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G. C. Setua · R. Kar · J. K. Ghosh · K. K. Das
S. K. Sen

Influence of arbuscular mycorrhizae on growth, leaf yield and phosphorus uptake in mulberry (*Morus alba* L.) under rainfed, lateritic soil conditions

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Abstract A field experiment was conducted to study the effect of arbuscular mycorrhizal association in mulberry saplings var. S_1, together with two levels of phosphate, on growth characters, leaf yield and phosphorus uptake. The experiment was conducted in the period 1995 to 1997 at the Technical Service Centre, Kashipur, Purulia (West Bengal) under rainfed, lateritic soil conditions. Pooled data analysis revealed that there was no significant variation among the treatments in most of the characters except height of plant and leaf moisture percentage. Plant height in both treatments with *Glomus mosseae* was significantly less than in the others, while there was significant reduction in moisture percentage in *G. fasciculatum* with 25 kg of phosphate and *G. mosseae* with 10 kg of phosphate ha^{-1} $year^{-1}$ than in other treatments. There was no significant season–treatment effect on any of the parameters. Considering all the quantitative and qualitative characters, *G. fasciculatum* together with the most economic dose of phosphate showed more promising performance than *G. etunicatum* and *G. mosseae* with different doses of phosphate fertilizer. At 80% of the recommended dose of inorganic phosphate fertilizer, Rs 534.0 were earned as net profit.

Key words Mulberry · Phosphate mobilization · *Glomus etunicatum* · *Glomus fasciculatum* · *Glomus mosseae*

Introduction

In plants, phosphorus (P) is required for maximization of root proliferation, quality leaf production, and also for resistance towards some diseases (Patnaik 1980). Total P exists in abundant quantities in the soil in most places in West Bengal, but little is in a form available to plants (Panda 1996).

The lateritic soil of the dry area of West Bengal, particularly of Purulia, contains only 10 kg P ha^{-1} in an available form while more than 600 kg ha^{-1} of P is present in bound or non-available form. Application of phosphate to soil is restricted by its high price. Moreover, continuous use of these chemical fertilizers deteriorates the soil texture and properties. It is a primary aim to exploit maximum use of biofertilizers and/or organic manures in any cultivation to reduce the use of chemical fertilizers. It is now well established that arbuscular mycorrhizas (AM) are capable of solubilizing the non-available form of phosphate. Also, the diffused path for transport of the plant-available form of phosphate is shortened, favouring the growth and yield of crop plants (Iqbal and Qureshi 1977; Sulochana and Manoharachary 1989; Nye and Tinker 1977).

There were also reports that AM are more active particularly in phosphorus- and moisture-deficient soil (Daft and Nicolson 1972, 1974; Black and Tinker 1977; Iqbal and Qureshi 1977; Saif and Khan 1977; Sieverding 1981). Extensive work (Heyler and Godden 1977; Bagyaraj and Sreeramulu 1982; Howeler et al. 1987; Bolan 1991; Katiyar et al. 1995) was done with the inoculation of various strains of AM in higher plants to improve growth and yield, while the utilization of inorganic phosphate fertilizer was substantially decreased in respect to variation within AM strains.

The present study was undertaken to ascertain the best strain of AM for quality and quantity leaf production, growth characters and P uptake in leaf of mulberry under the rainfed, lateritic soil conditions of West Bengal, in view of partial reduction of inorganic phosphate fertilizer.

G. C. Setua (✉) · R. Kar · J. K. Ghosh · K. K. Das
S. K. Sen
Mulberry Agronomy and soil Science Section,
Central Sericultural Research and Training Institute,
Berhampore 742101, Murshidabad, West Bengal, India
Tel.: +91-3482-51046, Fax: +91-3482-51046

Table 1 Initial physico-chemical properties of the experimental soil

Parameters	Value	Reference to the analytical methods followed
Sand (kg kg^{-1})	0.65	International pipette method
Silt (kg kg^{-1})	0.12	(Chopra and Kanwar 1982)
Clay (kg kg^{-1})	0.19	
Texture	Sandy loam	
Bulk density (mg m^{-3})	1.52	Core cutter method (Blake and Heritage 1986)
Water holding capacity (kg kg^{-1})	0.28	"Keen" box method (Chopra and Kanwar 1982)
pH (H_2O)	5.30	Soil water ratio 1:2.5 (Jackson 1973)
Cation exchange capacity [Cmol (p^+) kg^{-1}]	3.45	1M NH_4OAc equilibriation method (Black 1965)
Organic carbon (g kg^{-1})	2.50	Chromic acid digestion method (Black 1965)
Total N (g kg^{-1})	0.30	Kjeldahl digestion method (Jackson 1973)
Total P (g kg^{-1})	0.30	Tri acid digestion method
Total K (g kg^{-1})	1.80	(Jackson 1973)
Available N (kg ha^{-1})	146	Alkaline $KMnO_4$ method (Subbiah and Asija 1956)
Available P (kg ha^{-1})	10	Bray's extraction method (Jackson 1973)
Available K (kg ha^{-1})	170	NH_4 OAc extraction method (Jackson 1973)

Materials and methods

Soil and leaf analysis

The experiment was undertaken at the Technical Service Centre, Kashipur, Purulia (West Bengal) under rainfed, lateritic soil conditions. The initial characteristics and nutrient status of soil of the experimental field along with the relevant references for the analytical method followed are presented in Table 1. Uptake of P by leaf was determined with triacid (HNO_3:$HClO_4$:H_2SO_4) digested samples followed by spectrophotometric analysis with vanadomolybdate reagent (Jackson 1973). Moisture content in leaf was estimated by the oven drying method and leaf area was determined by the method of Satpathy et al. (1992). Atmospheric outdoor temperature was obtained by maximum-minimum thermometer and relative humidity was measured by dry and wet bulb thermometer. Total rainfall was measured by rain gauge and soil moisture by a gravimetric method.

Raising of mycorrhizal saplings

AM inocula were grown in earthen pots containing sterilized soil – FYM (Farm Yard Manure) (2:1) mixture under glasshouse conditions with maize plants. Nursery soil was surface sterilized twice by burning dry leaves, twigs etc. Saplings of mulberry var. S1 with the association of *Glomus etunicatum* (GE), *G. fasciculatum* (GF) and *G. mosseae* (GM) were grown separately in the nursery by using an AM inoculum at 200 kg ha^{-1} containing 100 spores per 5 g of rhizosphere soil, applied in furrows between the rows of cuttings. AM colonization in root was estimated by the method of Phillips and Hayman (1970) while percentage of root colonization was calculated by the root slide technique (Nicolson 1960). Spore density in rhizosphere soil was measured by the wet sieving and decanting technique (Gerdemann and Nicolson 1963).

Treatments

Six-month-old mulberry saplings with intact AM colonized root were transplanted to the field at 90×90 cm^2 spacing and maintained for 1 year for establishment. The experiment was initiated with pruning to a height of 15 cm above ground and was laid out in completely randomized block design with three strains of AM and two levels of phosphate (25 and 10 kg ha^{-1} $year^{-1}$) with three replicates in each treatment combination. AM-free saplings with 50 kg ha^{-1} $year^{-1}$ phosphate were treated as controls. Recommended cultural practices for rainfed mulberry, N (150 kg ha^{-1} $year^{-1}$) and K (50 kg ha^{-1} $year^{-1}$) were followed (Ullal and Narasimhanna 1987).

Data on growth characters, leaf yield, leaf area, moisture content and P uptake in leaves were obtained during August, October, February and May. Analysis of variance was performed on four seasons of two consecutive years. Seasonal effect on growth, yield and quality parameters were studied. However, the overall mean of each of the seven treatments and critical difference value ($P \leq 0.05$) were calculated. Economic gain due to reduction in dose of phosphate to 10 kg ha^{-1} $year^{-1}$ through the use of AM biofertilizer was also calculated.

Results

Table 1 shows that the soil was acidic with poor water and cation retention capacity. It was also low in organic C, available N and P content. A "climate diagram" corresponding to month-wise average for 2 years is presented in Fig. 1.

Analysis of variance in relation to effect of AM on growth characters, leaf yield and quality (Table 2) revealed that there was no significant difference in effects

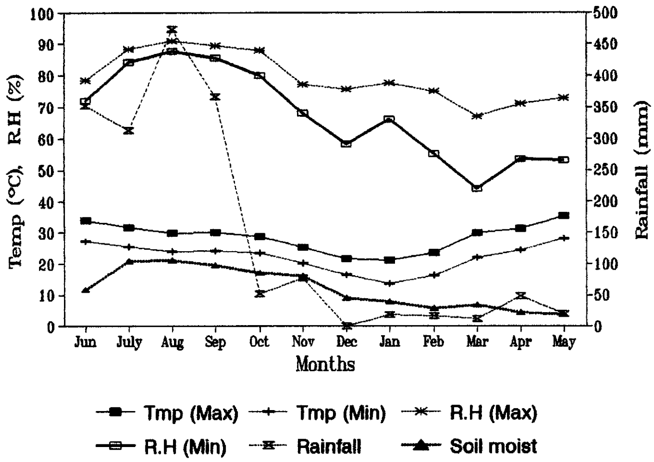

Fig. 1 Month-wise average climate diagram during the experimental period

Table 2 Analysis of variance (Mean Sum of Squares) for different plant growth and quality parameters. *Repl.* replicates, *Treat.* treatment, Trt × Sea = Treatment × Season, *NS* not significant

Source of var.	Degrees of freedom	Plant height (cm)	No. of branches plant $^{-1}$	No. of leaves branch $^{-1}$	Leaf yield (kg ha $^{-1}$ year $^{-1}$)	Leaf moisture (%)	Leaf area (cm ^{2})	Phosphorus uptake in leaf (mg g $^{-1}$)
Repl.	2	3321.23** 70.18	15.26** 16.12	25 217.90** 8.42	1 704 950.00** 10.72	2.63NS 0.90	56.56NS 0.8361	144 764.00 2.99
Treat.	6	159.91** 3.37	0.55NS 0.58	1322.73NS 0.44	81 364.80NS 0.51	7.58* 2.60	86.30 1.28	30 306.80NS 0.63
Season	3	4084.15** 86.31	13.40** 14.16	365 984.00** 122.26	41 830 400.00** 263.11	2148.20** 737.64	59 034.50** 872.69	11 019 400.00** 227.94
Trt × Sea	18	17.51NS 0.37	0.42NS 0.44	937.05NS 0.31	29 601.90NS 0.19	5.30NS 1.82	42.00NS 0.62	56 323.50NS 1.16
Error	54	47.32	0.90	2993.48	158 985.00	2.91	67.63	48344.10
Total	83	22 725.00	132.63	1 334 840.00	138 507 000.00	6748.03	182 143.00	37 154 000.00

* = $P < 0.05$
** = $P < 0.01$

Table 3 Effect of AM on growth attributes and leaf yield under rainfed, lateritic-soil conditions (2 years pooled data)

Treatment (phosphate · kg ha $^{-1}$ year $^{-1}$)	AM colonization rate (%)	Plant height (cm)	No. of branches per plant	No. of leaves per plant	Leaf yield (kg ha $^{-1}$ year $^{-1}$)
50 (Cont.)	30.14	180.4	9.8	376.9	7337.2
25 with GE	63.25	179.0	9.9	368.3	7167.2
10 with GE	66.84	177.6	10.1	360.6	6996.3
25 with GF	60.39	178.2	9.6	362.1	6627.6
10 with GF	64.71	175.0	10.1	372.5	6890.0
25 with GM	57.33	173.5	9.6	352.0	6622.6
10 with GM	60.55	169.9	9.6	348.2	6414.0
CD at 5%	1.99	5.63	NS	NS	NS

Table 4 Effect of AM on leaf quality under rainfed, lateritic soil conditions (2 years pooled data)

Treatment (P kg ha $^{-1}$) (year $^{-1}$)	Moisture (%)	Leaf area (cm ^{2})	P uptake in leaf (mg g $^{-1}$)
50 (Cont.)	68.0	69.1	2.7
25 with GE	67.7	64.5	2.7
10 with GE	67.6	63.2	2.7
25 with GF	66.0	63.3	2.7
10 with GF	68.3	68.6	2.8
25 with GM	67.7	68.7	2.7
10 with GM	66.7	64.6	2.8
CD at 5%	1.4	NS	NS

of treatment ($P \leq 0.05$) in respect of parameters under study, except plant height and leaf moisture percentage. However, the statistical analysis of data collected over eight seasons revealed that all the three strains of AM at reduced doses of phosphate were found to be similar in respect to number of branches per plant, number of leaves per plant and leaf yield over control, except plant height (Table 3). Plant height was found to be statistically significant and attained maximum (180.4 cm) in control (50 kg ha $^{-1}$ year $^{-1}$ phosphate) followed by 25 kg ha $^{-1}$ year $^{-1}$ phosphate each with the association of GE (179.0 cm) and GF (178.2 cm).

With regard to leaf quality, there was no significant variation among the treatments, except for leaf moisture percentage. However, moisture content, P uptake in leaf and leaf area were maximum or comparable to the association of GF at 10 kg ha $^{-1}$ year $^{-1}$ phosphate over control (Table 4). The effect of season × treatment was not found to be significant in any of the characters (Table 2; Figs. 2, 3). A comparative economic potentiality of AM application over inorganic phosphate fertilization is presented in Table 5. It was observed that the application of AM could supplement a substantial quantity of phosphate fertilizer and could also save Rs 534.0 per ha per year on the input cost.

Discussion

From the above findings it was evident that 80% of the total phosphate fertilizer requirement could be supplemented by the inoculation of AM fungi. It was also interesting to note that introduction of AM inoculum produced a similar effect on growth and yield parame-

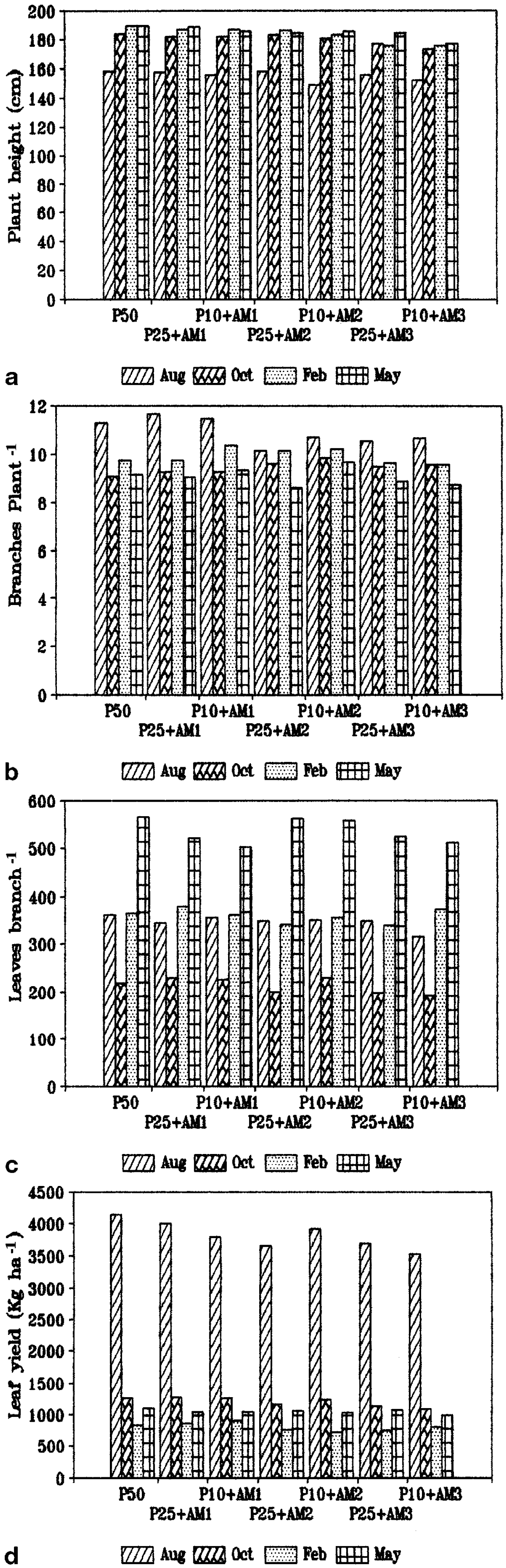

Fig. 2a–d Seasonal effect of AM on different growth characters in mulberry

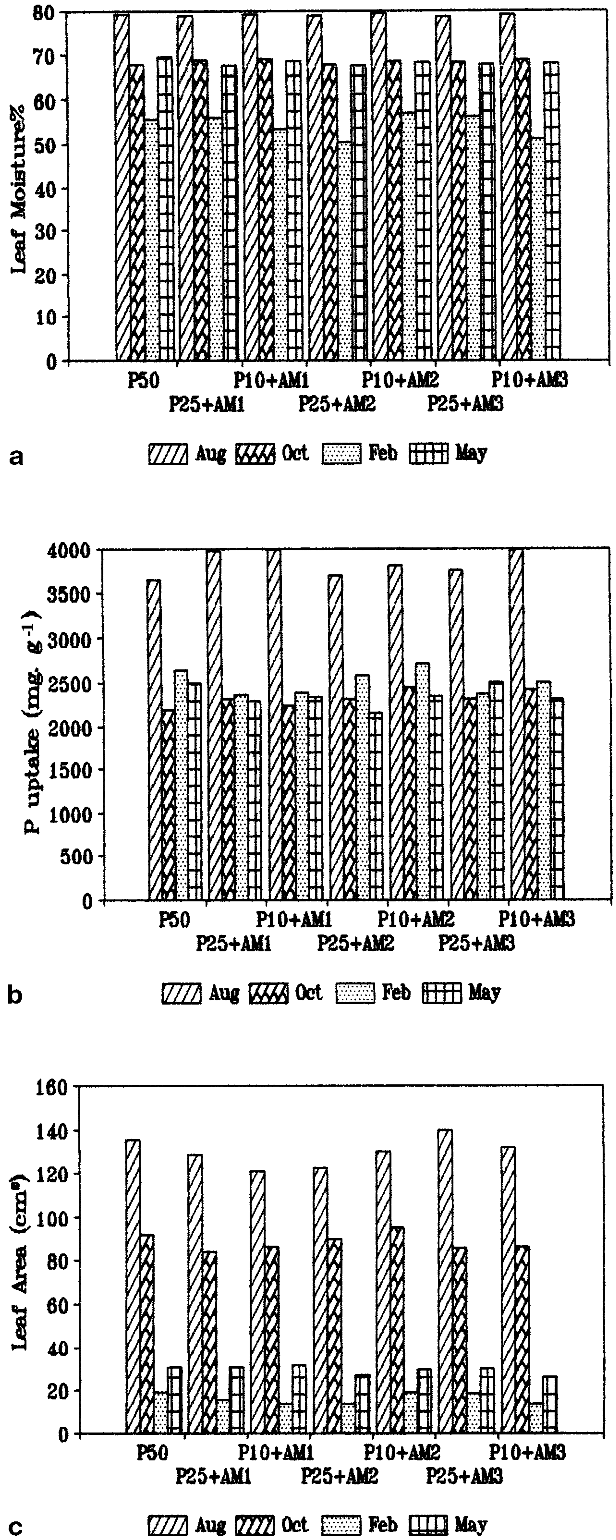

Fig. 3 Seasonal effect of AM on different leaf quality characters in mulberry

Table 5 Economic gain on application of AM biofertilizer

Nature of mulberry saplings Var S_1	Cost of AM inoculum (Rs ha^{-1})	Dose of phosphate (kg ha^{-1} $year^{-1}$)	Quantity of SSP reqd. (kg ha^{-1} $year^{-1}$)	Cost of SSP (Rs)	Profit (Rs)	Leaf yield (kg ha^{-1} $year^{-1}$)
Normal	–	50 (Recommended)	312.5	775	–	7337.2
Mycorrhizal	86	10 (Reduced)	62.5	155	534	6890.0

Rate of *SSP* (Single Super phosphate) (16% P_2O_5): Rs 2.48 per kg
Rate of AM inoculum: RS 10.00 per kg

ters at reduced doses of phosphate fertilizer over control. It is apparent that the phosphate solubilizing effect, coupled with shortening of P diffusion pathways due to AM inoculation, might be a plausible method for reducing phosphate fertilizer requirement (Hattingh et al. 1973; Nye and Tinker 1977).

Leaf quality, moisture content, P uptake in leaf and leaf area were found to be maximum or comparable with the association of GF at 10 kg ha^{-1} $year^{-1}$ phosphate over control. Season-treatment interaction was also not found to be significant on any of the parameters. The reason for maintenance of leaf quality with reduction of phosphate fertilizer, associated with GF inoculation, may be explained in a similar manner to that discussed above. Moreover, profuse mycelial growth of AM resulted in an increase in mycelial absorbing surface for diffusion of $H_2PO_4^-$ (Rhodes and Gerdemann 1975) and maximum solubilization of colloidal phosphate by producing organic and hydroxy acid, as influenced by AM, and phosphate solubilizing bacteria in rhizosphere soil, resulting in more P uptake (Hattingh et al. 1973). It is pertinent to mention here that P uptake in leaf was not reduced at the low dose of phosphate fertilizer, as mycorrhizal hyphae could absorb iron and aluminium phosphate (Nye and Tinker 1977), prevalent in the lateritic soil under study (Panda 1996). The result corroborated the findings on the improvement of growth, yield and quality in different crop plants (Black and Tinker 1977; Iqbal and Qureshi 1977; Bagyaraj and Sreeramulu 1982; Sulochana and Manoharachary 1989; Bolan 1991; Katiyar et al. 1995). Moreover, the activity of endomycorrhiza enhanced due to low phosphate and moisture content, as found in the experimental soil, and thus confirmed earlier findings (Daft and Nicolson 1974; Sieverding 1981).

Biofertilizers are eco-friendly and help to preserve the biodiversity and also improve soil condition to a great extent. Biofertilizers as a partial alternative to chemical fertilizer are now being used in many crop plants (Bagyaraj and Sreeramulu 1982; Bolan 1991). Earlier attempts (Setua et al. 1995) established that out of three strains, GM-associated mycorrhizal saplings variety S_1 was found to have the best potential for similar growth characters, leaf quality and similar or more leaf yield at 80% reduced dose of costly phosphate fertilizer over the recommended dose (180 kg per ha per year) in Gangetic alluvium under irrigated conditions, and thus earned Rs. 2325.0 per ha per year as net profit.

It is concluded from this study that out of three species of *Glomus* and two reduced levels of phosphate, GF-associated S_1 mulberry sapling was found to have the best potential and performed consistently better in respect to number of branches per plant, leaf yield, leaf area, moisture content and P uptake at the most economic dose of phosphate fertilizer (10 kg ha^{-1} $year^{-1}$) with comparable quality of mulberry leaves. This particular treatment produced a 6.09% loss of yield over control, but saved 80% phosphate (40 kg) over the recommended dose and thereby could save Rs. 534.0 per ha per year. This conclusion is helpful in the commercial exploitation of new mulberry plantations in rainfed, lateritic soil conditions.

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