

## Effect of Fertilizer, Inoculation and Sowing Time on the Uptake of Phosphorus, Potassium and Sodium Content of Field Grown Mature Soybean Seeds

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**Abstract:** The study was conducted on two different sowing dates in response to various level of added N fertilizer on the uptake of  $PO_4\text{-P}$ , K and Na content of mature soybean cv. Williams-82 seeds. Seven fertilizer treatments were applied to both non-inoculated (non-inoc) and inoculated (inoc) crop. Fertilizer treatments significantly increased the K and Na content of early planting, but decreased in late plantings, while P content is significantly reduced in both plantings when compared with their control treatment. Statistically a maximum significant amount of K is recorded in  $T_7$  ( $22.70 \text{ g kg}^{-1}$ ) and Na in  $T_6$  ( $9.667 \text{ g kg}^{-1}$ ) level of fertilizer. Result also showed that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation in general significantly increased the P and K content of both plantings and Na of early plantings. Statistically a maximum significant level of P, K and Na is recorded in  $T_2$  ( $4.997 \text{ g kg}^{-1}$ ),  $T_4$  ( $28.02 \text{ g kg}^{-1}$ ) and  $T_2$  ( $7.767 \text{ g kg}^{-1}$ ), respectively. Results further showed that early plantings produced 0.029 and 0.090% greater P and K, but 0.070% lesser Na over late planting seeds, respectively. The simple correlation coefficient (r) studies revealed that P content of early planting exhibited significant positive association with K (0.533), Na (0.653), soluble-sugars (0.615) and grain yield (0.448). While, in case of late plantings they only exhibited significant positive association with protein contents (0.548).

**Key words:** Soybean seeds, fertilizer, inoculation, sowing time, phosphorus, potassium, sodium

### 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.

A very little is known about the  $PO_4\text{-P}$ , K and Na level of mature soybean seeds in response to various level of added N fertilizer (with and without inoculation) and plantings dates. Recent work 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. and average of 1.7% K in control and 2.42% K in K fertilized treatment). Moreover, K in the non-nodulated plants was 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 was influenced by different level of applied N and significant positive response were observed by added N up to  $40 \text{ kg ha}^{-1}$ .

This study was therefore, undertaken with a view to find out the effect of fertilizer, inoculation and sowing time on the elemental composition of soybean seeds, which are highly desired by human nutrition and animal feedings.

### Materials and Methods

Two-year field experiment on soybean *Glycine max* L. Merrill cv. Williams-82 were carried out during the 1st week of July, 1996 (late sowing) and June, 1997 (early sowing) in Agricultural Research Institute (ARI), Quetta, respectively. Seven different treatments (T) of fertilizer were applied to both non-inoculated (non-inoc) and inoculated (inoc) set of experiments.  $T_1$  was kept control;  $T_2$  contained  $23 + 60 + 30 \text{ kg N, P}_2\text{O}_5$  and  $\text{K}_2\text{O ha}^{-1}$  and from  $T_3$  to  $T_7$  N fertilizer in the form of urea was added @ 25, 50,

75, 100 and  $125 \text{ kg ha}^{-1}$  along with combination of the same constant dose of P and K, respectively. The source, time and methods of fertilizer application have already explained by Achakzai *et al.* (2002). The seeds of each treatment were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown colouration. 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 Buhler 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  $4.0^\circ\text{C}$ , and then used for the determination of the following chemical contents.

**Phosphate determination ( $\text{g kg}^{-1}$ ):** Phosphate was spectrophotometrically determined following the procedure of Charlot (1964). Sulfuric acid (0.2 M), ammonium molybdate (0.016 M), hydroquinone (1.5% w/v), carbonate-sulite mixture (13:4) and  $\text{KH}_2\text{PO}_4$  solutions were used in the experiment and the absorbance was monitored at 720 nm.

**Potassium determination ( $\text{g kg}^{-1}$ ):** Potassium was determined by flame photometry following the procedure of Rehmani *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 solution containing 0, 5, 15, 20, 25 and 30 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 (Fig. 1). Similarly the samples were aspirated simultaneously and their readings were noted. The standard calibration graph was then used for the concentration K in seed samples.

**Sodium determination ( $\text{g kg}^{-1}$ ):** 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 seeing the sodium filter, the remaining procedure is the same as

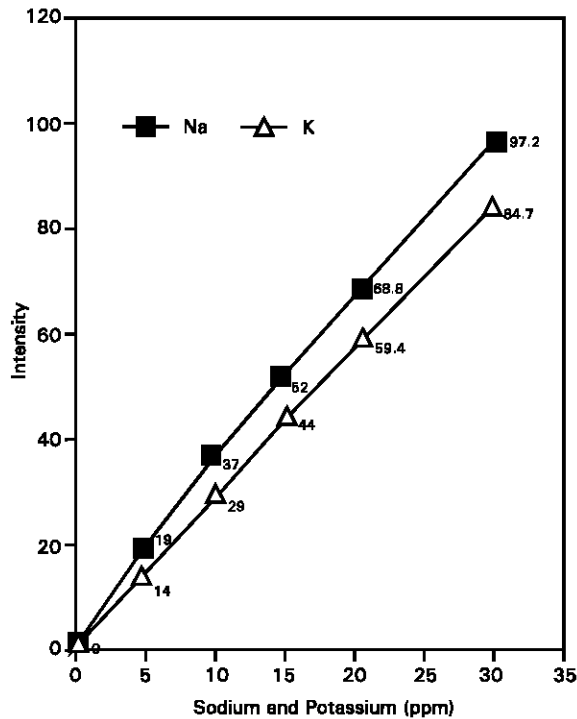


Fig. 1: Calibration graphs for Sodium and Potassium

that adopted for potassium. A plot of intensity vs sodium concentration was also drawn (Fig. 1) 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 under two factor randomized complete block design (RCBD) for statistical analysis was used for calculation of analysis of variance (ANOVA) and least significant difference test (LSD). Simple correlation coefficient (*r*) studies were also worked out for all mentioned nutrients as well as other chemical components and grain yield  $\text{plot}^{-1}$  in mature soybean seeds, which has been already explained by Achakzai and Kayani (2002a) and Achakzai *et al.* (2002).

## Results and Discussion

**PO<sub>4</sub>-P:** Data pertaining to mean values (Table 1) showed that fertilizer treatments in general significantly reduced the P content (except T<sub>2</sub>) of both planting (BP) seeds when compared with their respective control treatment (T<sub>1</sub>). Statistically a maximum significant level is recorded in T<sub>2</sub> (3.457 g kg<sup>-1</sup>) of late planting seeds. Research revealed that half of the P content of mature soybean seeds translocated from the above ground plant parts, and the remaining half were 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 at 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Beyond this a significant reduction in nutrient uptake was noticed. Moreover few others stated that added N fertilizer generally decreased the P level and the average P level in soybean seed was recorded as 0.60%. While in this study the highest P uptake is recorded in T<sub>2</sub> dose (viz., 23 + 60 + 30 kg NPK ha<sup>-1</sup>). Beyond this a gradual reduction was observed. Therefore, these findings in term of slight dose of applied fertilizer (T<sub>2</sub>) are in conformity with the results obtained by Hanway and Weber (1971b), Sharma and Dixit (1987), Kacha *et al.* (1990) and Achakzai and Kayani (2002b). Though DAP (diammonium phosphate) was mainly used P

content, but it also possess 18% N in addition to 46% P<sub>2</sub>O<sub>5</sub>. Therefore, added T<sub>2</sub> dose of fertilizer is quite enough to maximize the uptake of P content of seeds without using urea source of N. Data also suggested that by comparing the inoculated (inoc) with non-inoculated (non-inoc) in particular doses of fertilizer, inoculation significantly increased the P content of BP seeds. Statistically a maximum significant level is recorded in T<sub>2</sub> inoc (4.997 g kg<sup>-1</sup>) of EP and T<sub>6</sub> inoc (4.147 g kg<sup>-1</sup>) of LP seeds. Though nodules were not established in any level of treatment (Achakzai *et al.*, 2002a), but still our findings are in agreement with the results obtained by earlier workers (Hamel and Smith, 1991; Pongsakul and Jensen, 1991; Dubey, 1993) and in disagreement with Achakzai and Kayani (2002b). Whereas on the basis of marginal mean values the inoculation effects were found as 0.071 and 0.086% greater over non-inoc treatments of BP seeds, respectively. While on the basis of grand mean values, EP produced 0.0294% greater P content over LP seeds, which clearly signifies the importance of early planting over late plantings (Table 1).

**Potassium:** Data regarding mean values (Table 1) showed that fertilizer doses alone in general significantly and positively influenced the K content of EP (except T<sub>5</sub> and T<sub>6</sub>), but reverse is true in case of LP seeds when compared it with treatment not receiving fertilizer (T<sub>1</sub>). Statistically a maximum significant level of K is recorded in T<sub>1</sub> (24.70 g kg<sup>-1</sup>) of LP and T<sub>7</sub> (22.70 g kg<sup>-1</sup>) of EP seeds. Some researchers revealed that K uptake by mature soybean seeds significantly influenced by added N upto 40 kg ha<sup>-1</sup>. On the other hand few others stated that fixation capacities of K was not significantly reduced by sustained high rates of N fertilization. Therefore, these results of EP in term of K uptake by seeds are in agreement and that of LP are in disagreement with the results obtained by Ramakrishna *et al.* (1990) and Moraghan and Hammond (1996). The K content of LP is also in conformity with the pot culture studies of Achakzai and Kayani (2002b).

Data also suggested that by comparing the inoculated (inoc) with non-inoculated (non-inoc) in particular doses of fertilizer, inoculation in general significantly increased the K level of LP (except T<sub>1</sub> and T<sub>5</sub>) and EP seeds. Statistically a maximum significant level is recorded in T<sub>2</sub> (24.46 g kg<sup>-1</sup>) of EP and T<sub>4</sub> (28.02 g kg<sup>-1</sup>) of LP seeds. However, on the basis of marginal mean values, the inoculation effect in recorded as 0.178 and 0.169% greater in BP when compared with non-inoc treatments, respectively. Though nodules were not formed in any combination of fertilizer levels (Achakzai *et al.*, 2002a), but inoculation still showed their silent positive effect in this regard. Therefore in term of inoculation, these present findings are strongly in support of the reports recorded by Hanway and Weber (1971a,b), Hamel and Smith (1991) and Achakzai and Kayani (2002b). While on the basis of grand mean values the EP seed produced 0.09% greater K content over LP seeds, which also clearly signifies the importance of early planting over late planting (Table 1).

**Sodium:** Fertilizer treatments in general significantly increased the Na content of EP (except T<sub>3</sub> and T<sub>6</sub>), but adversely influenced in LP (except T<sub>2</sub>, T<sub>6</sub> and T<sub>7</sub>) seeds, when compared with treatment non receiving fertilizer (T<sub>1</sub>). Statistically a maximum significant amount in recorded in T<sub>6</sub> (9.667 g kg<sup>-1</sup>) of LP and T<sub>7</sub> (7.000 g kg<sup>-1</sup>) of EP seeds (Table 1).

Results also showed that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation significantly and positively affected the Na content of EP (except T<sub>7</sub>), but reverse is found in case of LP (except T<sub>3</sub>, T<sub>5</sub>) seeds. Statistically a maximum significant level is recorded in T<sub>2</sub> (7.767 g kg<sup>-1</sup>) of LP and T<sub>4</sub> (7.667 g kg<sup>-1</sup>) of EP seeds. Whereas, on the basis of marginal mean values, the inoculation effects are noted as 0.0785% greater in EP and 0.0529% lesser in LP seeds when compared with their non-inoc treatments. However, on the basis of grand mean values, the LP seeds produced 0.0702% greater Na content over EP seeds. This

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Table 1: Effect of various levels of added fertilizer without inoculum (non-inoc) and with inoculum (inoc) on the elemental composition of mature soybean cv. Williams 82 seeds grown on two different sowing dates

Treatments	Element composition (g kg <sup>-1</sup> )					
	PO <sub>4</sub> -P		Potassium content		Sodium content	
	LP	EP	LP	EP	LP	EP
T <sub>1</sub> (non-inoc)	2.96f	3.800f	24.70b	21.50k	7.500d	6.167g
(inoc)	3.307c	3.203e	21.79f	23.50d	7.233e	6.367f
T <sub>2</sub> (non-inoc)	3.183de	3.457d	20.07i	22.02j	8.033b	6.500e
(inoc)	3.450b	4.997a	22.40d	24.46a	7.767c	6.733d
T <sub>3</sub> (non-inoc)	2.757g	2.507hi	22.08e	22.50h	6.467h	5.067j
(inoc)	2.870f	3.397d	22.99c	22.80f	6.467h	7.533b
T <sub>4</sub> (non-inoc)	2.227i	2.553h	20.50k	22.20i	7.000f	6.467ef
(inoc)	2.937f	4.783b	28.02a	23.70c	7.067f	7.667a
T <sub>5</sub> (non-inoc)	2.337h	3.133ef	21.66g	20.70m	6.467h	6.633d
(inoc)	3.203cd	3.590c	21.20i	23.00e	6.433h	7.467b
T <sub>6</sub> (non-inoc)	2.887f	1.947j	16.48m	21.01l	9.667a	5.467i
(inoc)	4.147a	2.447j	21.30h	24.00b	7.467d	6.000h
T <sub>7</sub> (non-inoc)	1.717j	2.903g	20.50k	22.70g	7.700c	7.000c
(inoc)	3.080e	3.183e	20.81j	23.00e	7.667c	7.033c
LSD (5%)	0.1061	0.0751	0.0750	0.0530	0.0919	0.1061
CV (%)	2.11	1.24	0.22	0.13	0.76	0.94
MM (non-inoc)	2.5816	2.7971	20.86	21.80	7.543	6.186
MM (inoc)	3.2887	3.6571	22.64	23.49	7.014	6.971
Grand mean	2.933	3.227	21.750	22.650	7.281	6.579

Mean values in a column followed the different letters differ significantly at P < 0.05. LP = late plantings and EP = early plantings, CV = coefficient of variance, and MM = marginal mean, while non-inoc = non-inoculated and inoc = inoculated.

T<sub>1</sub> = 0+0+0 kg NPK ha<sup>-1</sup>; T<sub>2</sub> = 23+60+30 kg NPK ha<sup>-1</sup>; T<sub>3</sub> = 25+60+30 kg NPK ha<sup>-1</sup>; T<sub>4</sub> = 50+60+30 kg NPK ha<sup>-1</sup>; T<sub>5</sub> = 75+60+30 kg NPK ha<sup>-1</sup>; T<sub>6</sub> = 100+60+30 kg NPK ha<sup>-1</sup> and T<sub>7</sub> = 125+60+30 kg NPK ha<sup>-1</sup>

Table 2: Correlation coefficient (r) studies of various chemical components of field-grown mature soybean seed in response to various level of added N fertilizer (without and with inoculum) at two different sowing dates

Chemical components (g kg <sup>-1</sup> )	PO <sub>4</sub> -P	Potassium	Sodium	Soluble protein	Oil Contents	Soluble sugars	Starch	Grain yield (Kg Plot <sup>-1</sup> )
	LP EP	LP EP	LP EP	LP EP	LP EP	LP EP	LP EP	LP EP
PO <sub>4</sub> -P	1.000							
LP	1.000							
EP		1.000						
Potassium								
LP	0.091NS	1.000						
EP	0.533**	1.000						
Sodium								
LP	0.090NS	-0.502**	1.000					
EP	0.653**	0.336NS	1.000					
Soluble protein								
LP	0.548**	-0.143NS	0.395*	1.000				
EP	0.043NS	0.149NS	0.147NS	1.000				
Oil contents								
LP	0.205NS	0.228NS	0.072NS	0.501**	1.000			
EP	0.036NS	-0.041NS	-0.004NS	-0.279NS	1.000			
Soluble sugars								
LP	-0.357NS	0.254NS	-0.312NS	-0.507**	-0.064NS	1.000		
EP	0.615**	0.636**	0.574**	0.042NS	-0.63NS	1.000		
Starch								
LP	-0.371NS	0.587**	0.542**	-0.290NS	-0.272NS	0.242NS	1.000	
EP	0.187NS	0.259NS	0.527**	0.273NS	-0.679**	0.464	1.000	
Grain yield								
LP	0.188NS	0.053NS	0.066NS	0.138NS	-0.199NS	-0.108NS	-0.099NS	1.000
EP	0.448*	-0.098NS	-0.047NS	0.683**	-0.247NS	-0.211NS	0.331NS	1.000

\*P < 0.05 \*\* P < 0.01 and NS = non-significant at both probabilities. While LP = late plantings and EP = early plantings.

suggests that EP did not encourage the uptake of Na by soybean seeds. Though Na is not need by plants in general and C3 plants (are those plants in which phosphoglycerate, an organic acid, is the 1st stable product of photosynthesis) in particular (e.g., soybean). However, it is essentially needed by human diet and animal nutrition. A very little is known about the Na level of mature soybean seeds. Whereas, Moraghan and Hammond (1996) reported that flax seed [*Linum usitissimum* L.] contain Na in the range of 148-754 mg kg<sup>-1</sup> seeds. While, in present studies the mean minimum and mean maximum level of Na of BP are ranges between 5.067 to 9.667 g kg<sup>-1</sup>, which are far greater than flax seeds (Table 1).

**Correlation:** The correlation coefficient (r) studies (Table 2) revealed that PO<sub>4</sub>-P of LP seed are highly significantly (P < 0.01) and positively correlated with soluble proteins only (0.548), whereas in case of EP, they were significantly and positively correlated with K (0.533), Na (0.653), soluble sugars (0.615) and grain yield (0.448). While the K content of LP are significantly but negatively associated with Na (-0.502) and positively with starch content (0.587), whereas in case of EP they only significantly correlated in soluble sugars (0.636). Data also showed that Na content of LP exhibited significant positive correlation with protein (0.395) and starch (0.542), but in EP they only exhibited significant positive correlation with soluble sugar (0.542), but in EP they only

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exhibited significant positive correlation with soluble sugar (0.574) of seeds. However except of P content, the K and Na contents are non-significantly correlated with grain yield as well as with remaining chemical attributes. Research revealed that mineral composition of mature soybean seed has got least attention in term of their association with different biochemical attributes and grain yield. However, few researchers reported that there was a reciprocal K-Na relationship or ion antagonism at high K rates. But in this study such relationship is only found in case of LP seeds. Therefore, in term of K-Na relationship, our LP seeds are in accordance with the reports described by Ragab (1979). Therefore, it can be safely concluded that soybean is not only producing a good quality protein and a considerable amount of essential oils, but it could also be used as rich source of P, K and Na contents in human diet and animal nutrition. It is worth to mention that added fertilizer, inoculation and proper planting time and could also increased the level of aforementioned cations.

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