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Soybean Growth and Nitrogen Fixation as Affected by Sulfur Fertilization and Inoculation under Rainfed Conditions in Pakistan

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ABSTRACT

Seed yield and nitrogen fixation of legumes can be enhanced by supply of adequate amount of nutrients and inoculation. A field experiment was carried out at the Research Farm of Arid Agriculture University Rawalpindi (Pakistan) during summer 2009 using soybean (*Glycine max*) as test crop. The objective of this study was to investigate the effect of inoculation in combination with different sulfur rates (0, 15 & 30 kg S ha^{-1}) on soybean growth, nitrogen fixation and soil nitrogen balance. Experiment was laid according to randomized complete block design in triplicate. Combined application of inoculation and sulfur (30 kg ha^{-1}) resulted in significant increase in seed yield and yield attribute relative to control. Soybean plant height, number of pods per plant, straw yield, seed yield and dry matter yield increased up to 14, 56, 25, 20 and 26% as compared to control, respectively. Nitrogen uptake and fixation increased from 180 to 271 and from 83 to 143 kg ha^{-1} in the treatment of inoculation +30 kg S ha^{-1}. Soil nitrogen balance after harvest of soybean crop was negative in all the treatments and this deficit increased with increase in dry matter yield. © 2011 Friends Science Publishers

Key Words: *Glycine max*; Legume; Seed yield; Nitrogen fixation; Soil nitrogen balance

INTRODUCTION

Sulfur (S) deficiency has become a limiting factor for crop yield in many areas of world (Mascagni *et al.,* 2008). Situation is not different in Pakistan, where very little attention has been paid to this important element (Khalid *et al.,* 2009). Sulfur is becoming deficient in soils due to introduction of high yielding varieties, use of high grade S free fertilizers and reduced emission of S from industrial units (Scherer, 2009). It is therefore, very important to study the respose of different crops to S application under different agroecological conditions and cropping systems and its effect on soil nutrient balances.

Soybean (*Glycine max*) is one of the most important oil seed crops in the world that accounts for 30% of the world's processed vegetable oil and has also been employed as a source of bio-diesel fuels (Graham & Vance, 2003). Legume growth and nitrogen (N) fixation rates could be markedly increased by highly efficient, competitive and persistent strains of *Rhizobium* (Amanuel *et al.,* 2000) and by supply of adequate amount of nutrients such as phosphorus and sulfur (Olivera *et al*., 2004; Scherer *et al*., 2008).

Sulphur is one of the 16 elements essential for plant growth and is a component of amino acids (cystein & methionine) needed for protein synthesis (Jan *et al.,* 2002). Sulphur is also a vital part of the ferredoxin, an iron-sulphur protein occurring in the chloroplasts. Ferredoxin has a significant role in nitrogen dioxide and sulphate reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer *et al.,* 2008). Average S removal for producing 1 tonne of food grain is estimated to be 3-4 kg by cereals (wheat & rice), 5-8 kg by sorghum and millet, 8 kg by pulses and legumes and 12 kg by oilseeds (Kanwar & Mudahar, 1985).

Sulfur application and inoculation have immense potentional of increasing the amount of N fixed by legumes, thus improving fertility status of soil (Habtemichial *et al.,* 2007). However in Pakistan, research regarding S is limited to oil seeds and their oil contents only and very little attention has been paid towards investigating the role of S in the growth of legumes and subsequent soil N balance (Islam & Ali, 2009). Hence, present experiment was conducted to study the effect of inoculation and S application on growth, N fixation of soybean and soil N balance after crop harvest.

MATERIALS AND METHODS

A field experiment was carried out on soybean variety NARC-1 at the research farm of Arid Agriculture

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University, Rawalpindi during summer (July-October) 2009. Physical and chemical properties of soil of experimental site are given in Table I. The objective of trial was to investigate the effect of co-inoculation of *Bradyrhizobium japonicum and Azotobacter* on soybean growth and N fixation at three S levels (0, 15 & 30 kg ha^{-1}). Inoculum of *Bradyrhizobium japonicum and Azotobacter* was obtained from Soil Bacteriology Section, Ayub Agriculture Research Institute, Faisalabad. The starter dose of nitrogen (30 kg ha^{-1}) and phosphorus (80 kg P_2O_5 ha^{-1}) was applied in the form of urea and triple super phosphate, respectively in all the treatments. Sulfur was applied in the form of gypsum (15% S). All the fertilizers and inoculum were applied before sowing. Experiment was laid according to randomized complete block design in triplicate. The net plot size was 25 m^2. The treatments comprised of three levels of S (0, 15 & 30 kg ha^{-1}) with and without inoculum application. Crop was sown in first week of July maintaining row to row distance of 45 cm and plant to plant distance of 4 cm. Crop was grown under rainfed conditions and no supplemental irrigation was given throughout the growing season. Rainfall data was also recorded. Total amount of rainfall during crop growing season (July to October) was 336 mm with 60% (202 mm) of the rainfall received during the month of August. Nitrogen fixation was estimated by Xylem Solute Technique (Peoples *et al.*, 1989). Sap was collected at the pod-fill stage by Vacuum Extraction Method and stored in the freezer at -15°C, then concentrations of ureides, nitrate and amino-N were determined to calculate the relative ureide- N (%) and percent nitrogen derived from atmosphere (% N_{dfa}) by the following formula:

Relative ureide-N (%) = [4 × ureides/(4 × ureide + nitrate + amino-N)] × 100

After getting the value of relative ureide- N, % N_{dfa} was estimated.

% N_{dfa} = 1.6 [Relative ureide- N (%) -15.9] for plants during pod fill.

Legume N uptake (kg ha^{-1}) = legume above ground biomass (kg ha^{-1}) × N in plant tissue (%).

Amount of N fixed (kg ha^{-1}) = legume N uptake (kg ha^{-1}) × % N_{dfa} (Rochester *et al.*, 1998).

At physiological maturity, crop from an area of one square meter in the middle of each plot was harvested separately. Data for plant height, plant density and number of pods per plant were also recorded. The plant samples were dried and data were recorded for seed, straw and total dry matter yield. Harvest index was computed as ratio between seed yield and above ground biomass multiplied by 100. Representative samples of 100 g from both seed and straw were collected from bulk sample, oven dried and ground and analyzed for N (Ryan *et al.*, 2001). Soil N balance after soybean production was obtained by subtracting N output from N input as follows (Amanuel *et al.*, 2000).

$B = (N_f + N_{dfa}) - N_g$

Where B is soil N balance, N_f is the applied N, N_{dfa} is the total fixed N and N_g is the N removed by soybean.

Data on all observations were subjected to analysis of variance by using software MSTATC. Treatment means were compared by least significant difference (LSD) test. Correlation analysis was also done to study the relationship between different parameters.

RESULTS

Plant height: There was a significant increase in plant height with S application, while effect of inoculation was not significant (Table II). The maximum plant height (56 cm) was observed in 30 kg S ha^{-1} + inoculated, which was 14% higher than that of control. The treatments 15 kg S ha^{-1} + inoculated and 30 kg S ha^{-1} increased plant height significantly.

Number of pods per plant: There was significant effect of different treatment application on number of pods per plant (Table II). Maximum number of pods per plant (28.33) was recorded in 30 kg S ha^{-1} + inoculated which was at par with 15 kg S ha^{-1} + inoculated but significantly higher than all other treatments. Inoculation resulted in increase in number of pode per plant by 39%, where as combined application of S and inoculation resulted in increase in number of pods per plant up to 56 %. The data also revealed that number of pods per plant in 30 kg S ha^{-1} and inoculated was statistically similar to each other but significantly higher than those of control and 15 kg S ha^{-1}.

Straw, seed and dry matter yield: There was significant effect of inoculation and S application on straw, seed and dry matter yield of soybean (Table II). Data revealed that maximum straw yield was recorded in 15 kg S ha^{-1} + inoculated, which was at par with 30 kg S ha^{-1} + inoculated. The data also revealed that straw yield of 15 kg S ha^{-1} and inoculated were similar to each other but they were higher than those of control and 15 kg S ha^{-1}.

The results indicated that maximum biomass yield (16.9 Mg ha^{-1}) was recorded in 30 kg S ha^{-1} + inoculated, which was statistically similar to 15 kg S ha^{-1} + inoculated (Table II). The data also revealed that biomass yield in treatments 30 kg S ha^{-1} and inoculated was statistically similar to each other but significantly lower than those in both 15 kg S ha^{-1} + inoculated and 30 kg S ha^{-1} + inoculated treatments.

The maximum seed yield (4.8 Mg ha^{-1}) was observed in 30 kg S ha^{-1} + inoculated which was at par with 15 kg S ha^{-1} + inoculated, inoculated and 30 kg S ha^{-1} (Table II). The data also revealed that differences in yield among the treatments 15 kg S ha^{-1}, 30 kg S ha^{-1} and inoculated were not significant. There was an increase up to 23, 20 and 26% in straw, seed and dry matter yield, respectively due to combined application of S and inoculation relative to control. The treatments did not show any variation regarding harvest index.

Table I: Physical and chemical properties of soils of experimental site

Parameter	Unit	Value
Altitude	m	500
Latitude	N	33 28´
Longitude	E	73 05´
Mean annual rainfall	mm	900
Cropping season rainfall	mm	336
Sand	%	54
Silt	%	34
Clay	%	12
Texture	-	Sandy loam
pH $_{(1:2..5)}$	-	7.8
E.C. $_{(1:10)}$	dSm^{-1}	0.35
Total Organic Carbon	%	0.39
Total N	%	0.02
$N\text{-}NO_3$	µg g^{-1}	11.2
Available Phosphorus	µg g^{-1}	4.5
Available Potassium	µg g^{-1}	124
Bulk Density	Mg m^{-3}	1.40
Calcium carbonate	%	11.63
Sulphate Sulfur ($CaCl_2$ Extractable)	mg kg^{-1}	9.57

Table II: Effect of inoculation and different levels of sulfur application on seed yield and yield attributes

Treatments	Plant height (cm)	Number of pods per plant	Straw yield (Mg ha^{-1})	Seed yield (Mg ha^{-1})	Dry Matter Yield (Mg ha^{-1})	Harvest Index (%)
Control	49.3 b	18 d	9.7 c	4.0 c	13.4 d	29.9
15 kg S ha^{-1}	50.0 b	21 c	9.6 c	4.3 bc	13.7 cd	31.4
30 kg S ha^{-1}	55.0 a	25 b	10.9 b	4.6 ab	15.1 b	30.5
inoculated	53.0 ab	25 b	10.9 b	4.5ab	14.6 bc	30.8
15 kg S ha^{-1}+inoculated	55.0 a	27 ab	12.1a	4.7a	16.4 a	28.7
30 kg S ha^{-1}+inoculated	56.0 a	28 a	11.9 a	4.8 a	16.9 a	28.4
LSD value†	4.2	2.7	0.84	0.35	0.9	NS

Table III: Effect of inoculation and different levels of sulfur application on nitrogen uptake and fixation by soybean and soil nitrogen balance

Treatments	Nitrogen uptake (kg ha^{-1})	Amount of nitrogen fixed from atmosphere (kg ha^{-1})	Amount of nitrogen taken from fertilizer and soil (kg ha^{-1})	Soil nitrogen balance (kg ha^{-1})
Control	180 d	83 e	97 d	-67
15 kg S ha^{-1}	204 c	99 d	105 c	-75
30 kg S ha^{-1}	233 b	117 c	116 b	-86
inoculated	224 b	110 c	114 b	-84
15 kg S ha^{-1} + inoculated	263 a	133 b	130 a	-100
30 kg S ha^{-1} + inoculated	271 a	143 a	128 a	-98
LSD value†	14.1	7.7	6.0	-

†Means with different letters differ significantly according to Least Significant Difference (LSD) test (*$P<0.05$*)

Table IV: Correlation matrix of different yield attribute and nitrogen fixation in soybean

Parameters	Plant height	Number of pods	Straw yield	Seed yield	Dry matter yield	Harvest Index	Nitrogen uptake	Nitrogen fixed	Nitrogen from fertilizer and soil
Number of pods per plant	.958**								
Straw yield	.927**	.935**							
Seed yield	.959**	.990**	.904**						
Dry Matter Yield	.930**	.932**	.966**	.929**					
Harvest Index	-.601	-.546	-.769	-.520	-.799				
Nitrogen uptake	.940**	.972**	.955**	.974**	.984**	-.687			
Nitrogen fixed	.939**	.967**	.938**	.974**	.984**	-.683	.997**		
Nitrogen from fertilizer and soil	.929**	.967**	.972**	.960**	.972**	-.684	.992**	.979**	
Soil nitrogen balance	-.929**	-.967**	-.972**	-.960**	-.972**	.684	-.992**	-.979**	-1.00**

**denote $P < 0.01$

Nitrogen uptake and fixation: There was significant difference between treatments regarding N uptake (Table III). Compared with control, all the treatments increased N uptake, while inoculation along with S (30 kg ha^{-1}) resulted in maximum N uptake, which was statistically similar to inoculated+ 15 kg S ha^{-1}. Almost similar trend was observed regarding amount of N fixed with the exception that amount of N fixed was significantly higher in inoculated+ 30 kg S ha^{-1} as compared to inoculated+ 15 kg S ha^{-1}. There was increase of 29 and 51% with inoculation and combined application of S (30 kg ha^{-1}), respectively in N uptake relative to control. Corresponding increase in amount of N fixed was 41 and 72%.

Amount of N taken by plant from soil and fertilizer was significantly higher due to inoculation and S application as compared to control (Table III). Maximum amount N taken from soil and fertilizer was observed in 15 kg S ha^{-1} + inoculated, which was statistically similar to 30 kg S ha^{-1} and inoculated but significantly higher than all other treatments.

Soil nitrogen balance: Soil N balance was negative in all the treatments (Table III). Deficit of N in soil increased with both S and inoculation. This deficit was lowest (67 kg ha^{-1}) in control and maximum (100 kg ha^{-1}) in inoculated+ 15 kg S ha^{-1} followed by inoculated + 30 kg S ha^{-1}.

DISCUSSION

Sulfur plays important role in vegetative growth of plant as it is a component of ferredoxin in chloroplast and is involved in photosynthetic process (Fukuyama, 2004). Not only this, but it also increases seed yield and its quality as low supply of S leads to abortion of pods in rapeseed (Fismes *et al.,* 2000). There was 20% increase in the seed yield of soybean due to combined application of S and inoculum. The results are in conformity with the previous findings (Habtemichial *et al*., 2007; Togay *et al.,* 2008; Jamal *et al.,* 2010a). Habtemichial *et al.* (2007) recorded much higher response to combined application S (30 kg ha^{-1}) and inoulation using faba bean as test crop in Ethiopia under rainfed conditions. Lower response to S in present study might be due to difference in climatic conditions, soil and genotype used. Effect of inoculation and S application on harvest index was non-significant, which indicate similar role of S in both vegetative and reproductive phase of plant life cycle. Response to inoculation was due to lack of presence of indigenous soil rhizobia. Yield attributes such as plant height and number of pods per plant correlated positively with seed yield (Table IV).

Sulfur availability has a role in regulating nitrate redutase and ATP- sulphuurylase and increases protein and chlorophyll content in legumes (Jamal *et al.,* 2006 & 2010a; Ahmad *et al.,* 2007). There was increase in N uptake and fixation with both S application and inoculation, which is in line with the findings of earlier research work (Habtemichial *et al*., 2007; Varin *et al.,* 2009; Jamal *et al.,* 2010b). In fact, there is close link between S supply and N requirement of plant. There was accumulation of one nutrient in plant when other nutrient was lacking and accumulated nutrient was used in protein synthesis when treatments were reversed (Jamal *et al.,* 2010a). Dry matter yield and nitrogen uptake correlated positively with amount of N fixed (Table V). This shows the role of increased plant growth in enhancing N fixation besides accelerated nitrogenase activity. Similar relationship has been reported by Amanuel *et al.* (2000) and Habtemichial *et al*. (2007). Amount of N fixed was in range of 83 to 146 kg ha^{-1}, which is much lower as compared to that reported earlier (Amanuel *et al.,* 2000; Habtemichial *et al*., 2007). However, some studies conducted in almost similar conditions have recorded even much lower (13 - 80 kg ha^{-1}) N fixation by mung bean and mash bean (Scherer *et al*., 2008).

Negative N balance was due to the fact that nitrogen input (fertilizer & nitrogen fixation) was not enough to meet crop demand. Second point is that N balance after legume harvest is positive when crop residues are returned to soil and only seed or grain is removed (Amanuel *et al.,* 2000). Habtemichial *et al.* (2007) reported positive soil N balance in range of 12 to 52 kg ha^{-1} after harvest of faba bean crop in Northern Ethiopia but major difference was that crop residues were returned to soil. Higher positive N balance with S application might be due to the fact that amount of N fixed increased from 49 to 147 kg ha^{-1} due to S application and inoculation. This increase was much smaller in our case (83 to 143 kg ha^{-1}) resultantly soil N balance became more negative (Table III). There was negative correlation of all the parameters except harvest index with soil N balance, which shows that accelerated plant growth resulted in depletion of soil N reserves (Table IV).

CONCLUSION

Sulfur application along with inoculation resulted in significant increase in overall plant growth and N fixation. It is apparent from this study that S fertilization to legumes should be recommended to soils with suboptimal S levels in order to obtain maximum seed yield. Increased amount of N fxation was mainly due to enhanced dry matter yield and N uptake. Legumes will improve soil N status in that situation when crop residues are returned to the soil and only seed or grain is removed, otherwise there will be N depletion of soil despite significant amount of N fixation from atmosphere.

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