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 (T1) of N=20, P=10, K=20, fertilizer @ 23+60+30 kg ha⁻¹ and five different level (T1 to T5) of N fertilizers @ 25, 50, 75, 100 and 125 kg ha⁻¹ plus the same constant dose of P and K fertilizer, respectively. Whereas T1 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 (T1). 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 T4 and T4 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 (Fe²⁺+Fe³⁺) and copper (Cu²⁺+Cu³⁺) 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 widespread 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 (T1).
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 lower 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 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. T1 was kept control, T2 contained 23+60+30
kg N, P2O5 and K2O ha−1 and from T1 to T5 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 Fifth 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°C 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.

\[
\text{Moisture content, g kg}^{-1} = \frac{(W1-W2) \times 1000}{W1}
\]

**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
HNO3 : HClO4 (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 (T0). 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 T6 (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 (T6 to T9) significantly increased the total iron content of seeds when compared with treatment not receiving fertilizer (T0). Statistically a maximum significant level is noted in T6 (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 T6 (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 g⁻¹ (or 0.35 ppm) of iron content and that for soybean the sufficient level of iron ranges 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 N₂ fixing enzymes (nitrogenase).

Copper content: Data regarding mean values (Table 3) depicted that fertilizer treatments in general (except T6 and T9) significantly but adversely influenced the copper content of soybean seeds when compared with their respective control treatment (T0). 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)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value of variables at an error of 26</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertilizer (A)</td>
<td>Inoculum (B)</td>
<td>Fertilizer (A)</td>
</tr>
<tr>
<td>Moisture (g kg⁻¹)</td>
<td>69.436</td>
<td>33.375</td>
<td>11.573</td>
</tr>
<tr>
<td>Total Fe (ppm)</td>
<td>11.497</td>
<td>6.849</td>
<td>1.916</td>
</tr>
<tr>
<td>Total Cu (ppm)</td>
<td>4.378</td>
<td>3.121</td>
<td>0.730</td>
</tr>
</tbody>
</table>

*Significant at 1% level of significance. DF= degree of freedom and CV = coefficient of variance
Table 2: Effect of various levels of fertilizer on moisture, iron and copper contents of inoculated and non-inoculated Pot culture mature soybean seeds

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture content (g kg⁻¹)</th>
<th>Total iron (ppm)</th>
<th>Total copper (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁ (non-in)</td>
<td>36.94</td>
<td>2.647h</td>
<td>1.533e</td>
</tr>
<tr>
<td>(inoc)</td>
<td>37.72</td>
<td>3.523e</td>
<td>2.506</td>
</tr>
<tr>
<td>T₂ (non-in)</td>
<td>35.61</td>
<td>2.107f</td>
<td>0.340h</td>
</tr>
<tr>
<td>(inoc)</td>
<td>36.31d</td>
<td>3.117f</td>
<td>1.545e</td>
</tr>
<tr>
<td>T₃ (non-in)</td>
<td>34.94j</td>
<td>2.000j</td>
<td>1.153g</td>
</tr>
<tr>
<td>(inoc)</td>
<td>38.14a</td>
<td>3.340f</td>
<td>1.643d</td>
</tr>
<tr>
<td>T₄ (non-in)</td>
<td>33.55k</td>
<td>2.593h</td>
<td>1.353f</td>
</tr>
<tr>
<td>(inoc)</td>
<td>35.53f</td>
<td>3.340f</td>
<td>2.017b</td>
</tr>
<tr>
<td>T₅ (non-in)</td>
<td>33.11l</td>
<td>3.607de</td>
<td>1.410f</td>
</tr>
<tr>
<td>(inoc)</td>
<td>35.12j</td>
<td>4.610a</td>
<td>2.256a</td>
</tr>
<tr>
<td>T₆ (non-in)</td>
<td>32.84n</td>
<td>3.637cd</td>
<td>1.550e</td>
</tr>
<tr>
<td>(inoc)</td>
<td>34.86g</td>
<td>3.723c</td>
<td>1.563e</td>
</tr>
<tr>
<td>T₇ (non-in)</td>
<td>32.20l</td>
<td>3.333f</td>
<td>1.947e</td>
</tr>
<tr>
<td>(inoc)</td>
<td>35.01h</td>
<td>3.923b</td>
<td>2.047b</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>0.0503</td>
<td>0.106f</td>
<td>0.07506</td>
</tr>
<tr>
<td>MM (non-in)</td>
<td>34.60</td>
<td>2.896</td>
<td>1.328</td>
</tr>
<tr>
<td>MM (inoc)</td>
<td>36.10</td>
<td>3.654</td>
<td>1.873</td>
</tr>
<tr>
<td>Grand mean</td>
<td>35.20d</td>
<td>3.250</td>
<td>1.601</td>
</tr>
</tbody>
</table>

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.

T₁ = 0+0+0 kg NPK ha⁻¹; T₂ = 25+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: 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)

<table>
<thead>
<tr>
<th>Variables</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-0.097NS</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.085NS</td>
<td>0.777**</td>
<td>1.000</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-0.301NS</td>
<td>0.141NS</td>
<td>-0.279NS</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-0.229NS</td>
<td>0.560**</td>
<td>0.184NS</td>
<td>0.716**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-0.794**</td>
<td>0.421*</td>
<td>0.190NS</td>
<td>0.137NS</td>
<td>0.325NS</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-0.014NS</td>
<td>-0.611**</td>
<td>-0.383*</td>
<td>-0.353NS</td>
<td>-0.591**</td>
<td>-0.148NS</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-0.474*</td>
<td>0.580**</td>
<td>0.360NS</td>
<td>0.207NS</td>
<td>0.408*</td>
<td>0.610**</td>
<td>-0.281NS</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* 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

T₇ (2.957 ppm) dose of fertilizer. The same were also reported by Naichu and Fillai (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 T₇ (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


