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Effect of Fertilizer and Inoculation on Phosphorus, Potassium and Sodium Content of Pot Culture Mature Soybean Seeds

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Abstract: One year pot culture experiment on soybean cv.Williams-82 in Soil + FYM (3:1) was conducted in medium textured and salt free soil of Quetta, Balochistan. Seven fertilizer treatments were applied @ zero (control); 23; 25; 50; 75; 100 and 125 kg N ha^{-1} plus a constant dose of 60 kg P_2O_5 and 30 kg K_2O ha^{-1} respectively (except control). These fertilizer doses were applied to both non-inoculated (non-inoc) and inoculated (inoc) pot culture crop. Results suggested that added fertilizer significantly and inconsistently influenced the PO_4-P content, but negatively influenced the K content of mature soybean seeds. While reverse was true in case of Na contents. Results also revealed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly increased the K and Na contents, but reverse was found for P contents. Statistically a maximum significant amount of P (6.86 g kg^{-1}) was recorded in T_3 non-inoc dose of fertilizer (viz., 25 + 60 + 30 kg NPK ha^{-1}). While K (29.50 g kg^{-1}) and Na^+ (7.667 g kg^{-1}) was noted in T_2 inoc dose of fertilizer (viz., 23 + 60 + 30 kg NPK ha^{-1}). The simple correlation coefficient (r) values revealed that P content was significantly and positively correlated with starch (0.764), but negatively with oil contents (-0.448). While K (-0.839) and Na contents (-0.638) showed significant negative association with soluble sugars. It was also noted that grain yield pot^{-1} established a significant negative association with K (-0.573), but non-significant with P and Na contents of mature soybean cv.Williams-82 seeds.

Key words: Soybean, fertilizer, inoculation, phosphorus, potassium, sodium, correlation

Introduction

Soybean (***Glycine max*** L.) is mainly produced for protein and oil contents. It contains 40-42% good quality proteins and 18-22% oil comprising up of 85% unsaturated fatty acid and is free from cholesterol. Soybean not only contains high quality protein, but their protein content is also much higher than that of other foods. So it is highly desirable in human diet and animal nutrition (Anonymous, 1994; Aslam ***et al.***, 1995; Haq ***et al.***, 2002). Even though the mineral composition of soybean is fairly good, but it is notorious for its low carbohydrate contents.

The elemental composition of food grains is important in human and animal nutrition. A great deal of work has been done and is known about the elemental composition of food grains consumed by humans, but much less is known about the genetic and environmental factors controlling the mineral composition of seeds. A very little is known about the PO_4-P, K and Na level of mature soybean seeds in response to various level of added N fertilizer (with and without inoculation). Recent research has indicated that soybean not only contain appreciable amount of protein, oil and carbohydrate to some extent, but also has a potential to become both marketable human food grain and an important poultry feed. Soybean seed is a rich source of N, P and K and also accumulate other essential macro and micronutrients or elements needed by human and animal nutrition. Hanway and Weber (1971a, b) stated that K fertilizer resulted significantly higher K in all plant parts and least in the seeds (i.e., an average of 1.7% k in control and 2.42% K in K fertilized treatment). Moreover K in the non-nodulated plants was similar to that in the nodulated plants with a similar level of K fertilization. On the other hand Liu ***et al.*** (1997) stated that fixation capacities of K was significantly reduced by sustained high rates of K fertilization. While, Ramakrishna ***et al.*** (1990) noted that K uptake by soybean is influenced in response to different level of applied N and significant positive response were observed by added N up to 40 kg ha^{-1}.

Materials and Methods

One year pot culture experiment on soybean cv.Williams-82 was conducted in Soil + FYM (3:1) in Botanical Garden, University of Balochistan, Quetta. FYM was also analyzed for their N, P and K compositions, which were found as 0.75%, 0.18% and 0.25% respectively. The following seven different treatments (T) of fertilizer were applied to both non-inoculated (non-inoc) and inoculated (inoc) set of pot culture experiments.

T_1 = 0 + 0 + 0 kg NPK ha^{-1} (control)
T_2 = 23 + 60 + 30 kg NPK ha^{-1}
T_3 = 25 + 60 + 30 kg NPK ha^{-1}
T_4 = 50 + 60 + 30 kg NPK ha^{-1}
T_5 = 75 + 60 + 30 kg NPK ha^{-1}
T_6 = 100 + 60 + 30 kg NPK ha^{-1}
T_7 = 125 + 60 + 30 kg NPK ha^{-1}

The source, time, methods of fertilizer application and physicochemical characteristics of soil and water have already explained by Achakzai and Kayani (2002). The seeds of each treatment were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown coloration. Finally the seeds of each treatment were ground in a grinder, sieved through Mesh No. 60 (Johnson and Firth Brown Ltd. London). Air-dried defatted soybean seed powder (0.2 g) from each sample was homogenized in 20.0 ml of tris-

HCl buffer solution (0.1 M, pH 7.2) at room temperature for 16 h, with continuous shaking at 300 rpm (Edmond BÜhler 7400 TÜbingen). The sample was then centrifuged at 5,000 rpm using IEC B-20A Centrifuge (Damon/IEC Division) for 20 min, filtered through Whatmann filter paper, stored at 4.0 ^{0}C and then used for the determination of the following chemical contents.

Phosphate determination (g kg^{-1})

Phosphate was spectrophotometrically determined following the procedure of Charlot ***et al.*** (1964). An aliquot of 0.5 ml buffer extracted seed sample was mixed to 1.25 ml of sulfuric acid (0.2 M) and 0.5 ml of ammonium molybdate (0.016 M). Hydroquinone (0.25 ml) was mixed in the mixture solution and allowed to stand for ten minutes at room temperature. After this 0.25 ml of carbonate-sulfite mixture (13:4) was added in it and the volume was made up to 3.0 ml with water. The solution was thoroughly mixed and the absorbance was monitored at 720 nm. A calibration graph was also prepared for phosphate standard solutions (0.2 mM to 1.0 mM) and phosphate stock solution (0.01 M) was prepared by dissolving 0.14 g of KH_2PO_4 in 100 ml of deionized water. Finally the amount of phosphate phosphorus in the seed sample was manually calculated by using the standard calibration curve.

Potassium determination (g kg^{-1})

Potassium was determined by flame photometry following the procedure of Remani ***et al.*** (2002) by using Corning 400 flame photometer. The stock solution (100 ppm) of potassium chloride was prepared by dissolving 0.095 g KCl in 500 ml water. A series of standard solutions containing 0.0, 5.0, 15.0, 20.0, 25.0 and 30.0 ppm of KCl were prepared. After setting the filter and stabilizing the system, the galvanometer reading was adjusted to zero with blank (tris-HCl buffer). Each standard working solution was aspirated and noted their readings separately. Finally a plot of intensity vs. potassium concentration was drawn. Similarly the samples were aspirated simultaneously and their readings were noted. The standard calibration graph was then used for the calculation of K concentration in seed samples.

Sodium determination (g kg^{-1})

Sodium was also determined by flame photometry. Sodium chloride stock solution (100 ppm) was prepared by dissolving 0.127 g of NaCl in 500 ml water. After setting the sodium filter, the remaining procedure is the same as that adopted for potassium. A plot of intensity vs. sodium concentration was also drawn and used for the determination of Na concentration in seed samples.

The data obtained were statistically calculated following the procedure described by Steel and Torrie (1980). MSTAT-C Computer software package for statistical analyses was used for calculation of analysis of variance (ANOVA) and least significant difference test (LSD). Simple correlation coefficient (r) values were also worked out for all mentioned nutrients as well as other chemical components and grain yield pot^{-1} of mature soybean seeds, which has been already explained by Achakzai and Kayani (2002) and Achakzai ***et al.*** (2002b).

Results and Discussion

Data presented in Table 1 showed that in response to various level of added fertilizer with and without inoculation and their interactions, the P, K and Na content of pot culture mature soybean seeds were found as highly significant ($P < 0.01$).

PO_4-P

Data pertaining to mean separation values (Table 2) showed that fertilizer treatments significantly decreased the P content of seed (except T_2, T_3 and T) when compared with treatment not receiving fertilizer (T_1). Statistically and numerically a maximum significant (6.860 g kg^{-1}) level of P was recorded in T_3 dose of fertilizer (25 + 60 + 30 kg NPK ha^{-1}) followed by T_2

Table 1: Analysis of variance (ANOVA) for phosphorus, potassium and sodium content of pot culture mature soybean seeds in response to fertilizer treatments alone (A) and in combination with inoculums (B)

	Mean Square			F-value of variables at an error of 26			
Variables (g kg^{-1})	Fertilizer (A)	Inoculum (B)	A x B	A df = 6	B df = 1	A X B df = 6	CV (%)
1) PO_4-P	2.4	24.9	3.9	145.4*	1485.0*	237.6*	3.1
2) K^+	19.7	15.1	1.4	418.2*	320.5*	31.4*	0.8
3) Na^+	4.0	7.5	0.3	1189.9*	2238.5*	93.1*	0.9

* Highly significant at 1% level of significance.

Table 2: Effect of various level of fertilizer on phosphorus, potassium and sodium contents of inoculated and non-inoculated pot culture mature soybean seeds

Fertilizer Treatments	PO_4-P (g kg^{-1})	K^+ (g kg^{-1})	Na^+ (g kg^{-1})
T_1 (non-inoc)	4.5c	25.9c	5.4g
(inoc)	3.3gh	27.5b	6.2e
T_2 (non-inoc)	5.0b	27.5b	6.5d
(inoc)	3.7e	29.5a	7.6a
T_3 (non-inoc)	6.8a	26.2c	6.2e
(inoc)	3.1h	26.2c	7.0b
T_4 (non-inoc)	4.1d	23.5e	5.6f
(inoc)	4.0d	26.2c	6.4d
T_5 (non-inoc)	3.4fg	23.2ef	5.0h
(inoc)	2.8i	24.7d	6.4d
T_6 (non-inoc)	3.6ef	24.5d	4.5i
(inoc)	3.6ef	24.7d	4.4i
T_7 (non-inoc)	6.7a	23.0e	5.6f
(inoc)	2.7i	23.5e	6.6c
LSD (5%)	0.2	0.39	0.1
MM (non-inoc)	4.9	24.8	5.5
MM (inoc)	3.3	26.0	6.4
Grand mean	4.1	25.4	6.0

Figures followed by the same letter(s) in a column are not significantly different with each other at 5% level of significance. MM = marginal mean, non-inoc = non-inoculated and inoc = inoculated.

Table 3: Correlation coefficient (r) studies of few nutrients with some biochemical components and grain yield of pot culture mature soybean seeds receiving various level of added fertilizer (with and without inoculation)

Variables	Phosphorus ($g\ kg^{-1}$)	Potassium ($g\ kg^{-1}$)	Sodium ($g\ kg^{-1}$)	Proteins ($g\ kg^{-1}$)	Oil contents ($g\ kg^{-1}$)	Sugars ($g\ kg^{-1}$)	Starch ($g\ kg^{-1}$)	Grain yield ($g\ pot^{-1}$)
1	1.0							
2	-0.0 NS	1.0						
3	-0.0 NS	0.5 **	1.0					
4	-0.1 NS	0.1 NS	0.0 NS	1.0				
5	-0.4 *	-0.0 NS	-0.1 NS	0.7 **	1.0			
6	-0.1 NS	-0.8 **	-0.6 **	0.1 NS	0.3 NS	1.0		
7	0.7 **	-0.0 NS	-0.0 NS	-0.3 NS	-0.5 **	-0.1 NS	1.0	
8	-0.3 NS	-0.5 **	-0.3 NS	0.2 NS	0.4 *	0.6 **	-0.2 NS	1.0

* and ** significant at $P < 0.05$ and $P < 0.01$ level of probability respectively and NS = Non-significant.

and T_1 respectively. Research revealed that approximately half of the P content of mature soybean seeds was translocated from the above ground parts and the remaining half was taken up from the soil and nodules during the course of seed development. Research also revealed that a slight dose of fertilizer application in general and N in particular increased the P level of mature seeds. The highest P uptake by soybean seed was recorded @ 30 kg N + 60 kg P_2O_5 ha^{-1}. Beyond this a significant reduction in nutrient uptake was noticed. Moreover few others stated that added N fertilizers generally decreased the P level and the average P level in soybean seeds was recorded as 0.60%. While in present study the highest P uptake was recorded in T_3 dose of fertilizer (viz., 25 + 60 + 30 kg NPK ha^{-1}). Beyond this a gradual reduction was observed (except T_7). Therefore our findings in term of added fertilizer are also in conformity with the results obtained by Hanway and Weber (1971b), Sharma and Dixit (1987) and Kacha ***et al.*** (1990). Results also suggested that added P and K alone (T_2) significantly increased the P level as compared with treatment not receiving fertilizer (T_1). This is also in conformity with the results recorded by Hamel and Smith (1991), but in contradictory with Pongsakul and Jensen (1991). By comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly but negatively influenced the P content of seeds. Therefore the present findings are not in conformity with the results obtained by earlier workers (Hamel and Smith, 1991; Pongsakul and Jensen, 1991; Dubey, 1993). This might be due to complete absence of apparent nodulations, which has been already discussed by Achakzai and Kayani (2002).

Potassium ($g\ kg^{-1}$)

Data regarding mean values (Table 2) showed that fertilizer doses in general significantly but negatively influenced the K content of mature soybean seeds (except T_2) as compared with treatment not receiving fertilizer (T_1). Some researcher revealed that K uptake by mature soybean seeds were significantly influenced by added N up to 40 kg ha^{-1}. On the other hand few others stated that fixation capacities of K was not significantly reduced by sustained high rates of N fertilization. Therefore our results in term of K level of seeds are not in support of the results obtained by Ramakrishna ***et al.*** (1990) and Liu ***et al.*** (1997). Results also indicated that added P and K fertilizer alone (T_2) resulted significantly higher K level of seeds, which are also in accordance with Hanway and Weber (1971a, b), Ramakrishna ***et al.*** (1990) and Moraghan and Hammond (1996). By comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general significantly increased the K level of seeds and statistically a maximum significant level (29.50 g kg^{-1}) was found in T_2 dose of fertilizer (23 + 60 + 30 kg NPK ha^{-})$^{1}_{.}$ However on the basis of marginal mean values the inoculation effect was recorded as 4.75% greater over non-inoc treatments of fertilizer. Though nodules were not established in any dose of fertilizer, but still inoculation showed their silent positive effect in this regard, which are in support of the reports recorded by Hanway and Weber (1971a, b), Hamel and Smith (1991) and Achakzai ***et al.*** (2002a).

Sodium

Data presented in Table 2 showed that N fertilizer application in general and inoculation in particular significantly and positively affected the Na content of mature soybean seeds. Statistically a maximum significant (7.6 g kg^{-1}) level of Na was found in T_2 inoc dose of fertilizer (23 + 60 + 30 kg NPK ha^{-1}). On the basis of marginal mean values, the inoculation effect was noted as 15.21% greater over non-inoc treatments. Though, Na is not needs by plants in general and C_3 in particular (e.g., soybean), however it is essentially needed by human diet and animal nutrition. A very little is known about the Na status of soybean seeds. However, Moraghan and Hammond (1996) reported that flax seeds (***Linum usitismum L.***) contain Na in the range of 148 to 754 mg kg^{-1} seeds. While in present studies the mean minimum and mean maximum value of Na ranges between 4.47 to 9.66 g kg^{-1}, which are far greater than flax seeds. Thus it can be safely concluded that soybean is not only producing a good quality of protein and oil, but it could also be used as a rich source of K and Na in human and animal nutrition.

Correlation

The simple correlation coefficient (r) values revealed that PO_4-P exhibited significant positive association (0.764) with starch, but negative with oil (-0.448) and non-significant with grain yield pot^{-1} and remaining chemical attributes (Table 3). While K exhibited significant positive association (0.594) with Na, but negative with soluble sugars (-0.8) and grain yield (-0.5) respectively. However, Na level was found significantly and negatively correlated with soluble sugars (-0.6) and non-significantly with grain yield and remaining other chemical attributes. Research revealed that mineral composition of mature soybean seeds has got least attention in term of their association with different biochemical attributes and grain yield. However few workers reported that there was a reciprocal K-Na relationship or ion antagonism at high K rates as explained by Ragab ***et al.*** (1979), but in present study such relationship was not found.

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