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Uptake of Moisture, Iron and Copper Contents by Field Grown Mature Soybean Seeds

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Abstract: The study was conducted on two different sowing dates i.e., late sowing (LS) and early sowing (ES) in response to various level of added N fertilizer on the uptake of moisture, total iron (Fe) and copper (Cu) content of mature soybean cv. Williams-82 seeds. Seven different fertilizer treatments (T) were applied to both non-inoculated (non-inoc) and inoculated (inoc) field crops. Results showed that fertilizer treatments in general significantly ($P < 0.05$) but adversely influenced the moisture, total Fe (except LS) and Cu content of both planting (BP) seeds, when compared with treatment receiving no fertilizer (T_1). Results also suggested that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation in general significantly and positively influenced the moisture, Fe (except LS) and Cu contents (except ES). Statistically and numerically a maximum amount of moisture content is recorded in T_4 inoc (49.93 g kg^{-1}), Fe in T_6 inoc (2.86 ppm) and Cu in T_7 non-inoc (2.957 ppm). Results further enumerated that on the basis of grand mean values, ES produced 2.39, 11.86 and 33.41% greater moisture, total Fe and Cu content over LS seeds, respectively. Results based on simple correlation coefficient (r) studies depicted that these three contents are non-significantly correlated with the yield of BP. However, total Fe and Cu did establish a significant negative association with oil content (-0.529 and -0.535) and soluble sugars (-0.537 and -0.715) of BP seeds, respectively. Therefore, it can be safely concluded that seed moisture, Fe and Cu contents could not be used a suitable selection criterion for predicting the grain yield, but total Fe and Cu could be used for reducing the oil and soluble sugars and increasing the starch contents in the mature seeds of soybean cv. Williams-82.

Key words: Soybean, fertilizer, inoculation, sowing time, moisture, iron, copper, 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 total iron ($\text{Fe}^{2+} + \text{Fe}^{3+}$) and copper ($\text{Cu}^+ + \text{Cu}^{2+}$) content of mature soybean seeds in response to various level of added N fertilizer (with and without inoculation). 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. Iron plays a crucial role in plant and animal metabolism especially in oxidation-reduction reactions. The whole body of a healthy adult human contains 3-5 g of iron and about two third of it is concentrated in the blood. It is generally one of the most wide spread deficient element in dietary food. However, the recommended daily dietary allowance for adult humans ranging between 10 to 18 mg (Harper, 1979). No extensive study on the nutritional status of soybean has been carried out yet. However, Baloch (1999) and Fazal (1999) analyzed various cereal grains (viz., barley, gram, kidney-bean, maize, pearl millet, pulses, rice, semolina and wheat). They examined that all grains having an average of $1.73 \mu\text{g}/5\text{g}$ of total iron. Among grains the highest amount of iron was recorded in gram seeds, kidney beans and pulses, respectively. Copper is a part of several enzymes and also participates in protein and carbohydrate metabolism. The deficient and sufficient level of copper in soybean was found to be in the range of 5-9 and 10-30 ppm, respectively (Tandon, 1993). Research also revealed that fertilizer treatments in general significantly ($P < 0.05$)

but adversely influenced the moisture and copper content of soybean seeds when compared with treatment receiving no fertilizer (T_1). While reverse was true in case of iron contents (Achakzai, 2003). Whereas, Naidu and Pillai (1993) obtained a maximum significant amount of total Fe and Cu contents in fertilizer dose of 100 kg N ha^{-1} . However, Hussain *et al.* (1981) noted that seed moisture content was not affected by any fertilizer treatment, while Kamal (1989) recorded a significant reduction in moisture content and viability percentages of soybean seed during late plantings. Research further revealed that by comparing the inoculated with non-inoculated treatments of fertilizer, inoculation in general significantly increased the moisture, iron and copper content of soybean seeds. Statistically a maximum amount of moisture (38.14 g kg^{-1}), iron (4.61 ppm) and copper content (2.25 ppm) is recorded in T_3 and T_5 inoculated dose of fertilizer, respectively (Achakzai, 2003). Whereas Achakzai *et al.* (2002a) stated that the moisture content of various plant parts of soybean were significantly increased in both non-inoculated and inoculated treatments of fertilizer as compared with control (T_1).

The study was therefore mainly aimed to determine the effect of added N fertilizer (without and with inoculum) on the moisture, Fe and Cu content of mature soybean seeds. The study was also initiated to furnish the information on the nature of association among them as well as with other chemical components and also with their grain yield to choose suitable selection criteria for predicting the quality and quantity of grain yield in soybean cv. Williams-82.

MATERIALS AND METHODS

Two-year field experiments 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 kg N, P_2O_5 and K_2O ha^{-1} ; and from T_3 to T_7 N fertilizer was added @ 25, 50, 75, 100 and 125 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.* (2002b). 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) and analysed for the following contents:

Moisture content (g kg^{-1}): Ten-gram air-dried soybean seed powder (W_1) from each sample is placed in an oven at 75.0°C for 24 h. After drying in an oven the sample is then kept in a desiccator and reweighed (W_2) and thereafter the moisture content of each sample is calculated as under:

$$\text{Moisture contents, g kg}^{-1} = \frac{(W_1 - W_2) \times 1000}{W_1}$$

Sample digestion: For total Fe and Cu determinations, seed sample was digested in diacid mixture following the procedure of Tandon (1993). Defatted oven-dried seed powder (1.0 g) was mixed with 10.0 ml diacid mixture of HNO_3 : $HClO_4$ (9 : 4) by swirling in a China crucible. The contents were heated carefully until red fumes ceased, after cooling, 20.0 ml of deionized water was added in each crucible, filtered through Whatmann filter paper No. 40 and the volume of each filtrate was made up to 100 ml with water. For decolorizing the solution, 0.25 g of activated charcoal was added and boiled for 5 min by using water bath and then filtered it again.

Iron determination (ppm): Total iron content was determined spectrophotometrically following the method of Marczenko (1976). The digested filtrate sample (0.5 ml) was mixed with 0.5 ml of 1,10-phenanthroline and 0.2 ml solution of hydroxylamine hydrochloride (10%). The mixture was diluted up to 5.0 ml with acetate buffer (2.0 M, pH 3.7) and mixed thoroughly. After 10 min, the absorbance of each sample was measured at 512 nm against blank. A standard calibration graph of ferric alum was also drawn (Fig. 1)

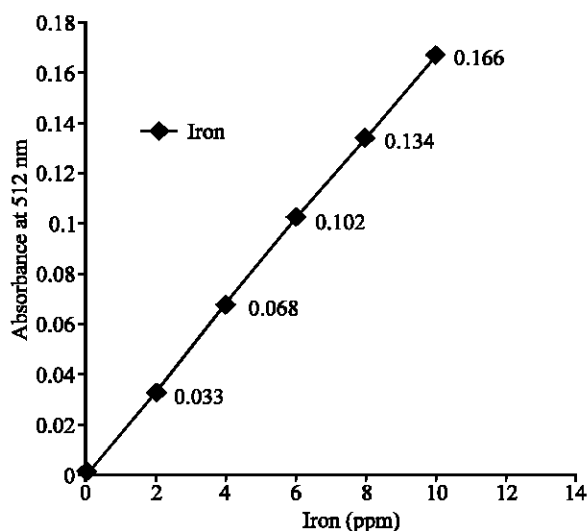


Fig. 1: Calibratin graph for total iron

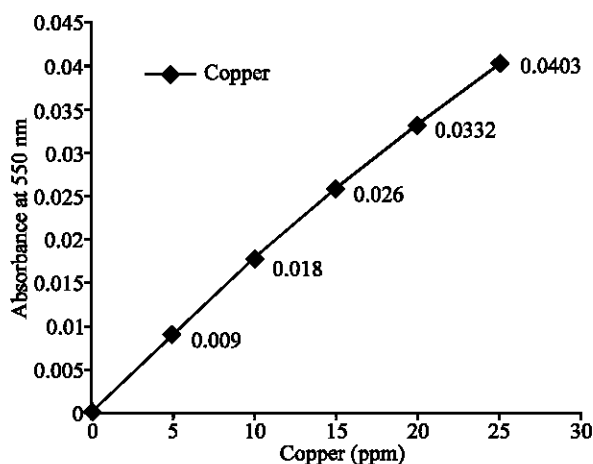


Fig. 2: Calibration graph for copper

Copper determination (ppm): Total copper content was also determined spectrophotometrically following the modified Biuret procedure as adopted by Harris and Angal (1989). The digested filtrate sample (0.5 ml) was mixed with 3.0 ml of sodium-potassium tartrate solution, 1.0 ml of protein solution and 0.5 ml of water. Mixed it well and heated at 37 °C for 10 min. Cooled the mixture and the absorbance was monitored at 550 nm against reagent blank. Copper sulphate solution was used for standard calibration graph (Fig. 2).

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) studies were also worked out for all mentioned nutrients as well as for other chemical components and grain yield in 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 deciphered that in relation to different level of added fertilizer (with and without inoculation) and their interaction significantly ($P < 0.01$) influenced the moisture, total iron and copper content of mature soybean seeds of both sowings.

Moisture content: Data regarding mean values (Table 2) showed that fertilizer treatments in general (except T_4 in LS) significantly but adversely influenced the moisture content of both sowings when compared it with their respective treatment receiving no fertilizer (T_1). Research revealed that seed moisture content was not significantly

affected by fertilizer doses. Therefore, our findings in this regard are not in support of the results gained by Hussain *et al.* (1981), but are strongly in line with the results of the same experiment conducted in earthen pots by Achakzai (2003). Results also showed that by comparing the inoculated (inoc) with non-inoculated (non-inoc) treatments in particular doses of fertilizer, inoculation significantly increased the moisture level of both year seeds. Statistically a maximum amount is recorded in T_1 (47.72 g kg⁻¹) of ES and T_4 (49.93 g kg⁻¹) of LS seeds. However, on the basis of marginal mean values the inoculation effect was recorded as 26.05 and 15.04% greater over non-inoc treatments in both year seeds, respectively. These results are also to great extent in conformity with the pot culture results of Achakzai (2003). While data based on grand mean values, ES produced 2.39% greater moisture content over LS seeds.

Total iron: Data presented in Table 2 enunciates that fertilizer treatments in general significantly and consistently increased the total iron content of LS seeds, but reverse was found in case of ES seeds (except T_6). Statistically a maximum significant level is noted (2.56 and 2.71 ppm) in T_6 dose of N-fertilizer. The same was also reported by Naidu and Pillai (1993) and Achakzai (2003) in their pot culture studies. Results also enunciates that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly and positively influenced the total iron content of ES, but significant reduction was noted in LS seeds. Statistically a maximum significant level is also recorded in T_6 (2.86 ppm) dose of added fertilizer. Whereas on the basis of marginal mean values, the inoculation effects are noted as 16.89% greater in ES, but 23.69% lesser in LS seeds. Therefore, in term of inoculation, our ES results are in accordance with the results explained by Achakzai (2003). While on the basis of grand mean values, ES produced 11.86% greater Fe content over LS seeds.

Copper content: Data concerned with mean values (Table 3) showed that fertilizer regime in general significantly but adversely influenced the total seed Cu content in both plantings (except T_7) when compared with treatment receiving no added fertilizer (T_1). Statistically and numerically a maximum level is noted in T_7 dose of fertilizer (i.e., 2.05 and 2.96 ppm) in both sowings, respectively. These achievements are in accordance with the results obtained by Achakzai (2003), but are conflicting with pot culture studies of Naidu and Pillai (1993). Data also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, the inoculation response was noted as positive only in case

Table 1: Analysis of variance (ANOVA) for few nutrients of field grown mature soybean seeds as influenced by Various levels of added fertilizer alone (A) and in combination with inoculum (B)

Variables	Mean square for fertilizer (A)		Mean square for inoculum (B)		F-value of variables at an error of 26					
					Fertilizer (A) (Df = 6)		Inoculum (B) (DF = 1)		A x B (Df = 6)	
	LS	ES	LS	ES	LS	ES	LS	ES	LS	ES
Moisture content, g kg ⁻¹	29.414	5.162	1007.73	390.76	54791.00*	11247.00*	1877213.00*	851408.00*	3452.00*	4616.00*
Iron content, ppm	0.281	0.871	1.73	1.18	98.93*	305.28*	609.50*	414.10*	84.44*	64.55*
Copper content, ppm	0.273	0.923	0.35	5.14	150.41*	520.45*	195.69*	2900.54*	60.43*	66.05*

*Significant at 1% level of probability. Df = degree of freedom. LS = late sowing. ES = early sowing

Table 2: Effect of various level of added fertilizer without inoculation (non-inoc) and with inoculation (inoc) on the moisture, iron and copper content of mature soybean cv. Williams-82 seeds grown on two different sowing dates

Treatments	Moisture content, g kg ⁻¹		Iron content, ppm		Copper content, ppm	
	LS	ES	LS	ES	LS	ES
T ₁ (non-inoc)	38.94i	42.52h	1.807g	2.257c	2.010b	2.903ab
(inoc)	46.93e	47.72a	2.093e	2.667b	2.133a	2.653c
T ₂ (non-inoc)	36.60m	41.76i	1.773g	2.013d	1.103i	2.850b
(inoc)	45.22f	46.15f	1.497hi	2.220c	1.833d	2.120g
T ₃ (non-inoc)	33.71n	39.62l	2.197cd	1.620gh	1.523h	2.243f
(inoc)	43.71g	44.75g	1.410i	1.743f	1.703e	1.147k
T ₄ (non-inoc)	40.93h	39.41m	2.263bc	1.680fg	1.550gh	2.453e
(inoc)	49.93a	46.92c	1.513h	1.843e	1.803d	1.607j
T ₅ (non-inoc)	38.66j	39.81k	2.317b	2.060d	1.603fg	2.537d
(inoc)	49.36b	46.82d	1.553h	2.252c	1.703e	1.747i
T ₆ (non-inoc)	37.75k	40.45j	2.557a	2.707b	1.667ef	2.717c
(inoc)	48.63c	47.51b	2.140de	2.857a	1.703e	1.957h
T ₇ (non-inoc)	37.29l	39.83k	1.960f	1.543h	2.050b	2.957a
(inoc)	48.13d	46.22e	1.823g	2.643b	1.913c	2.530d
LSD (5%)	0.05307	0.03753	0.09193	0.09193	0.07506	0.07506
CV (%)	0.05	0.05	2.77	2.48	2.45	1.82
MM (non-inoc)	37.62	40.49	2.125	1.983	1.644	2.666
MM (inoc)	47.42	46.58	1.718	2.318	1.827	1.966
Grand mean	42.517	43.535	1.922	2.150	1.736	2.316

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

T₁ = 0+0+0 kg NPK ha⁻¹; T₂ = 23+60+30 kg NPK ha⁻¹; T₃ = 25+60+30 kg NPK ha⁻¹; T₄ = 50+60+30 kg NPK ha⁻¹; T₅ = 75+60+30 kg NPK ha⁻¹; T₆ = 100+60+30 kg NPK ha⁻¹ and T₇ = 125+60+30 kg NPK ha⁻¹

Table 3: Correlation coefficient (r) studies of moisture, total Fe and Cu content with some other biochemical components and grain yield of field grown mature soybean seeds grown at two different sowing dates (LS and ES)

Variables	1 LS ES	2 LS ES	3 LS ES	4 LS ES	5 LS ES	6 LS ES	7 LS ES	8 LS ES
1 LS	1.000							
ES	1.000							
2 LS	-0.473*	1.000						
ES	0.539**	1.000						
3 LS	0.397*	-0.116NS	1.000					
ES	-0.506**	0.138NS	1.000					
4 LS	0.230NS	-0.272NS	0.059NS	1.000				
ES	0.010NS	0.165NS	0.048NS	1.000				
5 LS	0.037NS	-0.529**	0.110NS	0.501**	1.000			
ES	-0.303NS	-0.535**	-0.147NS	-0.279NS	1.000			
6 LS	-0.307NS	0.078NS	-0.537**	-0.507**	-0.064NS	1.000		
ES	0.482**	0.279NS	-0.715**	0.042NS	-0.063NS	1.000		
7 LS	-0.542**	0.762**	-0.218NS	-0.290NS	-0.272NS	0.242NS	1.000	
ES	0.631**	0.478**	-0.265NS	0.273NS	-0.679**	0.464*	1.000	
8 LS	0.264NS	0.016NS	0.096NS	0.138NS	-0.199NS	-0.108NS	-0.099NS	1.000
ES	-0.122NS	0.209NS	-0.041NS	0.683**	-0.247NS	-0.211NS	0.331NS	1.000

* And ** significant at P < 0.05 and P < 0.01 level of probability, respectively. NS = non-significant. (1) Moisture content g kg⁻¹ (2) Total iron, ppm (3) Total copper, ppm (4) Soluble-proteins, g kg⁻¹ (5) Oil-contents, g kg⁻¹ (6) Soluble-sugars, g kg⁻¹ (7) Starch, g kg⁻¹ (8) Yield Plot⁻¹, g

of LS, but negative for ES seeds. Whereas on the basis of marginal mean values, the inoculation effects are noted as 11.13% greater, but 35.61% lesser in both year field seeds, respectively. Therefore, in term of fertilization as well as

inoculation, LS results are in conformity with the pot culture studies of Achakzai (2003). While on the basis of grand mean values, ES produced 33.41% greater Cu content over LS seeds.

Correlation: The simple correlation coefficient (r) studies revealed that the aforementioned nutrients exhibited insignificant correlation with grain yield in both plantings, which are strongly in agreement with the results explained by Achakzai (2003). However, moisture content showed significant but inverse relationship with total Fe (-0.473 and 0.539) and Cu (0.397 and -0.506) as well as with starch content (-0.542 and 0.631) in both plantings, respectively. Whereas total Fe showed significant and consistent negative association with oil (-0.529 and -0.535), but positive with starch contents (0.762 and 0.478). While, total Cu exhibited significant negative association with soluble sugars only (-0.537 and -0.715). The associations of these chemical attributes are also in line with the pot culture studies conducted by Achakzai (2003). Whereas, the remaining chemical attributes showed either inconsistent or non-significant association among themselves. Therefore, it can be safely concluded that the aforesaid three contents could not be used a suitable selection criteria for predicting the grain yield, but total Fe and Cu could be used for reducing the oil and soluble sugars and increasing the starch contents without any concomitant loss in the protein content of mature soybean cv. Williams-82 seeds.

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