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Growth and biochemical response of *rajmash* to VAM fungal and inorganic P application

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ABSTRACT

Pot experiments were conducted on *rajmash* inoculated with three native vesicular-arbuscular mycorrhizal (VAM) fungi, namely *Gigaspora albida*, *Glomus mosseae* and *Sclerocystis sinuosa*. These were inoculated either alone or in combination with potassium phosphate (KH_2PO_4). Plants treated with VAM fungus+½dose KH_2PO_4 enhanced growth and yield of *rajmash*. Any of the three VAM fungi alone or P-fertilizers helped plants to grow better than control but independently these were lesser than those given combined inoculum. The combined inoculum also altered the biochemical constituents such as total soluble sugars, ortho-dihydric phenols and flavanols in the leaves of plants when inoculated with *G. mosseae*+½dose phosphorus. Wax content was maximum on leaf surface in the plants inoculated with *S.sinuosa*+½dose phosphorus. The extent of root colonization affected the plant growth, P- content, metabolites and yield.

Key words: Arbuscular-mycorrhiza, P-fertilizer, *Phaseolus vulgaris*, VAM-fungi

Vesicular-arbuscular mycorrhizal (VAM) fungi play a pivotal role in the establishment and growth of plants under natural as well as stressed conditions, particularly in nutrient deficient soil. Arbuscular mycorrhizae (AM) form a critical link between plant and soil by influencing soil structure and plant nutrient cycling-especially phosphorus (Karandashov and Bucher 2005) and directly contribute to soil fertility and quality through soil organic matter (Rilling *et al.* 2001). The role of VAM fungi in plant growth and productivity has been well documented (Bolan 1991). The number of AM fungal (AMF) propagules in relation to physico-chemical characteristics of soil revealed that lower levels of N, P and K in soil accorded better support to AM fungal growth whereas high levels of soil nitrogen showed negative effects on AMF spore numbers. However, optimum levels of phosphorus favoured the occurrence and distribution of AM fungi (Reddy *et al.* 2006). In legumes the P-requirement is high and, therefore, they respond more than cereals to mycorrhizal colonization, which indirectly enhances the biological nitrogen fixation through increased P availability especially in soils with low P content (Anilkumar and Muraleedhar 2003, Sieverding 1983). French bean (*Phaseolus vulgaris* L.), commonly known as *rajmash*, is grown in northern parts of Uttar Pradesh mainly for seeds. The present study deals with the relative influence of different VAM fungi and phosphorus fertilizer on growth, yield and biochemical constituents of *rajmash*.

MATERIALS AND METHODS

Pot culture experiments were conducted during the winters of 2005-06 in the natural climatic conditions of Rae Bareli on *rajmash* cv. Amber. The soil used for experiments contained low organic carbon (0.27%) and medium available phosphorus (24.2 Kg/ha) and its pH was 7.4. The soil based cultures of VAM fungi were raised and maintained on either *Sorghum vulgare* or *Zea mays* roots. It consisted of both spores and chopped, colonized root fragments. Chemical phosphorus (P) was given in the form of KH_2PO_4 (full dose @ 2.5 g/8kg soil) amendments. Besides uninoculated control plants raised in unsterilized soil and P full dose amended soil, six different treatments were given to plants. These were- *G. albida*, *G. albida* + ½ P, *G. mosseae*, *G. mosseae* + ½ P, *S. sinuosa* and *S. sinuosa* + ½ P. For each treatment, 15 pots were maintained and each pot initially contained eight seedlings for destructive sampling. After sowing, the seedlings were allowed to grow for 30 days and thereafter, ten plants from each treatment were harvested after every 15-days interval up to 90 days. Plant growth *i.e.*, average lengths of shoot and root per plant, fresh and dry weights of shoot, root and leaves per plant were recorded fortnightly. Dry weights were recorded after drying roots, shoots, and leaves at 80°C in hot-air oven for 48 hours. The percentage of mycorrhizal root colonization was calculated after clearing 1 cm long root segments with 10% KOH and staining with 0.05% Trypan Blue (Phillips and Hayman 1970).

Respective phosphorus concentrations in different plant organs were estimated by Olsen method (Olsen and Sommers 1982). One gram of hot air oven dried powdered plant tissue samples were digested in tri-acid (10ml HNO_3, 4ml $HClO_4$ and 1ml HCl) for 6-8 hours in Kjeldatherm gradually raising the temperature to 450°C till the solution became yellow or colourless. After cooling, the solution volumes were made-up to 100 ml with glass distilled water. The resultant solutions were used as aliquot for the estimation of P. Besides growth parameters, certain biochemical constituents including total soluble sugars (Dubois *et al.* 1956), ortho-dihydric (OD)-phenols (Mahadevan 1966), flavanols (Swain and Hillis 1956) and wax content on leaf surface (Ebercon *et al.* 1977) were also estimated. Former three constituents were estimated in oven-dried tissues of plant parts and epicuticular wax contents were estimated in fresh leaf tissues at the age of 75 days.

RESULTS AND DISCUSSION

Plant growth and yield: The inoculations of *rajmash* plants with different VAM fungi and chemical P-fertilizer improved the plant growth and health. It significantly increased the root and shoot length; fresh and dry biomass per plant as compared to those of uninoculated control (Fig.1). It was similar to an increase in total biomass in *Trigonella-foenum graecum* L. plants treated with different VAM fungi (Neeraj and Chauhan 2006). Rashmi and Roy (2003) also observed that growth performance of mycorrhizal finger-millet was much better than non-mycorrhizal plants. In our experiments, soil inoculation of VAM fungi in combination with phosphorus recorded as the best supporting bio-fertilizer.

The average root length produced by half dose of KH_2PO_4 combinations with either *S. sinuous* or *G. mosseae* or *G. albida* at 90 days were 12.6, 12.1 and 11.9 cm, respectively, whereas respective shoot lengths were 15.9, 15.1 and 14.9 cm obtained by these treatments. These findings are in conformity with inoculation of *Casuarina* seedling with either *G. fasciculatum* or *G. mosseae* or *G. etunicatum* with phosphatic fertilizer (Rajeshwari *et al.* 2001). Yield attributes' pattern was studied in terms of average pods/plant, seeds/pod, fresh and dry weight of pods. The best values of these parameters were recorded where AM-fungus was supported with ½ dose of P. Plants treated with *S. sinuosa*+ ½ P and *G.mosseae* + ½ P showed 9 pods per plant, the highest average number of pods/plant. On an average, maximum number of seeds/pod recorded in VAM+ ½ P was 5 as compared to control plants where it was 4 seeds/pod. These findings were similar to those reported by Neeraj and Singh (2004, 2005).

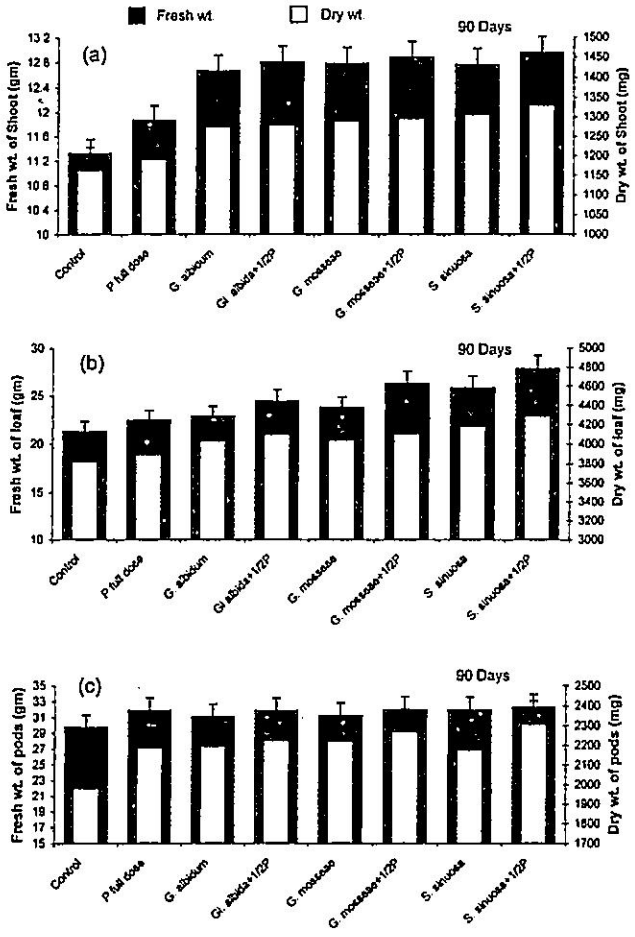

Fig. 1. Effect of inoculations with AM fungi and phosphorus fertilizer on fresh (outer columns) and dry (inner columns) weights of (a) shoots, (b) leaves and (c) pods of *Phaseolus vulgaris* L. at the age of 90 days

The maximum dry weight of pods/plant was recorded in plants treated with *S.sinuosa*+½ P (2.31 g) followed by *G. mosseae* + ½ P (2.27 g) and *G. albida* + ½ P (2.22 g). These values showed significant increase as compared to those plants which were treated with either of the VAM fungus alone (Fig.1), but later treatment too gave yields better than the uninoculated control plants.

Different species of AM-fungi varied in their respective abilities to stimulate plant growth, yield and colonization of roots. Amongst three VAM fungi, *G. mosseae* + ½ P showed highest percentage of colonization (70%) followed by *G. albida* + ½ P (68%) and *S. sinuosa* + ½ P (67%). The results are in agreement with the findings of Sharathbabu and Manoharachary (2006), who observed in *Tylophora indica* that the combination of *G. fasciculatum* and rock-phosphate gave better mycorrhizal colonization as compared to single inoculation either with *G. fasciculatum* or rock-phosphate. This may be due to adequate and steady supply of phosphorus from rock-phosphate, which is efficiently utilized by AM fungi. In our experiment, *G. mosseae* + ½ P showed better colonization than *S. sinuosa* + ½ P, but plant growth was better in the later treatment reconfirming that extent of root colonization was not always correlated with the particular species of AM-fungus (Mc Gonigle *et al.* 1990).

Uptake of P content in mycorrhizal plants was altered with mycorrhizal colonization. Higher colonization in plants showed maximum uptake of P. AMF treated plants with one-half dose of phosphorus fertilization showed greater shoot and root dry weights than their corresponding single treatments. Total P content in root, shoot and leaves of *G. mosseae* + ½ P treated plants were 0.7, 0.7 and 0.8 mg/g, respectively. In *S. sinuosa* + ½ P treated plants, the P content was 0.6 mg/g dry weight in root and 0.7 mg/g dry weight in shoot and leaves, which were significantly higher than the VAM alone treated and non-mycorrhizal plants (Table 1). AM fungi are thought to transfer inorganic P to the host plant across the arbuscule-cortical cell interface utilizing the phosphorus transporters which have been identified in *Glomus versiforme* (GvPT) and *Glomus intraradices* (GiPT) (Yadav *et al.* 2005).

Biochemical constituents: Biochemical constituents *viz.*, total sugars, orthodihydric (OD) phenols and flavanols

in plant tissues and epicuticular wax on leaf surface were altered due to AM inoculations. There were considerable differences in the levels of different biochemical constituents due to AM-inoculations in different plant parts like roots, shoots and leaves as shown in Table 1. Increased amounts of biochemicals in mycorrhizal *Neem* plants have been observed by Sumana and Bagyaraj (2002). Nagesh and Reddy (2004) also reported enhanced biochemical constituents in *Lycopersicon esculentum* roots colonized by *G. fasciculatum*. Our results showed that increase in biochemical constituents of mycorrhizal plants was further enhanced by addition of inorganic P with AM fungi.

Maximum total sugar enhancement was observed in the leaves of plants treated with *G. mosseae*+½ P after 75 days. It was 9600 µg/g dry tissue as against 2080 µg/g in uninoculated control plants. Amongst shoots, *S. sinuosa*+½ P supported plants had 3600 µg/g total sugars and amongst roots those colonized by *G. mosseae*+½ P had 840 µg/g total sugars as compared to respective uninoculated controls which had 1400 µg/g in shoots and 700 µg/g in roots.

The concentration of OD phenols 75 days after sowing recorded in the dry leaves of control plants was 432 µg/g and P-full dose given plants had 486 µg/g. Maximum phenolic concentration was observed in the dried tissues of leaves supported by *G. mosseae* + ½ P and *S. sinuosa* + ½ P (both 688 µg/g), whereas shoots of *G. albida*+½ P treated plants had 296 µg/g and roots colonized *S. sinuosa* + ½ P contained 80 µg/g phenols, which was 432, 164 and 40 µg/g, respectively in the leaves, shoots and roots of control plants. Concentration of flavanols in dried tissues of leaves, shoots and roots of *G. mosseae*+½ P given plants increased to 3320 µg/g, 2520 µg/g and 1900 µg/g, respectively, from 1900, 1280 and 840 µg/g found in uninoculated controls.

Epicuticular wax deposition on leaves imparts drought resistance and increase protection against foliar fungal infections (Sunkad and Kulkarni 2006). Plants inoculated with VAM fungi showed thicker wax coatings on leaves than the control plants. Both the VAM fungus alone and VAM+ ½ P also increased leaf surface wax contents. Among the three VAM fungi treated plants, maximum content was observed in fresh leaves of plants treated with *S. sinuosa*+½ P (104 µg/g) and minimum in *G. albida* + ½ P (72 µg/g). But later treatment was also better than control, which had 57 µg/g wax. Sunkad and Kulkarni (2006) reported that the natural protective covering of the epidermal cells of leaves of groundnut consists of the cuticle with its waxy coating and wetting-ability of leaves is related to early infection process of *P. arachidis*. As wax is negatively charged, it could not be easily wetted. Wax content was minimum at initial stage and increased consistently upto the last stage of sampling in all the varieties of groundnut (Gupta *et al.* 1985). Our results showed that wax content was increased due to AM inoculations. It was further increased by the addition of phosphorus.

The results of the present investigation reveal that *rajmash* plants are benefited when raised in the AM fungus inoculated soil with one-half dose of phosphorus. It is in conformity with the findings of Neeraj and Singh (2004) who obtained better biomass production in *Eleucine coracana plants with* VAM+half doses of chemical P fertilizer. Co-inoculation of VAM fungus+BGA also increased plant growth and yield of *Eleucine*. Similar results were obtained by Anilkumar and Muraleedhara (2003) who showed that VAMF associated leguminous plants grew better and enriched in nutrient levels than the ones without VAM association. In our experiments maximum growth was observed in plants due to *S. sinuosa* plus 1/2 dose of phosphorus. Likewise, *Gl. aggregatum* inoculated *Mucuna pruriens* (Biradar and Reddy 2007) and *Glomus sp.* inoculated rice (Ahanthem and Jha 2007) also had significant effects on the growth, when grown in unsterile soil.

Variations in concentrations of all the biochemicals were observed in different plant parts like roots, shoots and leaves of mycorrhizal or mycorrhiza plus ½ P given plants. Schaarschmidt *et al.* (2007) observed that P-uptake and transfer by the AM-fungus was dependent upon carbon transfer from the root to the fungus. Under normal growth conditions the

Table 1. Mycorrhizal colonization, phosphorus contents and biochemical constituents of different plant parts and foliar wax contents of *Phaseolus vulgaris* L. inoculated with different VAM fungal treatments

Treatment	Mycorrhizal colonization %	Total phosphorus (mg/g dry wt.)			Total soluble sugars (µg/g dry wt.)			Orthodihydric phenols (µg/g dry wt.)			Flavanols (µg/g dry wt.)			Epicuticular wax (µg/g fresh wt.)
		Root	Shoot	Leaves	Root	Shoot	Leaves	Root	Shoot	Leaves	Root	Shoot	Leaves	
Control	10	0.2	0.2	0.3	700	1400	2080	40	164	432	840	1280	1900	57
P-full dose	33	0.3	0.4	0.4	740	1400	4320	48	200	486	960	1640	1940	62
G. albida	63	0.4	0.4	0.5	640	2080	6320	52	236	496	1100	2120	3000	62
G. albida+½ P	68	0.5	0.6	0.6	736	2528	8342	52	296	664	1140	2040	2780	72
G.mosseae	65	0.5	0.5	0.7	640	2080	7232	48	168	356	1030	1680	2920	80
G.mosseae +½ P	70	0.7	0.7	0.8	840	2800	9600	64	240	688	1900	2520	3320	85
S. sinuosa	62	0.5	0.6	0.6	720	2528	8000	48	168	336	1320	1580	2340	102
S. sinuosa+½ P	67	0.6	0.7	0.7	736	3600	8342	80	280	688	1680	1760	2960	104

*P-full dose was given @ 2.5 g/8kg unsterilized soil.

supply of carbon to the fungal symbiont is already optimal. The carbohydrate supply (specifically hexose levels) in AM cannot be improved by root, implying that under normal conditions sufficient carbon is available to mycorrhizal roots. These findings suggested that, mycorrhizal plants possess more carbohydrates than the non-mycorrhizal plants. These observations were similar to our results that mycorrhizal plants possess higher content of total soluble sugars than non-mycorrhizal control plants which was further increased due to addition of one-half dose P. Amongst different plant organs, leaves contained more sugar content. Plants treated with *G. mosseae* + ½ P had more sugar content than other treatments. Kushwaha and Narain (2005) reported that total and reducing sugars were significantly higher in healthy leaves of resistant variety of *Cajanus cajan* than the susceptible ones. Influence of VAMF on the carbohydrate status of infected roots suggests that carbohydrate level in root depends on the balance between 'C' demand of the fungus and 'C' status the host (Harley and Smith 1983). Ocampo and Azcon (1985) reported that fungus demand for carbon did not significantly affect the quantity of sugar in the roots during the first stage of fungus development. But when the endophyte was well established, an increase in the amount of sugar in the infected roots was found. Heavy metal influence of nutrients like P, N, K, Zn etc. and water from soil, in mycorrhiza-colonized plant induce better growth and metabolism. Also, P-esters are instrumental in carbon metabolism and the energy status of cell, which is a reflection of C-metabolism, may influence membrane transport (Yadav *et al.* 2005).

OD phenols are important in disease resistance reactions. They are easily oxidized by phenol oxidases and the resulting quinones are highly reactive and toxic to pathogens and their enzymes. Increased production of OD phenol in resistant plant has been noted by Khilare *et al.* (2004) and Lodha *et al.* (1993). Present study also reveals that these were increased in plants due to inoculation of VAMF also. In our experiments amongst roots, shoots and leaves, more content of OD phenols was observed in the leaves of plants treated with *G. mosseae* + ½ P and *S. sinuosa* + ½ P as compared to plants treated with P full dose and untreated control plants (Table 1). These findings are in agreement with those of Neeraj and Singh (2007), who observed higher phenol contents in the leaves of *Phaseolus vulgaris* plants treated with AM Fungi + ½ P as compared to single VAM inoculations and it was further increased by addition of *Rhizobium*.

Like ortho-dihydric phenols, flavanols were also increased in plants inoculated with VAMF (Table 1). Flavanols also participate in disease resistance reactions. Our results showed that flavanol concentration was maximum in plants treated with *G. mosseae* + ½ P. Leaves of mycorrhizal plants had higher content of flavanols as compared to leaves of control plants. *Verticillium* resistant terminal leaves of cotton plants possessed higher concentration of flavanols than the susceptible leaves in response to infection (Swain and Hillis 1959). Identification of host and fungus metabolic pools (Bago *et al.* 2003) and biochemical pathways may lead to characterize the genes encoding key enzymes and transporters (Lammers *et al.* 2005). Amalgamation of knowledge of uptake and transfer of phosphorus with that of C and other metabolites will help us to get a clear picture of the mechanism of endomycorrhizal mutualism.

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