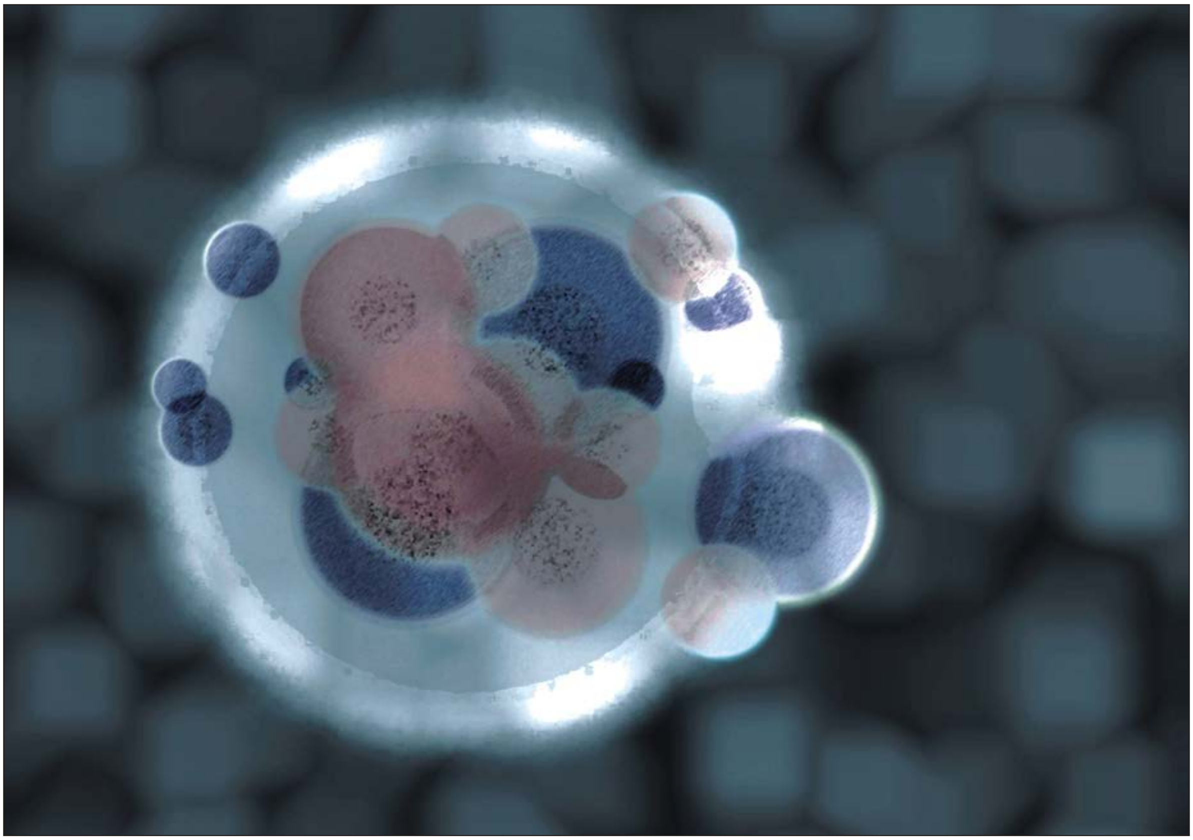

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Uptake of Moisture, Iron and Copper Content by Pot Culture Mature Soybean Seeds

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Abstract: A pot culture experiment on soybean cv.Williams-82 was conducted in clay loam soil with one level (T_2) of $N+P_2O_5+K_2O$ fertilizer @ 23+60+30 kg ha^{-1} and five different level (T_3 to T_7) of N fertilizers @ 25, 50, 75, 100 and 125 kg ha^{-1} plus the same constant dose of P and K fertilizer, respectively. Whereas T_1 with zero level of added NPK was kept control. Whereas a constant dose of FYM was also mixed with soil (1:3). These fertilizer treatments were applied to both non-inoculated (non-inoc) and inoculated (inoc) pot culture crops. Results showed that fertilizer treatments in general significantly ($P<0.05$) but adversely influenced the moisture and copper content of soybean seeds when compared with treatment not receiving fertilizer (T_1). While reverse was true in case of iron contents. Result also showed that by comparing the inoc with non-inoc treatments of fertilizer, inoculation in general significantly increased the moisture, iron and copper content of 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. The correlation coefficient (r) studies depicted that iron content exhibited highly significant positive correlation with grain yield (0.580) and oil contents (0.566), while moisture content showed significant but negative association with grain yield (-0.474). However, copper content did not establish any significant correlation with grain yield (0.366). Therefore, it can be safely concluded that iron could be used a suitable selection criteria for improving the quality (in term of oil and soluble sugars) and quantity of grain yield in soybean cv. Williams-82.

Key words: Soybean, fertilizer, inoculation, 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 having low concentration of carbohydrates.

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 (Fe^{2+}+Fe^{3+}) and copper (Cu^{+}+Cu^{2+}) content of mature soybean seeds in response to receiving various level of N fertilizer (with and without inoculation). Recent investigation has indicated that soybean not only contain considerable amount of protein, oil and carbohydrates 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 necessary macro and micronutrients or elements needed by human and animal nutrition. Iron plays a vital role in plant and animal metabolism especially in oxidation-reduction reactions. The whole body of a healthy adult human contains nearly 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 10-18 mg (Harper *et al.,* 1979). No extensive study has been carried out on the nutritional status of cereal grains in general and of soybean in particular. However, Baloch (1999) and Fazal (1999) analysed some 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 μg $5g^{-1}$ of total iron. Among grains the highest concentration of iron was recorded in gram seeds followed by kidney beans and pulses. Copper is a part of several enzymes and also participates in protein and carbohydrate metabolism. The sufficient and deficient level of copper in soybean was found to be in the range of 10-30 ppm and 5-9 ppm, respectively (Tandon, 1993).

Water is the most abundant and naturally occurring solvent in plants. The actual water content will vary according to organ, tissue and cell type and it is dependent to some extent upon the environmental and physiological conditions, but water typically amounts for more than 70% by weight of non-woody plant parts. Although certain desiccation-tolerant plants may experience water contents of only 20% and dry seeds may contain as little as 5% water, both are metabolically inactive and resumption of significant metabolic activity is possible only after the water content has been restored to normal levels (Hopkins, 1995). Hussain *et al.* (1981) noted that seed moisture content was not affected by any fertilizer treatment. While, Kamal (1989) and Achakzai (2003) recorded a significant reduction in moisture and Cu contents and viability percentages of soybean seeds during late planting. Whereas Achakzai *et al.* (2002) 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 treatment receiving no fertilizer (T_1). However, Sugimoto *et al.* (1998) studied the effects of N application on the water contents of soybean seeds from N-dressed (NDS) with those from undressed (UDS) plants during maturation. They found that water contents of NDS were slightly lesser than those of UDS for each stage of maturation.

The study was therefore mainly designed to determine and investigate the effect of added N fertilizer (without and with inoculum) on the moisture, total 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

One year pot culture experiment on soybean cv.Williams-82 was carried out in Soil + FYM (3:1) in Botanical Garden, University of Balochistan, Quetta. The soil used was of medium textured and salt free. The FYM was also analysed for their NPK composition, which was found as 0.75, 0.18 and 0.25%, 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 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 colour appeared. Finally the seeds of each treatment were ground in a grinder, sieved through Mesh No. 60 (Johnson and Firth Brown Ltd. London) and analyzed for the following constituents:

Moisture content (g kg^{-1}): Ten gram air-dried resultant soybean seed powder (W1) from every sample was placed in an oven at 75.0^0C for 24 h. After drying in desiccators, each sample is then reweighed (W2) and their moisture contents are calculated by using the formula given below.

$$\text{Moisture contents, g kg}^{-1} = \frac{(\text{W1-W2}) \times 1000}{\text{W1}}$$

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 deionised 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 deionised water. For the removal of colour from the solution, 0.25 g of activated charcoal was added and boiled for 5 min. by using water bath and then filtered it once-again.

Iron determination (ppm): Total iron content was determined spectrophotometrically following the procedure adopted by 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%) and diluted up to 5.0 ml with acetate buffer (2.0 M, pH 3.7) and thoroughly mixed it. After 10 minutes, the absorbance of each sample was measured at 512 nm against the reagent blank. A standard calibration graph of ferric alum was drawn for calculating the total Fe content in resultant seed samples.

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 them well and heated at 37°C for 10 min. Cooled the solution and the absorbance was monitored at 550 nm against reagent blank. A standard calibration graph of copper sulphate solution was also drawn for calculating the total Cu content in resultant 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) studies were also worked out for all mentioned nutrients, as well as for grain yield and other chemical components in mature soybean seeds, which has been already explained by Achakzai and Kayani (2002) and Achakzai *et al.* (2003).

RESULTS AND DISCUSSION

Data presented in Table 1 showed that in response to various level of applied fertilizer (without and with inoculum) and their interaction significantly ($P<0.01$) influenced the moisture, total iron and copper content of mature soybean seeds.

Moisture content: Data pertaining to mean values (Table 2) showed that fertilizer treatments in general significantly but adversely influenced the moisture content as compared with treatment not receiving fertilizer (T_1). Research revealed that seed moisture content was not significantly affected by fertilizer treatments. Therefore, our findings are not in support of the results obtained by Hussain *et al.* (1981), but are strongly in support of the field results of the same experiment obtained by Achakzai (2003). Result also showed that by comparing the inoculated (inoc) with non-inoculated (non-inoc) treatments in particular doses of fertilizer, inoculation significantly and positively influenced the moisture content of soybean seeds. Statistically a maximum significant amount is recorded in T_3 (38.14 g kg^{-1}) dose of fertilizer. However, on the basis of marginal mean values, the inoculation effect is recorded as 4.34% greater over non-inoc treatments. Therefore, these results are to some extent are in conformity with two-year field studies of Achakzai (2003).

Total iron: Data presented (Table 2) showed that high level of fertilizer (T_5 to T_7) significantly increased the total iron content of seeds when compared with treatment not receiving fertilizer (T_1). Statistically a maximum significant level is noted in T_6 (3.64 ppm) dose of fertilizer. The same were also recorded by Naidu and Pillai (1993) in pot culture and Achakzai (2003) in field studies. Result also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general significantly and positively affected the total iron content. Statistically a maximum significant level is recorded in T_5(4.61 ppm) dose of fertilizer, whereas, on the basis of marginal mean values, the inoculation effect is noted as 28.39% greater over non-inoc treatments. Though no extensive studies have been carried out on the effect of fertilizer and inoculation on the iron content of cereal grains in general and soybean in particular. However, few workers reported that cereal grains (including kidney-bean and pulses) having an average of 1.73 μg $5g^{-1}$ (or 0.35 ppm) of iron content and that for soybean the sufficient level of iron rages from 51 to 350 ppm. Whereas in present studies the mean minimum and mean maximum level of iron content ranges between 2.11 to 4.61 ppm, which are far lesser than the sufficient level reported by Tandon (1993) for soybean, but are much greater than those reported by Baloch (1999) and Fazal (1999) in cereal grains. It can be safely concluded that the low level of iron content could be attributed either calcareous nature of soils (Ting, 1980; Hopkins, 1995) or owe to complete absence of nodules (Achakzai and Kayani, 2002), because iron is very much needed by biologically N_2 fixing enzymes (nitrogenase).

Copper content: Data regarding mean values (Table 3) depicted that fertilizer treatments in general (except T_6 and T_7) significantly but adversely influenced the copper content of soybean seeds when compared with their respective control treatment (T_1). Statistically and mathematically a maximum significant level is recorded in

Table 1: Analysis of variance (ANOVA) for moisture content, total iron and copper content of Pot culture mature soybean seeds in response to added fertilizer treatments alone (A) and in combination with inoculum (B)

	Sum of squares		Mean square		F-value of variables at an error of 26			
Variables	Fertilizer (A)	Inoculum (B)	Fertilizer (A)	Inoculum (B)	A Df=6	B Df=1	A×B	CV (%)
Moisture Content (g kg^{-1})	69.436	33.375	11.573	33.375	8396.9*	24216.5*	783.0*	0.11
Total Fe (ppm)	11.497	6.849	1.916	6.849	616.98*	1847.77*	63.29*	1.87
Total Cu (ppm)	4.378	3.121	0.730	3.121	331.12*	1416.38*	116.81*	2.93

*Significant at 1% level of significance. Df = degree of freedom and CV = coefficient of variance

Table 2: Effect of various level of fertilizer on moisture, iron and copper contents of inoculated and non-inoculated Pot culture mature soybean seeds

Treatments	Moisture content (g kg^{-1})	Total iron (ppm)	Total copper (ppm)
T_1 (non-inoc)	36.94c	2.647h	1.533e
(inoc)	37.72b	3.523e	2.50b
T_2 (non-inoc)	35.61e	2.107i	0.340h
(inoc)	36.31d	3.117g	1.543e
T_3 (non-inoc)	34.94i	2.000j	1.153g
(inoc)	38.14a	3.340f	1.643d
T_4 (non-inoc)	33.55k	2.593h	1.353f
(inoc)	35.53f	3.340f	2.017bc
T_5 (non-inoc)	33.11m	3.607de	1.410f
(inoc)	35.12g	4.610a	2.250a
T_6 (non-inoc)	32.84n	3.637cd	1.560e
(inoc)	34.84j	3.723c	1.563e
T_7 (non-inoc)	33.20l	3.333f	1.947c
(inoc)	35.01h	3.923b	2.047b
LSD (5%)	0.05303	0.1061	0.07506
MM (non-inoc)	34.60	2.846	1.328
MM (inoc)	36.10	3.654	1.873
Grand mean	35.204	3.250	1.601

Figures followed by the same letter(s) in a column are not significantly differing with each other at 5% level of significance. MM = marginal mean, non-inoc = non-inoculated and inoc = inoculated.
T_1 = 0+0+0 kg NPK ha^{-1}; 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} and T_7 = 125+60+30 kg NPK ha^{-1}

Table 3: Simple 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	1	2	3	4	5	6	7	8
1	1.000							
2	-0.097NS	1.000						
3	0.085NS	0.777**	1.000					
4	-0.301NS	0.141NS	-0.279NS	1.000				
5	-0.229NS	0.566**	0.104NS	0.716**	1.000			
6	-0.794**	0.424*	0.190NS	0.137NS	0.325NS	1.000		
7	-0.014NS	-0.611**	-0.383*	-0.353NS	-0.591**	-0.148NS	1.000	
8	-0.474*	0.580**	0.366NS	0.207NS	0.468*	0.610**	-0.281NS	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 Pot^{-1}, g

T_7 (2.957 ppm) dose of fertilizer. The same were also reported by Naidu and Pillai (1993) in pot culture and Achakzai (2003) in field studies. Data also depicted that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation in general significantly and positively affected the copper level of seeds. Statistically a maximum level is recorded in T_5 (2.25 ppm) dose of fertilizer. However, on the basis of marginal mean values, the inoculation effect is noted as 41.04% greater when compared with their respective non-inoc treatments. These findings are once-again in conformation with the late sowing, but are in contradiction with the early sowing field results of Achakzai (2003). Though a very little is known about the copper status of mature soybean seeds. However, Tandon (1993) stated that 10-30 ppm is the sufficient level of copper contents in soybean. But in present studies the mean minimum and mean maximum level of copper ranges between 0.34-2.25 ppm, which is far lesser than that reported by Tandon (1993).

Correlation: The correlation coefficient (r) studies revealed that seed moisture content established a significant but negative correlation with grain yield (-0.474) and soluble sugars (-0.794), but non-significant with remaining chemical attributes (Table 3). Whereas total iron showed highly significant positive association with grain yield (0.580), copper (0.777), oil content (0.566) and soluble sugars (0.424) but negative with starch content (-0.611) and insignificant with soluble proteins (0.141). However, total copper content exhibited significant but negative association with only starch content (-0.383). Research revealed that moisture content, macro and micro nutrients of mature soybean seeds has got least attention in term of their association with grain yield and among themselves as well as with other chemical attributes. However, few researchers reported that moisture content is non-significantly correlated with protein and oil contents. Therefore, our findings are in accordance with the results obtained by Kamal (1989), Achakzai *et al.* (2002) and Ackazai (2003). Thus it can be safely concluded that iron did furnish significant positive correlation with oil and grain yield, while moisture content exhibit significant but negative association with grain yield.

REFERENCES

Achakzai, A.K.K. and S.A. Kayani, 2002. Effect of fertilizer and inoculation on nodulation, growth, yield, yield attributes and correlation studies of pot culture soybean cv.williams-82. Pak. J. Agric. Res., (submitted).

Achakzai, A.K.K., S.A. Kayani, M.A. Wahid and S. Jehan, 2002. Effect of fertilizer on growth, moisture contents, yield, yield attributes and correlation studies of non-inoculated and inoculated soybean grown under Quetta climate. Sarhad J. Agric., 18: 317-322.

Achakzai, A.K.K., 2003. Uptake of moisture, iron and copper contents by field grown mature soybean seeds. Asian J. Plant Sci., (submitted).

Achakzai, A.K.K., S.A. Kayani, M. Yaqoob and A. Nabi, 2003. Chemical composition of soybean cv.Williams-82 seeds in pot culture experiments. J. Biol. Sci., 3: 882-891.

Anonymous, 1994. Vegetable Soybean. Grow meat in your fields. Oil seeds Programme, NARC, Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan.

Aslam, M., S.M. Mirza, S.M. Shah, N. Javed and Naeemullah, 1995. New early maturing and high yielding soybean varieties. Crop Production Bulletin, pp: 1-11.

Baloch, S., 1999. Estimation of bioavailability of iron in wheat flour of different places of Balochistan and availability of iron in grains, fruits and vegetables. M.Sc. Thesis. Inst. Biochem. Univ. Balochistan, Quetta, Pakistan.

Fazal, R., 1999. Estimation of iron in different vegetables, fruits, cereals and flour. M.Sc. Thesis, Inst. Biochem. Univ. Balochistan, Quetta, Pakistan.

Haq, I., I. Hussain, A.R. Khan, M. Sajid and S. Khan, 2002. Soybean genotypic response in Abbottabad. Asian J. Plant Sci., 1: 418-419.

Harper, H.A., V.W. Rodwell and P.A. Mayes, 1979. Review of Physiological Chemistry. Lange Med. Publ. (17th Ed.), Drawer L, Los Altos, California 94022, USA.

Harris, E.L.V. and S. Angal, 1989. Protein Purification - A Practical Approach. 1st Ed., IRL Press, Oxford, UK.

Hopkins, W.G., 1995. Introduction to Plant Physiology. John Wiley & Sons, Inc. New York.

Hussain, S., T. Hussain, J.K. Khalil and I. Ahmad, 1981. Effect of fertilizer application on yield chemical composition and oil content of soybean. Pak. J. Agric. Res., 2: 215-218.

Kamal, B., 1989. Effect of environmental factors on the yield, quality and viability of soybean. M.Sc. Thesis Deptt. Agron. Fac. Agric., NWFP Agric. Univ. Peshawar, Pakistan.

Marczenko, Z., 1976. Spectrophotometric Determination Of Elements. Ellis Horwood Ltd. Chichester, pp: 305-321.

Naidu, M.V.S. and R.N. Pillai, 1993. Effects of nitrogen and phosphorus levels on yield, dry matter production and uptake of cationic micronutrients in soybean. Journal of Maharashtra Agricultural Universities., 18: 302-303.

Steel, R.G.D. and V.H.J. Torrie, 1980. Principles and Procedures of Statistics. McGraw- Hill Publishers. U.K, pp: 481.

Sugimoto, T., K. Nomura, R. Masuda, K. Sueyoshi and Y. Oji, 1998. Effect of nitrogen application at the flowering stage on the quality of soybean seeds. J. Plant Nutr., 21: 2065-2075.

Tandon, H.L.S., 1993. Methods of Analysis of Soils, Plants, Waters and Fertilizers. Fertilizer Development and Consultation Organisation, New Delhi-110048 (India), pp: 58-65.

Ting, I.P., 1982. Plant Physiology. Addison Wesley Services in Life Sciences. New York, USA.