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RESPONSE OF BERSEEM *(TRIFOLIUM ALEXANDRINUM)* SHAFTAL *(TRIFOLIUM RESUPINATUM)* AND LUCERNE *(MEDICAGO SATIVA)* TO PHOSPHORUS APPLICATION FOR YIELD, NODULATION AND NITROGEN FIXATION

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In a pot culture experiment, increasing levels of phosphorus at 40, 60, 80, 100 Kg P_2O_5 ha^{-1} in the presence of uniform dressing of nitrogen and potash (K_2O) each applied at 10 and 40 Kg ha^{-1}, increased significantly the nodulation response (number and weight of nodules $plant^{-1}$), nitrogenase activity, N-uptake and dry matter yield of shoots and roots of berseem, shaftal and lucerne.

The increase in shoots N-uptake was in the range of 117 to 233 percent for berseem, 52 to 224 percent for shaftal,50 to 330 percent for lucerne; whereas the increase in the root N-uptake was in the range of 67 to 266 percent for berseem, 64 to 240 percent for shaftal and 23 to 114 percent for lucerne.

The improvement in the N-uptake of shoots and roots is obviously due to marked improvement in nodulation response (number and weight of nodules $plant^{-1}$) and nitrogenase activity of the test crops as a result of all the applied doses of phosphorus.

1. Introduction

Soils of Pakistan are deficient in nitrogen in particular and in available phosporus in general [4]. Addition of these nutrient elements in various amounts in the form of fertilizers is often recommended to improve the yield and quality of crops. Effectively nodulating legumes in general require only a small amount of combined nitrogen as a starter dose at the time of sowing [2]. This practice is recommended in order to alleviate the symptoms of N-hunger in the early stages of plant growth, before the nodules are able to supply sufficient fixed N to meet plant requirements. Most of the legumes on the other hand, are considered to require a high level of P nutrition but several show little response to phosphorus above a low level [5, 8, 10, 13].

Berseem *(Alexandrinum trifolium)*, Shaftal *(Trifolium resupinatum)*, and lucerne *(Medicago sativa)* are important winter fodder crops in Pakistan which are usually grown without the application of any fertilizers. Being legumes, these crops can fix atmospheric nitrogen symbiotically to meet their own requirement provided these are effectively nodulated. However, favourable growth, yield nodulation and nitrogen fixation responses of these crops are expected due to the applications of phosporus when grown in soil responsive to this nutrient element. The objective of this experiment is to study the response of berseem, shaftal and lucerne to phosphorus application for yield, nodulation and nitrogen fixation.

2. Materials and Methods

The experiments were conducted in earthen glazed pots in three sets each containing 10 kg throughly mixed soil (loamy clay in texture having pH 7.9; E_{ce} = 1.5ds^{-m}, N = 0.01%, $CaCO_3$ = 3.2%). First set of the experiment was for berseem, second set for shaftal and third for luceme. Strains of *Rhizobium trifolii* and *Rhizobium melilotii*, isolated from nodules of locally growing berseem and lucerne and maintained on yeast extract mannitol agar medium (containing L^{-1}, mannitol = 10.0g; yeast extract = 0.5 g; K_2HPO_4= 0.5g; $MgSO_4.7H_2O$ = 0.2g; NaCl = 0.1 g; Agar = 15g) were used for the seed inoculation. *Rhizobium trifolii* was used for seed inoculation of berseem and shaftal while *Rhizobium melilotii* for seed inoculation of lucerne.

Phosphorus (P_2O_5) as single super phosphate at 40, 60, 80, 100 kg ha^{-1} was applied. Nitrogen as urea at 10 kg ha^{-1} and potash (K_2O) as potassium sulphate at 40 kg ha^{-1} were applied as basal dressing to each pot. In all, 5 treatments, including control (Rhizobium inoculum + Nitrogen and potash (K_2O) at 10, 40, kg ha^{-1}) were designed for each experiment.

Each set of experiment was laid out in a randomized block design and all the treatments were replicated 3 times. Berseem, shaftal and luceme seeds of local cultivars were seeded on September 20, 1988 using 10 seeds per pot. After successful completion of seed germination, only 3 healthy plants were selected for detailed study and the rest pulled out. For purpose of Rhizobium inoculation of seeds, the cultures of R. trifolii and R. melilotii were introduced into a flask, composition as given above, except agar which was used at 2.5 g1^{-1}. The culture suspensions so prepared were incubated at 30°C for 72 hours. It was then shaken on a vibratory shaker for further 72 hours at 30°C till the viable count of each culture was 4 x 10^6 and 4 x 10^4 ml^{-1} of suspension, respectively. Seed inoculation of berseem and ashaftal was performed with the help of *R.Trifoii* and that of lucerne with *R. melilotii* at the time of sowing by adding 1 ml of cultural suspension to each seed.

The experimental plants were maintained by giving regular irrigations with canal water, hoeing and other cultural practices. Fresh fodder yield in 3 different cuts at suitable intervals of plant growth was recorded. Samples of fresh fodder were dried in oven at 70°C to a constant weight. Total of all the 3 cuttings of dried fodder was taken as an index of dry matter yield of shoots. At the time of third cutting, on March 5, 1989, the plants were uprooted carefully, washed with tap water and acetylene reduction assay as a measure of nitrogenase activity was carried out according to the previously described procedure [10]. Root samples were then dried in oven at 70°C to a constant weight to record dry matter yield of roots. Shoots and roots samples were then ground to pass through a 40 mesh screen for N and P analysis. Nitrogen in plant samples was estimated by means of micro-kjeldahl method[1]. Phosphorus (P) in plant samples was determined by direct dry ashing method [9]. Physicochemical analysis of the test soil was peformed according to the previously described procedures [10].

For statistical analysis the data were subjected to analysis of variance and upon obtaining a significant F. ratio, the Ducan multiple range test (6) was employed to test the level of significance among the treatment means.

3. Results and Discussion

Results showing the effect of phosphorus and *Rhizobium* inoculation on dry matter yield, nodulation response, acetylene reduction assay, N and P concentration and uptake by berseem, shaftal and lucerne are given in Tables 1, 2 and 3 respectively. The results revealed that increasing rates of phosphorus at 40, 60, 80 kg P_2O_5 ha^{-1} applied to the inoculated treatments in the presence of uniform dressing of nitrogen and potash (K_2O) at 10, 40 kg ha^{-1} increased significantly the shoots dry matter yield of berseem by 117, 157, 257, 252 percent, of shaftal 71, 121, 204, 197 percent and of lucerne 76, 134, 267, 226 percent respectively. Application of phosphorus increased significantly the dry matter of berseem roots by 17, 162, 267, 254 percent, of shaftal 76, 105, 184, 186 percent and of lucerne 34, 90, 88, 88 percent, respectively.

Nodulation response (number and dry weight of nodules $plant^{-1}$) and acetylene reduction assay as a measure of nitrogenase activity of berseem, shaftal and lucerne exhibited a significant increasing tendency as a result of all the applied doses of phosphorus.

N-concentration in the shoots of berseem, shaftal and lucerne did not show any significant improvement perhaps due to growth dilution effect. However, N-uptake in the shoots of berseem, shaftal and luceme exhibited a significant increasing trend as a result of phosphorus application. The increase in N-uptake in shoots* of berseem was 117, 117, 233, 175 percent, of shaftal 52, 129, 224, 200 percent and of lucerne 50, 170, 330, 270 pecent respectively with increasing doses of phosphorus. N- concentration in roots of berseem, shaftal and lucerne did not exhibit any significant improvement, perhaps due to translocation of N from roots to vegetative plant parts. However, N-uptake in roots of berseem, shaftal and lucerne exhibited a significant increasing trend as a result of phosphorus application. The increase in N-uptake in roots* of berseem was 67, 200, 266, 200 percent, of shaftal 64, 112, 240, 216 percent and of lucerne 23, 105, 113, 80 percent due to application of all the applied doses of phosphorus.

P-concentration in shoots and roots of berseem, shaftal and luceme did not show any significant increase as a result of phosphorus application. The insignificant P-concentration in shoots can be attributed to the growth dilution effect, while that in roots can be attributed to the translocation of P from roots to the vegetative plant parts. The P-uptake in shoots and roots of berseem, shaftal and lucerne exhibited a significant increasing trend as a result of phosphorus application. The increase in P-uptake by shoots of berseem ranged between 109 and 363 percent, of shaftal between 100 and 233 pecent and of lucerne between 101 and 201 percent as a result of phosphorus application. The increase in P uptake by roots of berseem ranged between 133 and 500 percent, of shaftal between 90 and 178 percent and of lucerne between 86 and 95 percent with respective phosphorus doses.

The improvement in the nitrogen fixation of berseem, shaftal and lucerne could be attributed to the beneficial effect of phosphorus on root development, allowing effective symbiosis by *Rhizobium*. Nitrogen fixation in legumes is a complex phenomenon, which is controlled by microbiological, nutritional and climatic factors and clearly if soil has insufficient phosphorus for normal development of

* The increase in N-uptake of shoots and roots is due to nitrogen fixation.

Table 1. Effect of phosphorus on dry matter yield, nodulation, acetylene reduction assay - N and P concentration and uptake by berseem

P_2O_5 applied	DM Yield (g pot $^{-1}$)		Nodulation		ARA	Shoot N		Root N		Shoot P		Root P	
Kg ha^{-1}	Shoot	Root	No plant	Dry wt. pl (g)		% N	N uptake (g pot^{-1})	% N	N uptake (g pot^{-1})	% P	P uptake (g pot^{-1})	% P	P uptake (g pot^{-1})
	1	2	3	4	5	6 NS	7	8 NS	9	10 NS	11	12 NS	13
0	5.4D (-)	2.1D (-)	131 C	0.21C	4.5D	2.23	0.12D (-)	1.47	0.03D (-)	0.173	9.9E (-)	0.1	1.5D (-)
40	11.7C (116.7)	3.5C (66.7)	169B	0.27B	8.5C	2.25	0.26C (117)	1.38	0.05C (66.7)	0.123	20.74D (109)	0.1	3.52C (133)
60	13.9B (157.4)	5.5B (161.9)	168B	0.30B	10.5B	1.89	0.26C (117)	1.64	0.09B (200)	0.194	27.3C (176)	0.1	6.01B (300)
80	20.9A (287.0)	7.7A (266.7)	197A	0.44A	12.8A	1.98	0.40B (233)	1.42	0.11A (266)	0.179	42.4B (328)	0.1	7.2B (380)
100	19.00A (251.9)	7.4A (254.4)	195A	0.42A	12.4A	1.76	0.33A (175)	1.30	0.09B (200)	0.240	45.8A (363)	0.09	9.02A (500)

1) Each value in column 1 to 13 is the average of 3.
2) NS indicates non-significant difference.
3) ARA = Acetylene reduction assay expressed in μ mole g^{-1} dry wt. of nodule hr^{-1}.
4) Values within parenthesis indicate % increase over control.
5) Values not sharing a letter in common differe significantly according to DMRT.

Table 2. Effect of phosphorus on dry matter yield, nodulation, acetylene reduction assay - N and P concentration and uptake by shaftal

P_2O_5 applied	DM Yield (g pot $^{-1}$)		Nodulation		ARA	Shoot N		Root N		Shoot P		Root P	
Kg ha^{-1}	Shoot	Root	No plant	Dry wt. pl (g)		% N	N uptake (g pot^{-1})	% N	N uptake (g pot^{-1})	% P	P uptake (g pot^{-1})	% P	P uptake (g pot^{-1})
	1	2	3	4	5	6 NS	7	8 NS	9	10 NS	11	12 NS	13
0	6.91D	1.9D (-)	145D	0.23C	2.5E	3.04	0.21E (-)	1.31	0.025D	0.22	15E (-)	0.21	4.1D
40	11.82C (71.06)	3.34C (75.79)	177C	0.34B	5.6D	2.84	0.32D (52.38)	1.26	0.041C (64)	0.25	30D (100)	0.23	7.8C (90.24)
60	13.3B (121.42)	3.90B (105.26)	234B	0.34B	8.5C	3.1	0.48C (128.57)	1.36	0.053C (112)	0.20	36C (140)	0.17	6.5C (58.54)
80	21.01A (204.05)	5.40A (184.21)	274A	0.56A	9.5B	3.26	0.68B (223.81)	1.43	0.085B (240)	0.21	47B (213.33)	0.19	15.3A (273.17)
100	20.5A (196.67)	5.43A (185.79)	271A	0.55A	12.5A	3.31	0.63A (200.00)	1.23	0.079A (216.00)	0.24	50A (233.33)	0.21	11.4B (178.05)

1) Each value in column 1 to 13 is the average of 3.
2) NS indicates non-significant difference.
3) ARA = Acetylene reduction assay expressed in μ mole g^{-1} dry wt. of nodule hr^{-1}.
4) Values within parenthesis indicate % increase over control.

Table 3. Effect of phosphorus on dry matter yield, nodulation, acetylene reduction assay - N and P concentration and uptake by lucerne.

P_2O_5 applied	DM Yield (g pot $^{-1}$)		Nodulation		ARA	Shoot N		Root N		Shoot P		Root P	
Kg ha^{-1}	Shoot	Root	No plant	Dry wt. pl (g)		% N	N uptake (g pot^{-1})	% N	N uptake (g pot^{-1})	% P	P uptake (g pot^{-1})	% P	P uptake (g pot^{-1})
	1	2	3	4	5	6 NS	7	8 NS	9	10 NS	11	12 NS	13
0	4.72E (-)	3.37C (-)	132C	1.83D	3.8D	3.04	0.10E (-)	1.31	0.044D	0.25	11.7E	0.25	8.11B
40	8.3D (75.85)	4.53B (34.42)	162B	2.13C	6.8C	2.84	0.15D (50)	1.26	0.054D (222.73)	0.29	23.5D (100.85)	0.34	15.11A (86.31)
60	11.03C (133.69)	6.41A (90.21)	177B	2.87B	8.5B	3.1	0.27C (170)	1.36	0.090B (104.55)	0.25	27.3C (133.33)	0.28	17.8A (119.48)
80	17.31B (266.74)	6.35A (88.42)	216A	3.70A	10.5A	3.26	0.43B (330)	1.43	0.094B (113.64)	0.19	31.7B (170.94)	0.27	16.6A (104.69)
100	15.4A (226.27)	6.35A (88.42)	198A	3.40A	10.2A	3.31	0.37A (270)	1.23	0.082A (86.36)	0.22	35.2A (200.85)	0.27	15.8A (94.82)

1) Each value in column 1 to 13 is the average of 3.
2) NS indicates non-significant difference.
3) ARA = Acetylene reduction assay expressed in μ mole g^{-1} dry wt. of nodule hr^{-1}.
4) Values within parenthesis indicate % increase over control.
5) Values not sharing a letter in common differe significantly according to DMRT.

the root nodules, the direct application of phosphorus to the soil will benefit plant development. This is clearly confirmed by our present investigation. The importance of phosphorus in the nutrition of nodulated legumes to improve yield, growth and nitrogen fixation has been well documented [3, 5, 7, 8, 11, 14, 15, 16].

Our results suggest that an acceptable potential production of shoots roots yield and nitrogen fixation of berseem, shaftal and lucerne can be obtained through *Rhizobium* inoculation and adequate level of phosphorus application, a fact that is relevant in most of our soils, where phosphorus is not adequately available (Wahab 1965). Application of phosphorus at 80 kg P_2O_5 ha^{-1} in the inoculated treatments in the presence of uniform dressing of nitrogen of 10 kg N ha^{-1}+40 kg K_2O ha_{-1} appears to be a well justified dose in the present experiment, which markedly improved the dry matter of shoots and roots pot^{-1}, nodulation response, nitrogenase activity and nitrogen fixing capacities of berseem, shaftal and lucerne.

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