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SHORT COMMUNICATION

EFFECT OF INOCULATION RATES AND PHOSPHORUS FERTILIZATION ON NITROGEN FIXATION IN SOYABEAN

T.M.M. MOHARRAM, M.S.A. SAFWAT and M.M. FARGHALY
Department of Agricultural Microbiology,
Faculty of Agriculture, Minia University, Minia, Egypt.

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ABSTRACT

Field experiments were conducted to study the effect of inoculatin rates (10^4 and 10^8 rhizobial cells 100 g^{-1} seeds) and phosphorus fertilization (0 and 20 kg P ha^{-1}) on N-fixation, yield and N and P uptake for two cultivars of soybean (Clark and Crowford). Inoculation stimulated nodule formation and N-fixation. Application of phosphorus at the inoculation rate of 10^8 r.c. 100 g^{-1} seeds significantly increased the amount of nodules and N-fixed. Cultivar Clark performed better than Crowford.

***Key Words*: Nitrogen-fixation, phosphorus, soybean**

RÉSUMÉ

Des essais ont été conduits en champ pour étudier l'effet des doses d'inoculation (10^4 et 10^8 cellules rhyzobiales pour 100 g de semences) et l'apport de phosphore (0 et 20 kg ha^{-1}) sur la fixation du nitrate, le rendement de l'assimilation de l'azote et du phosphore de deux variétés de soja (Clark et Crowford). L'inoculation a favorisé la formation de nodules et la fixation de nitrate. L'inoculation de 10^8 de cellules rhyzobiales en combinaison avec l'apport de phosphore accroît de façon significative le nombre de nodules et la quantité de nitrate fixée. Les performances de Clark ont été supérieures à celles de Crowford.

***Mots Clés*: Fixation de nitrate, phosphore, soja**

INTRODUCTION

Soybean (*Glycine max*) is a protein-rich legume crop. Its vertical increase in yield is required to contribute to the increasing demand for food security. Inoculation of legumes with efficient *Rhizobium* strains (Dennis, 1981; Bushby *et al.*, 1983; Hegazi and Metwally, 1985; Moharram, 1986) and phosphorus fertilization (Robinson *et al.*, 1981; Jacobsen, 1985; Israel, 1987; Hassan *et al.*, 1990) increases nodulation, N_2-fixation and yield. Low phosphorus limits total attainable population density and slows growth of most rhizobial strains (Keyser and Munns, 1979). The present work aimed at determining the influence of inoculation rates at two levels of phosphorus fertilization on biological nitrogen fixation under field conditions.

MATERIALS AND METHODS

The field experiment was conducted at the Research Farm, Faculty of Agriculture, Minia University, Egypt. The soil is clay loam with pH 7.8, total N 0.09%, available P 47 mg kg^{-1} and $CaCO_3$ 2.16%. Two cultivars of soybean (Clark and Crowford) were studied. Maize (variety F 9) was used as a reference crop in determining N-fixation using the N-15 methodology (Fiedler and Proksch, 1975). Seeds of soybean were inoculated with *Bradyrhizobium japonicum* USDA 110 in a cotton seed husk base (Moharram, 1986) at the rates of 104 and 108 rhizobial cells 100 g^{-1} of seeds.

A split plot design in four replicates, was used. Each plot contained a sub-plot (1.2 m^2) in which the soil was enriched with N-15, with a row spacing of 30 cm and a planting distance of 10 cm.

Phosphorus fertilizer was broadcast during field preparation at rates of 0 and 20 kg P ha^{-1} as superphosphate. N-15 labelled ammonium sulphate fertilizer was sprayed as an aqueous solution one week after planting at a rate of 20 kg ha^{-1} of N-15 1% a.e for maize (reference crop). After 60 days of growth, five plants from each sub-plot were carefully uprooted, washed with water and the number and weight of nodules, plant dry weight and plant height recorded.

At harvesting (125 days after sowing), plants (above ground) of each sub-plot were chopped into small fragments and dry weights of plants and seeds and their nitrogen and phosphorus contents were determined. Total N and the N-15/N-14 ratios in plants were determined using a mass spectrometer (Fielder and Proksch, 1975) at the IAEA Seibersdorf Laboratory, Vienna, Austria. Percentages of the nitrogen derived from the atmosphere (% Ndfa), the fertilizer (% Ndff) and soil (% Ndfs) as well as total N-fixed were calculated (Fried and Middleboe, 1977).

Phosphorus utilazation efficiency (PUE) was estimated according to the following equations:

$$\text{PUE for } N_2\text{-fixed} = \frac{\text{amount of N fixed}}{\text{amount of P taken up}}$$

$$\text{PUE for dry matter} = \frac{\text{amount of dry matter}}{\text{amount of P taken up}}$$

The physical and chemical propertiees of the soil and the total phosphorus in plant samples were determined at the Soil Laboratory, Faculty of Agriculture, Minia University, according to Piper (1950).

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that inoculation and application of fertiliser phosphorus significantly increased nodule formation during the initial 60 days of growth. The highest number and dry weight of nodules were recorded with the

TABLE 1. Effect of rhizobial inoculation and phosphorus application rates on nodulation and growth of soybean cultivars 60 days after planting

Cultivars	Inoculation rates	P rates kg P ha^{-1}	Nodule no. $plant^{-1}$	Nodule wt. $plant^{-1}$
	0	0	13	0.11
		20	25	0.16
Clark		0	48	0.63
	10^4	20	61	0.90
	10^8	0	29	1.06
		20	126	1.13
	0	0	7	0.07
		20	16	0.12
Crawford	10^4	0	31	0.34
		20	52	0.65
	10^8	0	62	0.93
		20	86	1.03
L.S.D. (5%) between:				
Inoculation rate (I)			2.3	0.07
P-rate (P)			2.6	1.01
I x P			4.4	1.05

treatments inoculated with 10^8 r.c. 100 g^{-1} seeds. Cultivar Clark had a higher number of nodules than Cultivar Crowford. Application of phosphorus to uninoculated treatments also encouraged nodule formation.

Inoculation with rhizobia and appliction of phosphorus singly or in interaction also resulted in significant increases in the growth of soybean plants, the maximum increase in plant height and dry weight per plant being when the plants were inoculated with 10^8 r.c. 100 g^{-1} of seed and fertilized (Table 2). These results agree with those obtained by Bushby *et al.* (1983) and Sherif *et al.* (1990)

Results of the percentage of nitrogen derived from the atmosphere (% Ndfa), the labelled fertilizer (% Ndff) and the soil (% Ndfs) in Table 2 reveal that the uninoculated treatments that did not receive phosphorus derived the lowest amount of atmosphoric nitrogen perhaps due to the small number of nodules formed by these plants. In contrast, the inoculated treatments derived most of their N requirement from the atmosphere. Appliction of phosphorus to the inoculated treatments significantly increased % Ndfa, indicating the importance of phosphorus to N-fixation and plant growth (Robson *et al.*, 1981; Jacobsen, 1985; Abu-Gyamfi *et al.*, 1989).

Data in Table 3 also show that both inoculation and phosphorus application significantly increased the amount of nitrogen and phosphorus content (in shoots and seeds) and N fixed. The maximum values of these parameters were recorded with the treatments inoculated with 10^8 r.c. 10 g^{-1} of seed and fertilised with P. Cultivar Clark fixed more N_2 than cultivar Crowford, indicating the importance of the host plant for N-fixation.

Data in Table 4 indicate that both inoculation and phosphorus application significantly decreased the phosphorus utilization efficiency (PUE) for both N-fixed and dry matter in all treatments due to the high amount of phosphorus taken up by the plants as a result improved crop growth. Consequently, both dry matter and seed yield significantly increased. Again, the maximum increase was recorded with the inoculation rate of 10^8 r.c. 100 g^{-1} of seed in the fertilizer treatment for both Clark and Crowford cultivars. However, cultivar Clark was the most superior.

From the above results it may be concluded that phosphorus fertilization enhanced the activity of the inoculum, and the overall N-fixation capacity also depends on the rate of inoculation. Evidence suggests that the phosphorus requirements for nodulation and maximum nodule activity are much greater than for host plant growth (deMooy and Pesek, 1966; deMooy *et al.*, 1973). Therefore, soybean seed yield is maximum only when the phosphorus requirements for N-fixation are fully met.

TABLE 2. Effect of inoculation rates and phosphorus application on nitrogen derived from atmosphere (% Ndfa), fertilizer (Ndff) and soil (% Ndfs) for soybean cultivars

Cultivars	Inoculation rates	P-rates kg ha^{-1}	% Ndfa	% Ndff	% Ndfs
	0	0	24	2.4	73.6
		20	46	2.0	52.0
Clark		0	43	1.8	55.2
	10^4	20	59	1.5	39.5
	10^8	0	56	1.4	42.6
		20	71	1.1	27.9
	0	0	24	2.4	73.6
		20	43	2.1	54.9
Crowford	10^4	0	40	1.9	58.1
		20	56	1.6	42.4
	10^8	0	52	1.5	46.5
		20	67	1.2	31.8
L.S.D. (5%) between:					
Inoculation rate (I)			2.1	N.S	3.1
P-rate (P)			2.4	N.S	3.5
I x P			5.6	N.S	6.9

TABLE 3. Effect of inoculation rates and phosphorus application on the N and P-uptake and N fixed (kg ha^{-1}) for soybean cultivars at harvesting

Cultivars	Incubation rates	P-rates kg P ha^{-1}	N-uptake Shoots	N-uptake Seeds	N-uptake Total	P-uptake Shoots	P-uptake Seeds	P-uptake Total	N_2-fixed
		0	38.7	287.6	326.3	9.4	15.7	25.1	78.3
	0	20	47.4	319.5	366.9	15.6	25.5	41.1	168.7
Clark		0	41.6	325.4	367.0	11.8	22.4	34.2	157.8
	10^4	20	57.3	349.3	406.6	22.5	43.2	65.7	239.8
		0	55.6	344.3	399.9	16.2	27.5	43.7	223.9
	10^8	20	392.6	471.1	35.1	59.3	94.4	334.4	
		0	35.2	279.4	314.6	18.1	13.1	21.2	75.5
	0	20	43.6	310.2	353.8	12.6	20.3	32.9	152.1
		0	42.1	309.3	351.4	10.3	19.1	29.4	140.5
Crowford	10^4	20	53.4	338.6	392.0	18.7	36.2	54.9	219.5
		0	51.3	330.2	381.5	13.2	22.3	35.5	198.3
	10^8	20	71.5	365.6	437.1	28.6	43.2	71.8	292.8
L.S.D. (5%) between:									
Inoculation rate (I)			1.1	6.2	8.0	0.8	1.2	1.9	7.3
P-rate (P)			2.0	7.7	10.2	1.1	1.5	2.3	8.6
I x P			3.1	17.0	17.5	1.7	2.6	4.2	16.1

TABLE 4. Effect of inoculation rates and phosphorus on phosphorus utilization efficiency (PUE), dry matter and seed yield (kg ha^{-1}), and phosphorus utilization efficiency (PUE) for both N fixed and dry matter of two soybean cultivars

Cultivars	Inoculation rates	P rates kg ha^{-1}	Dry matter	Seeds	PUE for N fixed	PUE for dry matter
Clark	0	0	1994	3224	3.1	79.4
		20	2624	3780	4.1	63.8
	10^4	0	2490	3610	4.6	72.8
		20	2951	4409	3.6	45.0
	10^8	0	2812	4380	5.1	64.3
		20	3691	5740	3.5	39.1
Crawford	0	0	1933	3107	3.5	91.2
		20	2515	3540	4.6	76.4
	10^4	0	2368	3516	4.7	80.5
		20	2765	4130	3.9	50.4
	10^8	0	2680	3785	5.5	75.5
		20	3290	4970	4.0	45.8
L.S.D. (5%) between:						
Inoculation rates (I)		61	73	0.1	2.6	
P-rate (P)			70	81	0.1	3.2
I x P			109	156	0.1	4.7

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