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INFLUENCE OF NITROGEN AND PHOSPHATE SOLUBILIZING BACTERIA AND PHOSPHORUS SOURCES ON GROWTH, CHLOROPHYLL AND YIELD OF MAIZE

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SUMMARY

Field experiments were conducted during *kharif* seasons of 2002 and 2003 at Indian Agricultural Research Institute, New Delhi to study the effect of different phosphatic fertilizers and biofertilizers on growth attributes and chlorophyll content of maize. Different doses of phosphatic sources (single super phosphate and rock phosphate with and without PSB and VAM) were applied either without nitrogen or in combination with nitrogen fertilizer. The treatments $N_{120}P_{60}$ gave the highest leaf area and dry matter/ plant during both the years and in second year it was at par with $N_{120}SSP_{30}VAM$. In case leaf area index (LAI), highest values were associated with $N_{120}P_{60}$ at 40 DAS in 2002 and 2003. In second year it was statistically at par with $N_{120}SSP_{30}VAM$ at 40 and 80 DAS. At knee high stage of maize, the highest chlorophyll content of 1.03 mg/g and 1.10 mg/g fresh weight were recorded in $N_{120}SSP_{30}VAM$ and $N_{120}RP_{30}VAM$ in 2002 and 2003 respectively. The treatments $N_{120}SSP_{30}VAM$ and $N_{120}RP_{30}PSB$ were statistically at par in both the years.

Key words: **Chlorophyll, growth attributes, maize, phosphatic source.**

INTRODUCTION

In India, most of the soils are either deficient or marginal in phosphorus (P) status. Adequate P fertilization is thus essential for economic and sustained crop production. Phosphorus deficient soils respond to heavy dose of phosphatic fertilizers for optimal yield. The requirement of P is met by the import of phosphatic fertilizers which proves expensive. Also immediate conversion of water soluble P, due to P fixation result in low fertilizer use efficiency. Among the different inorganic P sources, single super phosphate (SSP) is most widely used phosphatic fertilizers which supply P in water soluble form in the immediate vicinity of roots. Its importance as the most efficient P fertilizer source is well established. It also suffers from the problem of fixation in the long run. However, India has vast resources of indigenous rock phosphate (RP), unfortunately most of the RPs of Indian origin have the limitation of low P_2O_5 content and low reactivity and perform poorly when applied directly to neutral soil and are not suitable for manufacture of phosphatic fertilizer (Dwivedi and Dwivedi 1992). With the discovery of several deposits of RP in the country, interest in the use of this indigenous material as alternative phosphatic fertilizers has increased greatly. Although RP can effectively replace water soluble phosphates in acid soils, but its efficacy in neutral, alkaline and calcareous soils is extremely low (Marschner 1983). To make it effective in such soils it is being converted into water soluble form by mixing with SSP or by partial acidulation with mineral acids, for which sulphur is being imported. In all these cases, the

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underlying principle is to supply acid for conversion of insoluble P of RP into soluble P. In this context, biofertilizers like phosphate solubilizing bacteria (PSB) and vesicular arbuscular mycorrhiza (VAM) can prove effective. The phosphate solubilizing bacteria releases organic acids in the rhizosphere which decreases the rhizosphere pH and helps in dissolution of insoluble and fixed form of P thus rendering them available to the plant. Three principle mechanisms have been postulated as being responsible for the effect work of VAM: (i) the greater exploitation of the soil volume by the hyphal network; (ii) the higher affinity of hyphae for phosphate and the ability to absorb P at lower solution concentrations than the roots themselves and (iii) changes in the rhizosphere by VAM, such as exudation of acids or chelates (Bolan 1991). Maize is a mycotrophic plant and owing to its high nutrient demand often benefits from mycorrhizal symbiosis. In low P soils, improvement in plant growth by mycorrhizal fungi appears as high levels of mycorrhizal infection are attained (Hall 1978b, Lu *et al*. 1994). Keeping the above facts in view the present study was undertaken to analyse the effect of various sources of phosphorus with and without PSB, VAM and nitrogenous fertilizer on growth and yield of maize.

MATERIALS AND METHODS

Field experiments were conducted on maize (*Zea mays* cv.Ganga Safed 2) in the research farm of Division of Agronomy at Indian Agricultural Research Institute during the *Kharif* season of 2002 and 2003.The experiment was laid out in randomized block design (RBD) with twelve treatments and three replications. The twelve treatments were - T_1: SSP_{60}; T_2: RP_{60}; T_3: N_{120}; T_4: $N_{120}P_{60}$; T_5: PSB; T_6: VAM; T_7: N_{120}PSB; T_8: N_{120}VAM; T_9: $N_{120}SSP_{30}$PSB; T_{10}: $N_{120}SSP_{30}$VAM; T_{11}: $N_{120}RP_{30}$PSB; T_{12}: $N_{120}RP_{30}$VAM. The soil of the experimental field was sandy loam in texture, low in organic carbon, available nitrogen and available phosphorus contents and medium in potassium. The soil pH was 7.6. The cation exchange capacity of the soil was low. Total leaf area of the fresh green functional leaves of maize was measured at 40 and 80 DAS with the help of leaf area meter.

$$CGR = W_2 - W_1 / t_2 - t_1$$

Where, W_1 and W_2 are plant dry weights at time t_1 and t_2 respectively. It was expressed in terms of g /m^2 (land area) / day. Leaf Area Ratio reflects the leafiness of a plant. It is calculated by using the following formula:

$$LAR = LA / W$$

Where, LA and W are leaf area and dry weight, respectively. It is expressed as cm^2 of leaf area per g dry matter. Estimation of chlorophyll was done by using Dimethyl sulfoxide (DMSO) method of extraction (Hiscox and Israelstam 1979). All the data of treatments were subjected to statistical analysis following the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Growth attributes

The highest leaf area of 3105.8 and 3142.5 cm^2/plant was obtained in $N_{120}P_{60}$ at knee high stage of maize in 2002 and 2003, respectively. But in second year it was at par with $N_{120}SSP_{30}$VAM. During the year 2002, $N_{120}P_{60}$ gave highest LAI of 2.59 while during 2003, the LAI was maximum (2.62) in $N_{120}P_{60}$ but it was statistically at par with $N_{120}SSP_{30}$VAM (2.60).

The VAM treatment gave the highest leaf area ratio of 126.70 cm^2/g at 40 DAS during 2002 which was statistically at par with PSB, $N_{120}P_{60}$, N_{120}, SSP_{60}, N_{120}VAM, $N_{120}SSP_{30}$PSB, $N_{120}SSP_{30}$VAM. During the year 2003 the highest LAR value of 107.84 cm^2/g was obtained in N_{120}VAM at the same growth stage. The treatments SSP_{60}, RP_{60}, N_{120}, PSB, VAM, N_{120}PSB, $N_{120}SSP_{30}$PSB, $N_{120}SSP_{30}$VAM were statistically at par with that of N_{120}VAM but were significantly greater than $N_{120}P_{60}$, $N_{120}RP_{30}$PSB, $N_{120}RP_{30}$VAM. Mala and Thongchai (1995) also observed the positive effect of VAM on maize growth.

The highest CGR value of 9.91 g/m^2/day in 2002 and 9.75 g/m^2/day in 2003 were observed in treatments $N_{120}SSP_{30}$VAM and $N_{120}SSP_{30}$PSB. However, these values were statistically at par with $N_{120}P_{60}$. Heggo and Barakah (1993) also suggested shoot and root dry weight were higher with SSP than with RP. Rock phosphate alone had little effect on plant growth, while RP combined with both inoculants gave similar shoot and root dry weight to SSP alone. Application of either RP or SSP decreases phosphatase activity in soil, while inoculation (particularly VAM) increased the activity. Phosphatase activity in roots has been lowest after application of SSP (Mosse, 1973). Highest plant DW was given by SSP combined with both soil inoculant. Mishra *et al*. (1995) also indicated that VAM, PSB increased grain yield by 6 and 31 %, respectively compared with the control.

Chlorophyll content

During the year 2002, the highest chlorophyll content of 1.03 mg/g fresh weight was recorded in $N_{120}SSP_{30}VAM$ which was statistically at par with that of $N_{120}RP_{30}PSB$. During the year 2003, $N_{120}RP_{30}VAM$ gave highest value of 1.10 mg/g fresh weight but it was statistically at par with $N_{120}RP_{30}PSB$ and $N_{120}SSP_{30}VAM$. In treatments SSP_{60}, PSB, VAM the chlorophyll content decreased in the second year, while in all other treatments it increased during 2003. The magnitude of increase in chlorophyll content over that of the preceding year was highest in treatment $N_{120}RP_{30}VAM$ followed by $N_{120}RP_{30}PSB$ (Table 1).

Rathore and Singh (1995) reported that phosphorus application and VAM inoculation increased dry matter accumulation and uptake of N, P and K by maize shoots at 30 and 45 days of growth. He also reported that mycorrhizal inoculum may substitute phosphatic fertilizer equivalent to about 30 kg P / ha.

The leaves are the main photosynthesizing organ of a plant and one of the main contributors of final grain yield. The treatments $N_{120}P_{60}$ gave the highest leaf area and dry matter. The treatments $N_{120}SSP_{30}VAM$ gave comparable performance in the first year while in second year the treatment $N_{120}RP_{30}PSB$ gave similar performance as that of $N_{120}P_{60}$.

When the chemical P sources fertilizer like SSP, RP and the phosphatic biofertilizer were applied singly, the

Table 1. Effect of phosphorus on growth attributes at knee high stage.

Treatment	Leaf area (cm^2/plant)		Dry matter (g/plant)		Crop growth rate (g/m^2/day)		Leaf area index		Leaf area ratio (cm^2/g)		Chlorophyll (mg/g fw)	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
SSP_{60}	940.2^{gh}†	960.5^{fg}	7.52^{h}	9.15^{e}	6.33^{d}	6.33^{d}	0.78^{gh}	0.80^{fg}	125.03^{ab}	104.99^{a}	0.65^{efg}	0.63^{d}
RP_{60}	693.6^{i}	723.3^{h}	6.45^{i}	7.12^{f}	5.83^{d}	6.08^{d}	0.58^{i}	0.60^{h}	107.50^{d}	101.57^{abc}	0.54^{g}	0.61^{d}
N_{120}	957.6^{gh}	972.2^{fg}	8.12^{g}	9.35^{e}	7.41^{c}	7.50^{c}	0.80^{gh}	0.81^{fg}	118.02^{abc}	103.99^{ab}	0.73^{def}	0.76^{c}
$N_{120}P_{60}$	3105.8^{a}	3142.5^{a}	24.74^{a}	31.89^{a}	9.83^{a}	9.33^{ab}	2.59^{a}	2.62^{a}	125.54^{ab}	98.62^{bcd}	0.91^{b}	0.94^{b}
PSB	872.7^{h}	914.1^{g}	7.04^{h}	8.64^{ef}	6.25^{d}	6.41^{d}	0.73^{h}	0.76^{g}	123.94^{abc}	105.79^{a}	0.60^{g}	0.59^{d}
VAM	916.0^{gh}	952.3^{fg}	7.23^{h}	9.06^{e}	6.25^{d}	6.25^{d}	0.76^{gh}	0.79^{fg}	126.70^{a}	105.11^{a}	0.62^{fg}	0.57^{d}
$N_{120}PSB$	1053.8^{fg}	1081.5^{f}	9.25^{f}	10.46^{de}	7.25^{c}	7.75^{c}	0.88^{fg}	0.90^{f}	113.93^{cd}	103.37^{abc}	0.76^{cde}	0.82^{c}
$N_{120}VAM$	1170.0^{f}	1286.1^{e}	9.69^{f}	11.92^{d}	8.08^{b}	8.00^{c}	0.98^{f}	1.07^{e}	120.79^{abc}	107.84^{a}	0.79^{cd}	0.80^{c}
$N_{120}SSP_{30}PSB$	2181.2^{d}	1886.7^{d}	18.69^{d}	18.24^{c}	8.33^{b}	9.75^{a}	1.82^{d}	1.57^{d}	116.73^{abcd}	103.43^{abc}	0.87^{bc}	0.96^{b}
$N_{120}SSP_{30}VAM$	2788.8^{b}	3125.0^{a}	22.47^{b}	30.22^{a}	9.91^{a}	9.00^{b}	2.32^{b}	2.60^{a}	124.11^{abc}	103.73^{abc}	1.03^{a}	1.06^{a}
$N_{120}RP_{30}PSB$	2371.1^{c}	2966.0^{b}	20.54^{c}	31.63^{a}	9.33^{a}	9.41^{ab}	1.98^{c}	2.47^{b}	115.42^{bcd}	93.83^{d}	0.96^{ab}	1.09^{a}
$N_{120}RP_{30}VAM$	1522.7^{e}	2411.5^{c}	17.31^{e}	24.74^{b}	8.33^{b}	8.91^{b}	1.27^{e}	2.01^{c}	88.19^{e}	97.50^{cd}	0.85^{bc}	1.10^{a}
SEm±	47.73	48.63	0.193	0.577	0.242	0.216	0.041	0.043	3.135	1.902	0.037	0.032
CD (P = 0.05)	140.00	142.64	0.567	1.693	0.708	0.633	0.119	0.124	9.194	5.578	0.107	0.093

†Within a column, means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT).

growth was very low. This may be attributed to the absence of nitrogenous fertilizer. The element nitrogen play important role in affecting growth and development of plants and in the absence of N the treatments gave very poor growth performance. When phosphatic biofertilizers like PSB, VAM were applied in combination with N_{120}, at 80 DAS there was 46.79 % increase in leaf growth in 2002 and 57.27 % leaf growth in 2003 in N_{120}PSB and the corresponding increase was 75.38 % and 66.63 % respectively in N_{120}VAM. But in absence of any inorganic source of P fertilizer the treatments recorded very poor growth performance. With the addition of SSP along with N_{120} and PSB there was 32.67 % increase in dry matter yield at 80 DAS in 2002 and 37.29 % in 2003 over that of N_{120}PSB. Similarly, with addition of RP along with N_{120}PSB, the corresponding figures were 47.97 % and 61.71 % respectively. In case of application of SSP along with N_{120} and VAM, the dry matter yield at 80 DAS increased by 46.33 and 45.53 % in 2002 and 2003 respectively. When RP was applied along with N_{120} and VAM the corresponding figures were 18.32 and 34.58 % respectively. Thus the higher percent increase was obtained when SSP was applied with N_{120}VAM and when RP was applied with N_{120}PSB.

Grain yield

During the year 2002, the highest grain yield of 47.77 q/ha was recorded in $N_{120}SSP_{30}$VAM closely followed by $N_{120}P_{60}$, $N_{120}RP_{30}$PSB and all these treatments were statistically at par. During the year 2003, the treatment $N_{120}RP_{30}$PSB recorded the highest grain yield of 51.27 q/ha which was statistically at par with $N_{120}SSP_{30}$VAM but was significantly greater than $N_{120}P_{60}$. The grain yield during 2003 improved in most of the treatments except treatments SSP_{60}, PSB, VAM where the grain yield was lower in the year 2003. *Bacillus subtilis,* a PSB has been reported to enhance grain yield in maize (Malik *et al*. 1996). Dubey (1996) reported that in case of seed yield

Table 2. Effect of treatments on yield and harvest index of maize

Treatment	Grain yield (q/ha)			Stover yield (q/ha)			Harvest index		
	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled
SSP_{60}	34.96^{f}†	31.37^{f}	33.17	70.44^{g}	69.04^{e}	69.74	0.33^{ab}	0.31^{abc}	0.32
RP_{60}	25.93^{h}	27.75^{h}	26.84	65.79^{i}	65.17^{g}	65.48	0.28^{b}	0.30^{c}	0.29
N_{120}	36.35^{ef}	38.21^{e}	37.28	72.46^{f}	75.16^{d}	73.81	0.33^{ab}	0.34^{abc}	0.34
$N_{120}P_{60}$	45.36^{abc}	48.36^{b}	46.86	79.89^{c}	83.25^{b}	81.57	0.36^{a}	0.37^{a}	0.37
PSB	31.42^{g}	28.86^{gh}	30.14	67.78^{h}	66.71^{fg}	67.25	0.32^{ab}	0.30^{c}	0.31
VAM	33.87^{fg}	30.17^{fg}	32.02	68.30^{h}	67.92^{ef}	68.11	0.33^{ab}	0.31^{bc}	0.32
N_{120}PSB	38.82^{de}	40.81^{d}	39.82	75.76^{e}	77.40^{c}	76.58	0.34^{ab}	0.34^{abc}	0.34
N_{120}VAM	40.05^{d}	41.63^{d}	40.84	76.16^{e}	77.55^{c}	76.86	0.34^{ab}	0.35^{abc}	0.35
$N_{120}SSP_{30}$PSB	44.10^{bc}	44.84^{c}	44.47	78.24^{d}	81.53^{b}	79.89	0.36^{a}	0.35^{abc}	0.36
$N_{120}SSP_{30}$VAM	47.77^{a}	49.95^{a}	48.86	84.76^{a}	86.02^{a}	85.39	0.36^{a}	0.37^{ab}	0.37
$N_{120}RP_{30}$PSB	46.52^{ab}	51.27^{a}	48.90	82.40^{b}	86.51^{a}	84.46	0.36^{a}	0.37^{a}	0.37
$N_{120}RP_{30}$VAM	42.77^{c}	45.86^{c}	44.32	77.64^{d}	81.71^{b}	79.68	0.35^{a}	0.36^{abc}	0.36
SEm±	0.877	0.455		0.496	0.642		0.018	0.018	
CD (P = 0.05)	2.574	1.334		1.456	1.883		0.054	0.054	

† Within a column, means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test (DMRT)

of soybean superphosphate is superior to rock phosphate, but when rock phosphate at the rate of 60 kg P_2O_5 / ha was applied with *Pseudomonas striata* (PSB) inoculant, it remained at par with superphosphate at the rate of 60 kg P_2O_5 / ha. Koreish *et al.* (1998) reported the effect of VAM and phosphate dissolving bacteria on yield and yield components. Grain and straw yield as well as N, P uptake improved with inoculation by PSB or VAM and their combination along with Mussorie rock phosphate amendment (Singh and Kapoor 1999).

The highest grain yield was obtained against treatment T_{11} ($N_{120}RP_{30}PSB$) but it was closely followed by T_4 ($N_{120}P_{60}$) and T_{10} ($N_{120}SSP_{30}VAM$). The treatments T_1 (SSP_{60}), T_5 (PSB), T_6 (VAM) showed reduction in grain yield during 2003. The treatments T_2 (RP_{60}), T_{11} ($N_{120}RP_{30}PSB$) and T_{12} ($N_{120}RP_{30}VAM$) registered sharp increase during 2003. This may be attributed to greater P availability from RP with the increase in time after application unlike the case of SSP where it undergoes a steady decline. The reduced grain yield of T_1, T_5 and T_6 during 2003, may be attributed to the prevalence of imbalanced nutrient supply experienced by the crop in the second year. Since no nitrogenous sources were added, the soil available nitrogen gradually got depleted which caused reduction in growth and development of plant and finally drastic reduction in grain yield. However, in case of RP_{60} also no nitrogenous sources were added yet yield increase was observed. This may be due to the fact that in the first year maize crop, rock phosphate by virtue of its poor solubility was almost unavailable to the maize plant and slowly become available with passage of time. This contributed to greater available P sources in the second year as compared to the available P source of first year maize crop. This greater availability of P might have resulted in greater root growth and thus better N, P uptake along with enhanced growth and yield components.

Addition of SSP_{30} along with $N_{120}VAM$ increased grain yield 11.78 % and 19.98 % in 2002 and 2003, respectively over $N_{120}VAM$. Similarly addition of RP_{60} along with $N_{120}PSB$ resulted in 19.83 % and 25.63 % increase in grain yield over $N_{120}PSB$ in 2002 and 2003, respectively.

The treatments $N_{120}SSP_{30}VAM$, $N_{120}RP_{30}PSB$ gave similar performance as that of $N_{120}P_{60}$ in case of growth parameters in maize. The chlorophyll content was highest in treatments $N_{120}SSP_{30}VAM$ and $N_{120}RP_{30}VAM$ during 2002 and 2003, respectively. Thus it can be concluded that $N_{120}SSP_{30}VAM$ and $N_{120}RP_{30}PSB$ gave higher growth attributes and grain yield. So this combination of chemical fertilizers and biofertilizers may prove economical and sustainable.

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