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Reducing Phosphorous Requirement Using AM Fungi in Mulberry Grown under Alkaline Conditions

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Abstract: Most of the soils fix phosphorus (P) and make it unavailable for plant growth. Tropical soils, specifically salt affected, are deficient in P. It is estimated that major portion (~75%) of super phosphate applied is fixed and only 25% is available for plants. India has a large area of alkaline soils and to reclaim these soils heavy doses of chemical fertilizers are applied. The use of chemicals is neither economical nor ecofriendly. Biofertilizer like mycorrhizal fungi could play a very important role in this regard. They have been recognized as potential biotechnology tool for enhancing plant growth as well as for reclamation of wastelands. Arbuscular Mycorrhizal Fungi (AMF) are well known for phosphorus solubilization, increased plant nutrient uptake and in control of root diseases. Mulberry (*Morus alba*), an important cash crop for sericulture, does not grow well in alkaline soils due to their high pH and deficiency of nutrients and requires heavy doses of NPK for its proper growth. To propagate sericulture in salt affected area, it is important to meet the nutrient requirement of mulberry. Exploitation of AMF can meet great demand of phosphorus besides other nutrients in alkaline soils. A study was conducted to evaluate potential of AMF in reducing the amount of P required for proper growth of *M. alba* var. Sujan Puri in alkaline soils. Four concentrations i.e., 0, 25, 50, 75 and 100% of recommended dose of P were applied with AMF. The results suggested a net saving of 25% of the recommended dose of inorganic P i.e., triple super phosphate, with AMF in alkaline soils for the proper growth of mulberry.

Key words: Arbuscular mycorrhizal fungi, alkaline, biofertilizer, mulberry, *Morus alba*, phosphorus

INTRODUCTION

Phosphorus (P) occurs both as inorganic phosphate and in the organic matter in soils. The dominant characteristics of soil phosphate are very low solubility of phosphate minerals and strong binding on to particle surfaces giving small soil solution concentrations (Nye and Tinker, 1977). As a result phosphate deficiencies in crops are common (Rowell, 1994a). Because of strong adsorption and small solution concentrations, the contribution of mass flow uptake of phosphate by roots is very small and diffusion rates are slow. Thus roots take up phosphate only from their immediate vicinity. An extensive root system is therefore important for adequate uptake. Mycorrhizal roots due to their extramatrical hyphae, capable of absorbing and translocating nutrients, can explore more soil volume than the non-mycorrhizal roots (Bieleski, 1973; Trappe, 1981; Joner and Jakobsen, 1995; Marschner, 1995) and thus increase the supply of slowly diffusing ions such as phosphate to the plant (McArther and Knowles, 1993). In other words arbuscular mycorrhizae increase nutrient uptake and enhance plant growth by shortening the distance that nutrients must diffuse through soil to the roots (Hattingh *et al*., 1973; Rhodes and Gerdemann, 1975; Cuenca *et al*., 1990; Michelsen and Rosendahl, 1990). In return, the plant meets the carbon requirement of the fungus (Jakobsen and Rosendahl, 1990).

Mulberry (*Morus alba*), a slightly acidic soil loving plant, does not grow well in alkaline soils due to their high pH and deficiency of nutrients. It requires relatively high rates of fertilization, especially for P, which is the element, most limiting to its growth. In India, an area of 10.1 mha is salt affected (Yadav, 2000). It is a paradox that on the one hand there is acute shortage of cultivable land, while on the other sizeable fertile areas are going out of cultivation due to the malady of salinity and sodicity. Haryana region alone has 0.5 mha of area affected with salt problems i.e., salinity, alkalinity or both (HOA, 1997). Conventional farmers overcome this problem by applying fertilizers containing readily soluble P (single or triple super phosphate). But the excessive use of chemical fertilizers is neither economic nor ecofriendly.

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Arbuscular Mycorrhizal Fungi (AMF) have been reported to occur naturally in saline/alkaline environment (Pond *et al.*, 1984; Sidhu and Behl, 1990). Inoculation with AMF has been recommended to help supply P from the soil (Hoepfner *et al.*, 1983; Cuenca *et al.*, 1990; Michelsen and Rosendahl, 1990) and assist in uptake of micronutrients such as zinc, copper, manganese and iron (Harley and Smith, 1983; Miller *et al.*, 1986; Sreenivasa, 1993; Smith *et al.*, 1994) and enhance uptake of macronutrients such as nitrogen and magnesium (Krishna *et al.*, 2005). Mycorrhizal infection may counteract adverse soil factors such as high salinity-alkalinity and also increase growth and yield of the plants on wastelands (Madan *et al.*, 1995; Sidhu and Behl, 1995). The interaction of mycorrhizal fungi and P solubilizing bacteria; the production of phosphatases by the mycorrhizal fungi; and organic acids produced by the mycorrhizal hyphae are the three mechanisms of mycorrhizal activity that have been proposed to contribute weathering of soil P as well as simple transport to the host plant (Raman and Mahadevan, 1996). Azcon *et al.* (1976) proposed that the bacteria associated with mycorrhizal plants could improve the P acquisition of added rock phosphate in alkaline soils. Therefore, colonization of the plant species by an efficient AM fungus, opens up the possibility of reducing the quantity of P fertilizer applied, without significant reduction in yield (Mallesha *et al.*, 1996). There have been studies (Padma and Kandasamy, 1990; Sharma and Adholeya, 2004) that have found that the use of arbuscular mycorrhizal fungi can reduce the phosphorus fertilization.

The present study was conducted to evaluate potential of AMF in reducing the amount of P required for proper growth of *M. alba* var. Sujan Puri in alkaline soils.

MATERIALS AND METHODS

To evaluate potential of AMF in reducing the amount of P required for proper growth of *M. alba* var. Sujan Puri in alkaline soils, an experiment was conducted with AMF in combination with different concentration levels i.e., 0, 25, 50, 75 and 100% of recommended dose of P. The study was conducted in 2002 at Indian Institute of Technology, Delhi, India.

Culturing of AM fungi and inocula production: AM fungal spores were isolated from the rhizosphere of maize, *Zea mays*, by sieving and decanting technique (Gerdemann and Nicolson, 1963). The spores were surface sterilized with 2% Cholramine T (w/v) and 200 ppm Streptomycin sulphate for about 15 min followed by successive washings in sterilized water. The spores were then used to infect seedlings of castor, *Ricinus communis*, grown in soil sterilized by formalin (0.1%). Well infected (90-100%) root system together with adhering soil was chopped and used as starter inoculum to scale up the production of inoculum in bulk by infecting fresh seedlings of castor raised in sterilized soil.

Table 1: Characteristics of the soil used in experiment

Properties	Values
Texture	Sandy
pH	8.8
Electrical conductivity (m/mho)	0.39
Organic carbon (%)	0.47
Available nitrogen (kg ha^{-1})	410.2
Available phosphorus (kg ha^{-1})	7.0
Available potassium (kg ha^{-1})	235.0

Soil analysis: The soil used in the experiment was collected from the village Farukhnagar, Haryana, India. The pre and post analysis of soil was done for its physico-chemical properties (Table 1) i.e., pH (1:2 soil:water ratio) by pH meter, electrical conductivity (EC) (1:2 soil:water ratio) by EC meter (Singh *et al.*, 1999b), organic carbon by Walkley and Black method (Singh *et al.*, 1999a), organic N by Kjeldahl method (Rowell, 1994b), available (extractable) P by spectrophotometer (Rowell, 1994a) and available (exchangeable) K by flame photometer (Singh *et al.*, 1999a).

Inoculation of plants with mycorrhizae and phosphorus: A mixture of three parts field soil and one part farmyard manure (FYM) thoroughly mixed and disinfected with 1% formalin, was filled in earthen pots (25.4 cm height with 24.1 cm mouth diameter) at the rate of 6 kg/pot. The already prepared inoculum i.e., 100 g soil containing ~200 spores, infected root bits and mycelial fragments of the AM fungi was placed as a thin layer at about 4 cm below the surface of the soil in the pots. Three cuttings of mulberry (~20 cm long having 2-3 buds per cutting) were planted in each pot. After sprouting and rooting, the cuttings were thinned to one plant per pot only. The P was added in four levels viz., 0, 25, 50, 75 and 100% of recommended dose of P i.e., 100 kg ha^{-1} in the form of solution of triple super phosphate in two doses (Rangaswami *et al.*, 1988). Each treatment was replicated five times. The following combinations were made:

T_1 = Control i.e., without mycorrhiza and without phosphorus
T_2 = Mycorrhiza (M) only

T_3 = Mycorrhiza+25% Phosphorus
T_4 = Mycorrhiza+50% Phosphorus
T_5 = Mycorrhiza+75% Phosphorus
T_6 = Mycorrhiza+100% Phosphorus
T_7 = 100% Phosphorus only

The study was carried out for 10 months. After completion of the experiment, the data pertaining to plant growth i.e., fresh and dry shoot and root weight, shoot and root length, number of leaves and total biomass yields were recorded. Dry shoot and root weights were taken after oven drying (70°C until the stable weight was achieved). Mycorrhizal response in terms of Mycorrhizal Colonization Percentage (MCP), number of spores/100 g soil and Mycorrhizal Inoculation Effect (MIE) was recorded. MCP was determined as per the procedure outlined by (Philips and Hayman, 1970). The number of spores/100 g soil was estimated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Mycorrhizal Inoculation Effect (MIE) was calculated by the formula given by Bagyaraj *et al.* (1988). The plant samples of all the treatments were estimated for major nutrients content after drying completely at 70°C. C and N content were determined by dichromate method (Rowell, 1994b) and Kjeldahl method (Rowell, 1994b), respectively. P estimation was done by spectrophotometer (Singh *et al.*, 1999b) and K by flame photometer (Singh *et al.*, 1999b) after the digestion of plant samples in a tri-acid mixture (HNO_3, H_2SO_4, $HClO_4$ in 10:1:4 v/v). The data were analyzed statistically by one-way analysis of variance (ANOVA) and Critical Difference (CD) was calculated using MSTATE software programme.

RESULTS AND DISCUSSION

Data pertaining to the effect of inoculation of AMF and different doses of P on the mycorrhizal response in *M. alba* var. Sujan Puri are depicted in Fig. 1. The trend observed in percent infection in roots was in the order of; T_5 (70.6%) > T_6 (68.0) > T_4 (65.3) > T_3 (62.7) > T_2 (60.6). Similar trend was observed with number of spores/100 g soil. Percent root colonization and sporulation increased with increasing P levels upto 75% of the recommended dose and decreased thereafter. The decline effect observed with M+100% P could be due to depressed activity of AMF at increased availability of P (Bagyaraj and Powell, 1985), possibly through membrane mediated root exudation (Graham *et al.*, 1981). Present data, in this regard, are also in accordance with Padma and Kandasamy (1990), Naik *et al.* (1995), Singh and Singh (1995).

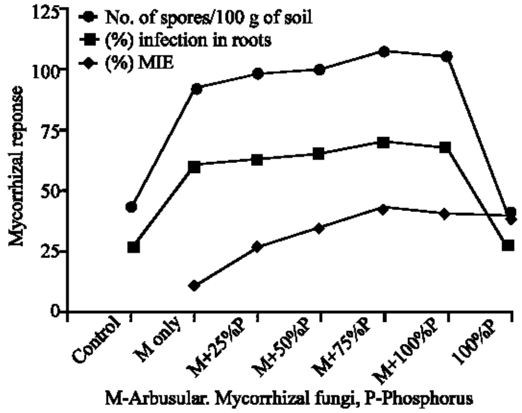

Fig. 1: Effect of chemical fertilizer (triple superphosphate) and AMF when combined together, on mycorrhizal response in *M. alba* var. Sujan Puri grown in alkaline soil

The data regarding post analysis of soil for pH EC, C, N, P and K are shown in Table 2. Although the treatments were found to be different from each other, the difference was not significant except in case of available P content. The slight decrease in soil pH after the experiment could be due to release of certain organic acids by AM fungi (Graham *et al.*, 1981). Localized enhanced excretion of organic acids increases the effectiveness of exudates for nutrient mobilization, not only of P but also of micronutrients such as Zn, Fe and Mn (Ohwaki and Hirata, 1992). Also it is well established that the presence of low molecular weight organic acids (e.g., citric acid, oxalic acid) in the rhizosphere soil enhances the solubility of mineral P by ligand exchange and complexation of metal ions such as aluminum (Al^{3+}), iron (Fe^{3+}) and calcium (Ca^{2+}) (Bar-Yosef, 1996). The added quantity of P i.e., 25, 50, 75 and 100% might have also added P in the soil. EC was found to be slightly increased in all the treatments, which could be due to increased release of nutrients (ions). A decrease in available P content was observed in treatment T_1 while in other treatments an increase was noticed. Available N and K contents were increased in the rhizospheric soil of all the treatments as compared to control which could have been due to rapid mineralization of underground organic matter resulting in more release of these nutrients.

The C, N, P and K contents of all the treatments were increased as compared to control plants (Table 3). Except the carbon percent, all the nutrient contents of all the treatments, increased significantly over control. AM fungi are known to enhance nutrient uptake, especially in P-deficient soil (Singh, 1990). Application of P in combination with *G. fasciculatum* had increased effect in

Table 2: Effect of different doses of triple super phosphate and AMF on chemical characteristics of rhizospheric soil of *M. alba* var. Sujan Puri grown in alkaline soil

Treatments	pH	EC (mmho/cm)	C (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
T_1	8.73±0.345	0.39±0.019	0.53±0.029	399.2±15.80	5.7±0.269	224.3±8.88
T_2	8.69±0.342	0.39±0.015	0.55±0.022	399.9±18.85	8.0±0.377	225.0±8.86
T_3	8.66±0.343	0.40±0.019	0.57±0.032	400.5±15.85	10.8±0.510	225.8±8.94
T_4	8.62±0.339	0.40±0.016	0.58±0.023	401.2±18.91	12.0±0.566	226.5±8.92
T_5	8.51±0.337	0.41±0.020	0.64±0.036	403.1±15.95	15.3±0.722	228.7±9.05
T_6	8.58±0.338	0.40±0.016	0.60±0.024	401.8±18.94	20.2±0.952	228.0±8.98
T_7	8.55±0.337	0.41±0.016	0.62±0.024	402.5±18.97	20.0±0.943	227.2±8.95
CD at 5%	0.536	0.027	0.043	27.84	1.050	14.08

All values are mean and standard deviation of five replicates

Table 3: Nutrient content of *M. alba* var. Sujan Puri grown on alkaline soils treated with triple super phosphate and AMF

Treatments	C (%)	N (%)	P (%)	K (%)
T_1	37.0±1.28	2.0±0.07	0.13±0.005	0.79±0.027
T_2	37.5±1.10	2.2±0.06	0.14±0.005	0.80±0.032
T_3	37.9±1.32	2.4±0.08	0.16±0.005	0.91±0.036
T_4	38.3±1.12	2.5±0.07	0.19±0.006	1.02±0.035
T_5	39.2±1.36	2.8±0.09	0.23±0.008	1.30±0.052
T_6	38.8±1.13	2.7±0.07	0.25±0.008	1.23±0.042
T_7	38.6±1.13	2.6±0.07	0.25±0.008	1.28±0.043
CD at 5%	1.85	0.11	0.01	0.058

All values are mean and standard deviation of five replicates

Table 4: Effect of AMF and phosphorus on the growth and yield of *M. alba* var. Sujan Puri grown in alkaline soil

			Shoot					Root			
				Weight (g)					Weight (g)		
Treatment	Sprouting of cuttings (%)	Survival (%)	Height (cm)	Fresh	Dry	No. of lateral branches/ plant	No. of leaves/ plant	Height (cm)	Fresh	Dry	Shoot: Root weight ratio
T_1	73.3±2.41	80.0±3.16	46.1±1.82	42.1±1.39	15.0±0.59	1.6±0.05	49.8±1.66	39.4±1.3	17.7±0.31	2.4±0.08	5.46±0.18
T_2	80.0±3.77	83.3±2.73	50.6±1.67	47.2±2.23	16.5±0.65	2.0±0.08	57.9±2.28	42.8±1.68	10.0±0.33	3.1±0.12	4.72±0.22
T_3	86.7±2.85	90.0±3.55	65.4±2.59	55.2±1.82	19.2±0.76	2.2±0.07	68.8±2.29	53.4±1.78	14.5±0.57	4.5±0.15	3.80±0.13
T_4	93.3±4.40	93.3±3.05	75.5±2.49	61.8±2.91	21.3±0.8	42.6±0.10	82.6±3.25	55.6±2.19	17.6±0.59	5.3±0.21	3.51±0.16
T_5	100.0	100.0	103.7±4.10	73.9±2.44	24.7±0.98	3.0±0.10	115.1±3.83	60.2±2.00	20.2±0.80	6.0±0.20	3.65±0.12
T_6	100.0	100.0	100.8±3.32	70.2±3.31	23.8±0.94	3.0±0.12	109.7±4.32	58.2±2.29	18.0±0.60	5.4±0.21	3.91±0.18
T_7	100.0	100.0	98.7±3.25	69.3±3.27	23.7±0.94	2.8±0.11	108.4±4.27	57.9±2.28	18.2±0.61	5.3±0.21	3.80±0.17
CD at 5%	6.04	5.21	4.51	4.05	1.302	0.147	5.17	3.09	0.891	0.276	0.263

enhancing N, P, K, Ca and Mg content in blackgram plants compared to uninoculated plant supplemented with P alone (Devi and Sitaramaiah, 1990). Krishna *et al.* (2005) reported that mycorrhizal inoculation accumulated higher N, P, Mg and Fe in grape plantlets. AMF stimulate growth of many tropical trees by promoting a more efficient uptake of nutrient elements such as P (Cuenca *et al.*, 1990; Michelsen and Rosendahl, 1990). It has been demonstrated that hyphal inflow of P (uptake of P per unit length of hypha per unit time) is about six times higher in AM infected roots that in uninfected roots and this hyphal inflow of P differs with AM species (Sreenivasa, 1993). Mycorrhizae are also known to enhance the phosphate uptake by enhancing phosphatases activity. Phosphatases play an important role in the solubilization of P from non-available P sources (Kumari *et al.*, 1990). There is evidence that phosphatase activity is higher in the rhizosphere around AM than in non-mycorrhizal roots (Dodd *et al.*, 1987). Acquisition of organically bound P is enhanced by acid phosphatases released as ectoenzymes from roots, or from microorganisms including AM fungi (Tarafdar and Marschner, 1994). The activity of acid phosphatases in the rhizosphere is enhanced under P deficiency (Ozawa *et al.*, 1995).

Data pertaining to the effect of inoculation of AMF and different doses of P on the plant growth parameters of *M. alba* var. Sujan Puri presented in Table 4 showed that the total biomass yield was higher in all mycorrhizae inoculated plants as compared to uninoculated control ones. The increase in all plant growth parameters and total biomass yield was significantly superior over the control. The best results pertaining to all plant growth parameters i.e., survival (100%), number of leaves/plant (115.1), shoot fresh weight (73.9 g), total biomass yield/plant (94.1 g) were obtained with M+75% P (T_5) followed by M+100% P (T_6), 100% P only (T_7), M+50% P (T_4), M+25% P (T_3), M (T_2) and control (T_1). The best growth and yield at 75% of the P level in the plants inoculated with AMF may be due to the availability of optimum amount of P required and other nutrients absorbed by AMF exhibiting higher percentage root colonization and number of spores observed at this level (Fig. 1). The present data of increased growth of plants with AMF and P inoculation in soils with low available P are also corroborated by Clarke and Mosse (1981), Powell (1981), Padma and Kandasamy (1990), Sharma and Adholeya (2004). Padma and Kandasamy (1990) also concluded that among the different levels of P, 75% of the recommended level of P+AM significantly enhanced the growth of papaya than the other P levels, thus indicating the optimum dose for the crop. Similarly M+75% P has been observed as the best combination in the present study. The increased growth of plants inoculated with AMF could also be due to increased uptake of Zn because it has been reported that alkaline soils are also deficient in Zn besides P.

CONCLUSION

The present study suggested a net saving of 25% of the recommended dose of inorganic P i.e., triple super phosphate, in alkaline soils for the proper growth of mulberry. By exploiting AM fungi not only the use of chemical fertilizer is minimized, soil fertility is also improved on sustainable basis.

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