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RESEARCH ARTICLE :

Enzyme activity and nodulation of soybean [*Glycine max* (L.) Merrill] as influenced by various levels of nitrogen and phosphorus

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SUMMARY : A field experiment was conducted at Main Agricultural Research Station, Dharwad on medium black soil during *Kharif*-2015. There were twelve treatment combinations consisted of three levels of nitrogen (20, 40 and 60 kg N ha^{-1}) and four levels of phosphorus (40, 60, 80 and 100 kg P_2O_5 ha^{-1}). Application of nitrogen @ 60 kg ha^{-1} recorded significantly higher chlorophyll content (44.32) and dehydrogenase activity (5.89 µg TPF formed g $soil^{-1}d^{-1}$). Among the phosphorus, application of phosphorus @ 80 kg ha^{-1} recorded significantly higher nodule numbers, dehydrogenase and phosphatase activity (31.60, 5.41µg TPF formed g $soil^{-1}d^{-1}$ and 8.33 µg PNP formed g $soil^{-1}h^{-1}$, respectively) compared to other treatments and it was on par with application of phosphorus @ 100 kg ha^{-1}. Application of nitrogen @ 60 kg, phosphorus @ 80 kg and potash @ 25 kg per hectare found optimum to soybean.

KEY WORDS :
Soybean, Nitrogen, Phosphorus, Enzyme activity

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BACKGROUND AND OBJECTIVES

Soybean [*Glycine max* (L.) Merrill], is an introduced and commercially exploited crop in India. The crop is also called as "Golden Bean" or "Miracle crop" of the 21st century on account of its multiple uses. It has highest protein 40 %, oil 20 %, rich in lysine and vitamins A, B and D and also rich in mineral salts. Among the nutrients; nitrogen is a major essential plant nutrient element. It has the quickest and most pronounced effect on plant growth and yield of crops. It tends primarily to encourage above ground vegetative growth and to impart deep green colour to the leaves. In all plants, nitrogen governs a considerable degree of utilization of potassium, phosphorus and other nutrients. Plants receiving insufficient nitrogen are stunted in growth with restricted root systems (Penas and Wiese, 1987). The leaves turn yellow or yellowish green and tend to drop off. Phosphorus stimulates rhizobial activity, nodule formation and thus, helps in N_2-fixation. It increases the water use efficiency, improves storage quality and hardiness of the bean seed coat. As phosphorus plays a role in photosynthesis, respiration, energy storage and transfer, cell division and enlargement, it has been shown

to be important for growth, development and yield of soybean (Kakar *et al*., 2002). It helps in uptake of more nutrients and balances the nitrogen deficiency in soil and assists in seed maturation. Thus, it is needed to find out proper amount of nitrogen and phosphorus required for achieving better yield of soybean. Hence, in order to verify and workout the optimum nitrogen and phosphorus dose the present investigation was undertaken.

RESOURCES AND METHODS

The field experiment was carried out at Main Agricultural Research Station, Dharwad, during *Kharif* 2015 to study the "Enzyme activity and nodulation of soybean [*Glycine max* (L.) Merrill] as influenced by various levels of nitrogen and phosphorus"

The experiment was replicated thrice in Randomized Complete Block Design in factorial concept. There were twelve treatment combinations consisted of three nitrogen levels (20, 40 and 60 kg N ha^{-1}) and four phosphorus levels (40, 60, 80 and 100 kg P_2O_5 ha^{-1}). One of the treatment combinations comprised the recommended dose of 40 kg N, 80 kg P_2O_5 and 25 kg K_2O per hectare. The soil was medium deep black with pH 7.10. The available N, P_2O_5 and K_2O contents were 252, 32.5 and 292.8 kg ha^{-1}, respectively. FYM @ 5 t ha^{-1} was applied 15 days before sowing of the crop. The gross plot size was 5.0 m × 3.6 m and net plot size was 4.8 m × 3.0 m.

Seeds were treated using *Rhizobium* and Phosphorus solubilizing bacteria @ 1250 g per hectare. Two seeds per hill were dibbled 5 cm deep in furrows at a spacing of 30 cm x 10 cm. Recommended dose of K_2O @ 25 kg ha^{-1} was applied at the time of sowing. N and P_2O_5 were applied as basal as per the treatments. The crop was harvested at its physiological maturity. The data was statistically analysed as per the procedure given by Gomez and Gomez (1984).

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Nodule number and nodule dry weight :

Application of nitrogen @ 40 kg ha^{-1} recorded significantly higher number of nodules and nodules dry weight $plant^{-1}$ at 50 DAS (33.41 and 0.55 g) compared to 20 (27.08 and 0.44 g) and 60 (31.52 and 0.51 g) kg N ha^{-1}. Among the phosphorus levels, application of phosphorus @ 80 kg ha^{-1} recorded significantly higher number of nodules and nodules dry weight (31.60 and 0.51 g) compared to 60 (29.90 and 0.49 g) and 40 (29.10 and 0.47 g) kg P_2O_5 ha^{-1} however, it was on par with 100 (32.07 and 0.53 g) kg P_2O_5 ha^{-1}. In combined application of nitrogen @ 40 kg ha^{-1} and phosphorus @ 80 kg ha^{-1} recorded significantly higher number of nodules (34.53) compared to other treatment combinations however, it was on par with application of nitrogen @ 40 kg ha^{-1} and phosphorus @ 100 (35.03) kg ha^{-1} (Table 1). With respect to nodules dry weight non-significant difference among treatment combinations noticed. It is mainly attributed to application of nitrogen and phosphorus accelerated the photosynthetic rate leading to more production of carbohydrates, helping in root formation and nitrogen fixation results in positive effect

Table 1: Number of nodules and dry weight of nodules (50 DAS) as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg ha^{-1})	Number of nodules				Dry weight of nodules (g)			
	Nitrogen (kg ha^{-1})							
	20	40	60	Mean	20	40	60	Mean
40	26.33	31.63	29.34	29.10	0.42	0.52	0.48	0.47
60	26.74	32.44	30.53	29.90	0.43	0.53	0.50	0.49
80	27.40	34.53	32.87	31.60	0.44	0.56	0.53	0.51
100	27.85	35.03	33.33	32.07	0.46	0.58	0.54	0.53
Mean	27.08	33.41	31.52		0.44	0.55	0.51	
	S.E.±		C.D. (P=0.05)		S.E.±		C.D. (P=0.05)	
Nitrogen	0.27		0.80		0.01		0.02	
Phosphorus	0.32		0.92		0.01		0.02	
Interaction	0.55		1.60		0.03		NS	

NS= Non-significant

on photosynthesis which in turn favors better nodules formation in the crop. These results are in line with the findings of Aziz *et al.* (2016); Bhattacharjee *et al.* (2013); Geetha and Radder (2015) and Son *et al.* (2006).

Chlorophyll content (SPAD meter) :

At 30 and 60 DAS, among the different levels of nitrogen significantly higher chlorophyll content was recorded with application of nitrogen @ 60 kg ha^{-1} (35.35 and 44.22, respectively) over 20 kg ha^{-1} (31.12 and 40.10, respectively) and 40 kg ha^{-1} (32.87 and 42.18, respectively). There was no significant difference observed with phosphorus treatments and combined application of nitrogen and phosphorus application on chlorophyll content at 30 and 60 DAS (Table 2). It's mainly due to nitrogen is required in the biosynthetic pathway and essential for the synthesis of chlorophyll. These results are in agreement with the findings of Sohrabi *et al.* (2012); Mohammadi *et al.* (2015) and Zhang *et al.* (2013).

Enzyme activity :

Application of nitrogen @ 60 kg ha^{-1} recorded significantly higher dehydrogenase activity at 50 DAS (5.89 µg TPF formed g soil^{-1}d^{-1}) compared to 20 (4.54 µg TPF formed g soil^{-1}d^{-1}) and 40 (5.46 µg TPF formed g soil^{-1}d^{-1}) kg N ha^{-1} and non-significance difference with respect to phosphatase activity. Among the phosphorus levels, application of phosphorus @ 80 kg ha^{-1} recorded significantly higher dehydrogenase activity and phosphatase activity at 50 DAS (5.41 µg TPF formed g soil^{-1}d^{-1} and 8.33 µg PNP formed g soil^{-1}h^{-1}) compared to 40 kg ha^{-1} (5.01µg TPF formed g soil^{-1}d^{-1} and 5.78 µg PNP formed g soil^{-1}h^{-1}) and 60 kg ha^{-1} (5.19 µg TPF formed g soil^{-1}d^{-1} and 6.07 µg PNP formed g soil^{-1} h^{-1}), however, it was on par with 100 (5.57 µg TPF formed g

Table 2 : SPAD meter reading soybean as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg ha^{-1})	SPAD 30 DAS				SPAD 60 DAS			
	Nitrogen (kg ha^{-1})							
	20	40	60	Mean	20	40	60	Mean
40	30.95	32.65	34.93	32.84	39.85	41.99	43.96	41.93
60	31.05	32.75	35.34	33.05	40.36	42.18	44.15	42.23
80	31.20	32.98	35.53	33.24	40.39	42.25	44.26	42.08
100	31.29	33.11	35.59	33.33	40.48	42.28	44.50	42.42
Mean	31.12	32.87	35.35		40.10	42.18	44.22	
	S.E.±		C.D. (P=0.05)		S.E.±		C.D. (P=0.05)	
Nitrogen	0.21		0.62		0.26		0.76	
Phosphorus	0.24		NS		0.30		NS	
Interaction	0.42		NS		0.52		NS	

NS= Non-significant

Table 3 : Dehydrogenase and phosphatase activity (50 DAS) as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg ha^{-1})	Dehydrogenase (µg TPF formed g soil^{-1}d^{-1})				Phosphates activity (µg PNP formed g soil^{-1}h^{-1})			
	Nitrogen (kg ha^{-1})							
	20	40	60	Mean	20	40	60	Mean
40	4.06	5.28	5.70	5.01	5.76	5.78	5.79	5.78
60	4.42	5.39	5.77	5.19	5.78	6.21	6.21	6.07
80	4.70	5.55	5.98	5.41	7.99	8.25	8.76	8.33
100	4.98	5.63	6.10	5.57	6.79	6.38	7.01	6.73
Mean	4.54	5.46	5.89		6.58	6.65	6.94	
	S.E.±		C.D. (P=0.05)		S.E.±		C.D. (P=0.05)	
Nitrogen	0.10		0.30		0.11		NS	
Phosphorus	0.12		0.34		0.13		0.38	
Interaction	0.20		NS		0.22		NS	

NS= Non-significant

soil^{-1}d^{-1} and 6.73 µg PNP formed g soil^{-1}h^{-1}) kg P_2O_5 ha^{-1}. In Interaction effect nitrogen and phosphorus application did not show any significant difference with respect to dehydrogenase and phosphatase activity (Table 3). These results are in line with the findings of Gabriel and Vasile (1996) and Klikocka *et al.* (2012).

Conclusion:

The investigation revealed that application of nitrogen @ 60 kg ha^{-1} and phosphorus @ 80 kg ha^{-1} and potassium @ 25 kg ha^{-1} found to optimum to soybean crop.

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