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

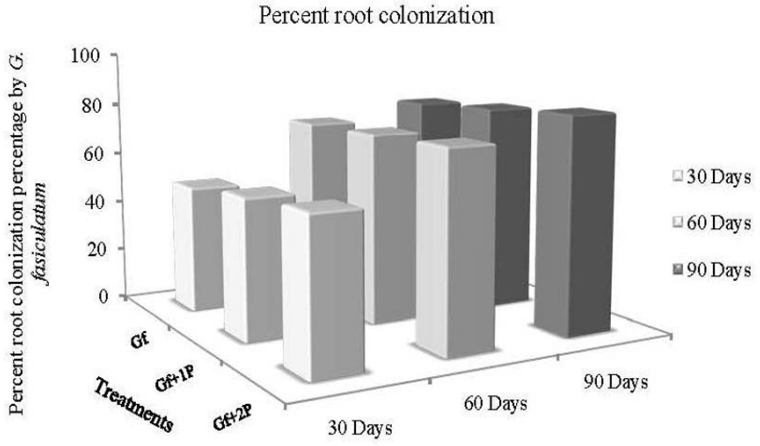

Fig. 2. Percent root colonization

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different (p<0.05) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

Discussion

It is well acknowledged that traditionally mycorrhizal colonization benefits (Francis and Read, 1995), can improve nutritional status of their host plants. The symbiosis develops in the roots where the AM fungi deliver phosphate and nitrogen to the root cortex and in return obtain carbon from the host plant (Smith and Read, 2008). Also, mycorrhizal infection is of particular value to legumes because it can increase the P (phosphorous) and restricted root system leads to poor competition for soil P (Carling *et al.,* 1978). Therefore, the plant has better growth and yield. In addition, it has been suggested that phytohormones, such as indole acetic acid (IAA) and cytokinins, released by mycorrhizal fungi may also contribute to the enhancement of plant growth (Frankenberger and Arshad, 1995).

In the present greenhouse trials, groundnut plants were tested for requirements with or without mycorrhiza and P nutrition. Groundnut plants were analyzed for their growth response to mycorrhizal inoculation and P nutrition. Two different levels of P were applied along with AM fungus (*G. fasciculatum*) and it was compared with control ones. The certain species or strains in combination with mycorrhizal fungus can show a discrepancy in their ability to take up P from soil and relocate it to the host plant (Smith *et al.*, 2000). The obtained results are in confirmation with similar kind of studies made by various researchers (Giri *et al.*, 2005; Sharma and Johri, 2002; Singh and Adholeya, 2002) that groundnut plant required a phosphate conc. in soil when they are colonized by AMF as they do without AMF for the same level of production. Without mycorrhiza, much higher amounts of P and/or

Tab. 5. Acid and alkaline phosphatase in *Arachis hypogaea* L. after 30, 60 and 90 days of AM inoculation

	Acid Phosphatase μ mole pnp-released/gm fresh weight			Alkaline Phosphatase μ mole pnp-released/gm fresh weight		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	1.03±0.10d	1.14±0.06d	1.30±0.06d	1.27±0.08d	1.53±0.12d	1.93±0.27c
C+1P	1.10±0.09d	1.23±0.06d	1.45±0.05cd	1.46±0.18d	1.68±0.19d	2.08±0.11c
C+2P	1.24±0.08cd	1.28±0.06d	1.57±0.05cd	2.02±0.25c	2.07±0.19c	2.26±0.25bc
Gf	1.45±0.10bc	1.51±0.07c	1.78±0.10b	2.04±0.22c	2.18±0.15c	2.69±0.30b
Gf+1P	1.66±0.15ab	1.81±0.08b	2.32±0.33a	2.25±0.06c	2.64±0.22b	3.37±0.26a
Gf+2P	1.90±0.28a	2.11±0.08a	2.46±0.31a	2.67±0.18b	3.02±0.08a	3.55±0.29a

C: control uninoculated; C+1P: control+50 mg kg^{-1} of phosphorous; C+2P: control+100 mg kg^{-1} of phosphorous; Gf: *G. fasciculatum*; Gf+1P: *G. fasciculatum*+ 50 mg kg^{-1} of phosphorous; Gf+2P: *G. fasciculatum*+100 mg kg^{-1} of phosphorous; values followed by same letter are not significantly different ($p<0.05$) by Duncan's multiple range test, each value represents means ± standard deviation (SD) of three replicates

Zn fertilizer are required to attain the same level of productivity as when plants are inoculated with mycorrhiza.

In this experiment as predicted all the growth parameters of inoculated peanut plants showed increased development. This may be due to enhancement in P uptake through the root as reported by Sharifi *et al.* (2007) in soybean. The green house trial lead to increase in overall plant growth. The significant increase in observed parameter such as shoot and root length which may be credited to mycorrhizal inoculation as it resulted into improvement of phosphate uptake (Al-Karaki, 2000).

Inoculation of groundnut plants with AM significantly enhanced fresh and dry weight of groundnut plants. These results are in accordance with data of (Torelli *et al.*, 2000; Trotta *et al.*, 1991) who reported the increase in shoot dry weight, root dry weight, of groundnut plants when they were inoculated with *G. fasciculatum*. The increase in shoot and root dry weights in mycorrhizal plants may be due to increase in supply of nutrients (Furlan *et al.*, 1983; Habte and Soedrajo, 1996; Marschner and Dell, 1994; Requena *et al.*, 1997). Increased overall plant growth, fresh and dry weight of shoot and root, mycorrhizal colonization in the plants inoculated with *G. fasciculatum* were showed by Selvaraj (1989). Groundnut plants when inoculated with *G. fasciculatum* resulted into considerable enhancement in the growth which suggests probable improvement in water and phosphorous uptake by mycorrhizal fungus. A similar study by Marschner and Dell (1994) showed increase in growth of *C. siamea* due to greater exploration capacity of extrametrical fungal hyphae by uptaking of nutrients. There was significant increase in pod number and nodule number of groundnut plants as compared to non-mycorrhizal control plants. The nodulation increased with mycorrhizal inoculation and P application. There is relatively significant increase in number of leaves in tested plants. Similar type of study was made by Copetta *et al.* (2006) who showed increased overall morphological parameters in soybean plant due to inoculation with AM fungi and Kapoor *et al.* (2007) demonstrated that enhanced phosphorous application had led to overall improvement in plant growth and concentration of artemisinin in annual wormwood (*Artemisia annua* L.).

Total chlorophyll content was higher in mycorrhizal plants and was the highest in plants where second level of P along with mycorrhiza was applied than non-mycorrhizal groundnut plants. As vesicular arbuscular mycorrhizal (VAM) association affects the host plants in terms of stomatal movement and photosynthesis of leaves and has been shown to increase chlorophyll content and the rate of transpiration and photosynthesis (Bethlenfalvay *et al.*, 1988, Panwar, 1991). The increase in this manner might be due to increase in the rate of photosynthesis and transpiration or increased growth rate (Sampathkumar and Ganeshkumar, 2003) or due to the presence of a large number of chloroplasts in the bundle sheath of inoculated leaves (Krishna and Bagyaraj, 1984). Higher content of chlorophyll in leaves of mycorrhizal plants under saline conditions has been suggested by several authors (Colla *et al.*, 2008; Sannazzaro *et al.*, 2006; Sheng *et al.*, 2008; Zuccarini, 2007). Thus, the study reveals the fact that there is an increase in the total chlorophyll content, because the association of VAM and P is supposed to affect the photosynthesis.

Mycorrhizal dependency is the degree to which a plant species is dependent on mycorrhizal association to produce its maximum growth or yield at a given level of soil fertility (Gerdemann, 1975; Giri *et al.*, 2007). In this investigation the mycorrhizal dependency in groundnut plants was generally high at first low level of phosphate application as compared second high level of phosphate application.

The activity of phosphatases increased in groundnut plant ('JL-24') inoculated with mycorrhiza when compared with uninoculated non-mycorrhizal ones. The increase of phosphatase activity in mycorrhizal plants which has been reported by several other researchers (Dodd *et al.*, 1987; Khalil *et al.*, 1994) and is most likely due to higher phosphatase activity of the internal hyphae produced by mycorrhizal fungi (Saito, 1995). An alkaline phosphatase activity was detected within the fungal vacuoles using histochemical technique (Gianinazzi *et al.*, 1979). Even Fries *et al.* (1998) associated levels of mycorrhizal colonization of roots to acid phosphatase and alkaline phosphatase activities in maize roots. Results in the present study showed that moderate application of soluble phosphorous along

with mycorrhiza proliferates mycorrhizal symbiosis and suggests P nutrition as an important profit.

Thus, potential of AM fungi to promote crop growth, crop production in general and as a biofertilizer has been established which not only covers an eco-friendly aspects but also reduces the use of costly and hazardous chemical fertilizers. As when almost 70% of phosphorous applied to crops are not used AM fungi helps in uptake of phosphorous. Hence, mycorrhizal technology will be useful for improving crop production and be of assistance in sustaining ecosystem.

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