

SOYBEAN (Glycine max L.) RESPONSE TO MICRO-NUTRIENTS

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ABSTRACT

Soybean (*Glycine max* L.) generally respond to micro-nutrients by enhanced nodulation and more seed yield. A field trial was carried out to evaluate the response of soybean to micronutrients viz. iron (Fe), molybdenum (Mo) and cobalt (Co). Results indicated that iron applied at the rate of 400 g ha⁻¹ and molybdenum @ 20 g ha⁻¹ had a significant effect on shoot length, shoot dry weight, number of nodules plant⁻¹, nodules fresh weight and thousand seed weight. The highest seed yield 42.28% over control, dry matter yield , seed nitrogen and seed protein content was recorded by combined application of Fe at the rate of 400 g ha⁻¹ and Mo @ 20 g ha⁻¹.

Key Words: Cobalt, Iron, Molybdenum, nodulation, soybean, yield.

INTRODUCTION

Soybean (*Glycine max* L.) is herbaceous, erect grown, annual, warm climate and legume plant. It is considered as highly nutritive food for human, livestock (Berglund, 2002; Akparobi, 2009). Soybean enhances the content of soil organic matter when rotated in farming systems (O'Hara et al., 2002). But poor availability of plant nutrients limits yield in many world's crop production areas (Heitholt et al., 2003).

Micronutrients deficiency is wide spread in calcareous soils (Ahmad et al., 2012) and all over the world (Gurmani et al., 2012). Metal such as iron is very important for normal growth of soybean (Fageria, 2007). It also plays an important role in the formation of some nodule proteins like nitrogenase and leghaemoglobin (Moran et al., 1997). Iron is necessary for plant growth because of its participation in most important metabolic processes such as ribonucleotides and molecular nitrogen reduction, and energy yielding electron transfer reactions of respiration and photosynthesis (Guerinot and Yie, 1994). Iron deficiency in soybean results in chlorosis (Rotaru and Sinclair, 2009). It was assumed that a single unit increase in chlorosis score resulted in 20% yield losses in soybean (Froehlich and Fehr, 1981). Similarly Molybdenum plays a role as a co-factor of proteins, responsible for electron transfer in synthesis of nitrogenase enzyme and conversion of N2 into ammonia in nitrogen fixation process (Martens and Westerman, 1991). Deficiency of molybdenum reduces chlorophyll content in soybean and corn (Liu and Yang, 2003). Along with iron and molybdenum, cobalt is an essential element for legume crops because it is utilized by micro-organisms for the process of atmospheric nitrogen fixation (Evan and Kliwer, 1964). Growth processes such as coleoptiles and stem elongation, bud development, leaf disc expansion and opening of hypocotyle hooks are enhanced by cobalt application in soybean (Klein 1959). High concentration of cobalt becomes toxic and has an adverse effect on physiological and biochemical functions of plant (Parmar and Chanda, 2005).

Little information about the effects of these micronutrients (Fe, Mo and Co) on growth, yield and quality of soybean is available. Hence a need was felt to devise an experiment with the aim to evaluate the interactive effect of application of various doses of iron, molybdenum and cobalt on growth, nodulation, yield and quality of soybean.

MATERIALS AND METHODS

The present study was carried out under field conditions at the experimental area of the Department of Agronomy, Faculty of Agriculture, Rawalakot, Azad Jammu Kashmir, Pakistan during 2010 and 2011. The study was aimed to find out the effect of cobalt, Iron and molybdenum application on nodulation, growth, yield and N nutrition of the soybean plants. The experimental area was between $33-36^{\circ}$ N and $73-75^{\circ}$ E with an elevation of 1633 m above sea level. Sub-mountainous topography of valleys and extended plains with temperate sub-humid

climate are the feature of the area. Average rainfall range between 500–2000 mm annually. Summer is mild with mean annual temperature of 27°C while moderately cold temperature even below freezing point prevails in winter (Tahir et al., 2009).

The trial was laid out in Randomized Complete Block Design (RCBD) having three replications with a net plot size of 2.5m x 2.5m. Main plot treatments consist of three Fe rates; 0, 200 and 400 g Fe ha⁻¹. Subplot treatments were two Mo rates; 0 and 20 g Mo ha⁻¹. The sub-subplot treatments were two rates of Co; 0 and 20 g Co ha⁻¹. Fe was applied as foliar spray, each consisting of 1.82 kg or 3.64 kg ha⁻¹ of FeEDTA (5.5% Fe and 2% EDTA) and 50 ml of surfactant in 200 ml ha⁻¹ of water, using a knapsack sprayer. The physico-chemical properties of soil are as follows.

Table 1. Soil physico-chemical properties before sowing of soybean during 2010 and 2011

| Year | Soil Bulk density | Soil nH | Soil total N | Soil organic matter | Gravimetric soil moisture content | Fe (mg/kg) | Mo (mg/kg) | Co (mg/kg) |
|------|----------------------------|------------|-----------------|------------------------|--------------------------------------|---------------|---------------|---------------|
| 2010 | $1.33 (g \text{ cm}^{-3})$ | 7.65 | 0.08 (%) | 1.20 (%) | 0.07 (%) | 2.99 | 0.09 | 0.115 |
| 2011 | $1.27 (g \text{ cm}^{-3})$ | 7.55 | 0.11 (%) | 1.29 (%) | 0.09 (%) | 3.21 | 0.11 | 0.235 |

Half of the Fe doses were applied at the 1–2 trifoliolate stage and application was repeated at the 4–5 trifoliolate stage as recommended by Goos and Johnson (2000). Plant growth stages were determined by the methods of Fehr and Caviness (1977). Mo and Co was applied as sodium molybdate (Na₂MoO₄.2H₂O) and cobalt chloride (CoCl₂) as seed treatment. Seeds were coated with Na₂MoO₄.2H₂O and CoCl₂ by using 10% sucrose solution. A basal dose of phosphorous and potassium was applied in all plots including control at sowing time at the rate of 90 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹ as single super phosphate (SSP) and sulphate of potash (SOP), correspondingly by broadcast method. The various treatment combinations were:

Soybean cultivar NARC-1 at the rate of 80 kg ha⁻¹ was planted as test crop on May 15th and 5th, (2010 and 2011 respectively) at 40 and 10 cm inter- and intra-row distance, respectively to obtain an appropriate population density ha⁻¹. Observations on shoot length (cm), shoot dry weight (g), number of nodules plant⁻¹, nodules fresh weight (g), 1000 seed weight (g), seed yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), seed nitrogen content (%) and seed protein content (%) were taken to determine the effect of treatments on the growth and yield of crop characteristics, analysis of variance (ANOVA) and least significant difference (LSD) tests was carried out for each trait individually using a MSTAT-C. Comparison of means for each treatment was done at the 5% probability level based on the F-test of the analysis of variance (Steel et al., 1997).

RESULTS AND DISCUSSION

The interaction of various micro-nutrients (Fe, Mo, Co) showed a positive and significant response to growth and yield parameters of soybean. The highest shoot length (76.70) was recorded in Fe₂Mo₁Co₀ where Fe was applied @ 400 g ha⁻¹, Mo @ 20 g ha⁻¹ and Co 0 g ha⁻¹. The percent increase over control was 25.06 %. While the lowest shoot length (61.33) was observed in control (Fe₀Mo₀Co₀). The significant increase in shoot length in Fe₂Mo₁Co₀ can be attributed to Fe and Mo application as Fe played a vital role in nitrogen fixation and photosynthesis (Bennett, 1993) that ultimately increased

shoot length (Ahmad et al., 2002). Goos and Johnson (2000) and Rashid and Ryan (2004) from their studies reported that application of Fe correct Fe chlorosis and improve vegetative growth in soybean. Abd EI-Hai (1995) observed significant response of Mo on plant height.

Likewise the lowest shoot dry weight (0.80) was recorded in control ($Fe_0Mo_0Co_0$) where no micro-nutrition was added and the maximum (1.50) was obtained in $Fe_2Mo_1Co_0$ (Table 2). Maximum shoot dry weight was probably due to integrated use of Fe+Mo+Co because these results favours the findings of Guerinot and Yie (1994); Bhuiyan et al., (2008) and Caliskan et al., (2008) who observed a significant increase in shoot and root dry weight in mungbean with Mo application and Fe that improves photosynthesis activity that contributed for better plant dry biomass.

Regarding number of nodules plant⁻¹ the maximum number of nodules plant⁻¹ (106.7) were observed in Fe₂Mo₁Co₀. The increase in number of nodules plant⁻¹ in Fe₂Mo₁Co₀ was 48.87 % over control. It was at par with Fe₂Mo₁Co₁, Fe₁Mo₁Co₁ and Fe₁Mo₁Co₀ where number of nodules plant⁻¹ were 103.3, 99 and 94.67 respectively and Fe was applied at the rate of 400 g ha⁻¹, 200 g ha⁻¹ while Mo and Co at the rate of 20 g ha⁻¹ each. Data also showed the lesser number of nodules $plant^{-1}$ (71.67) in control. Highest number of nodules plant⁻¹ might be attributed to Mo and Co application as Mo is utilized by specific plant enzymes which play a role in oxidative and reduction reactions and Co being a part of cobalamin (vit. B12 and its derivaties), a component of different enzymes in nitrogen fixing micro-organisms (Hegazi et al., 2011). Further more, application of Fe enhance number of nodules plant⁻¹ as Fe is an important element for rhizobia nutrition, nodulation, nodule activity and biological nitrogen fixation (O' Hara, 2001). Balachandar et al., (2003) and Subasinghe et al., (2003) reported that Mo and Co significantly enhance nodules number plant⁻¹ in black gram and cowpea, respectively.

Similar trend was observed for nodules fresh weight. Maximum nodules fresh weight (0.067) were noted in $Fe_2Mo_1Co_0$ and was at par with $Fe_2Mo_1Co_1$ and $Fe_1Mo_1Co_1$ where nodule fresh weight were 0.050 g each respectively while the lowest nodules fresh weight (0.023) were recorded in control. The maximum nodules fresh weight in $Fe_2Mo_1Co_0$ (Table 2) can be attributed to Fe and Mo application because these results supports the findings of Balachander et al., (2003) and Subasinghe et al., (2003)

who reported that nodules fresh weight can be increased by Mo and Co application in black gram and cowpea. Also Krouma and Abdelly (2003) from their studies suggested that Fe is necessary for overall growth of nodules.

 Table 2. Effect of micronutrients on growth, yield and nodulation of soybean (*Glycine max* L.) during 2010-11 (pooled over years)

| Treatments | Shoot length (cm) | Shoot dry weight (g) | Number of nodules plant ⁻¹ | Nodules fresh weight (g) | 1000 seed weight (g) | Seed yield (kg ha ⁻¹) | Dry matter yield (kg ha ⁻¹) | Seed nitrogen content (%) | Seed Protein content (%) |
|---|-------------------------|--------------------------------|---|-----------------------------------|----------------------------|---|--|------------------------------------|-----------------------------------|
| Fe ₀ Mo ₀ Co ₀ | 61.33 c | 0.80 d | 71.67 f | 0.023 c | 151.3 d | 1213 d | 1741 e | 2.50 e | 34.00 e |
| Fe ₀ Mo ₀ Co ₁ | 65.00 bc | 1.04 cd | 76.67 ef | 0.023 c | 161.7 cd | 1283 cd | 1843 e | 2.70 de | 35.00 de |
| Fe ₀ Mo ₁ Co ₀ | 66.73 bc | 1.08 bcd | 77.67 ef | 0.023 c | 163.0 bcd | 1289 cd | 1973 de | 2.80 de | 35.40 cde |
| Fe ₀ Mo ₁ Co ₁ | 67.03 bc | 1.17 abc | 81.67 def | 0.030 c | 165.7 bc | 1348 bcd | 2074 cde | 2.90 cde | 36.00 cd |
| Fe ₁ Mo ₀ Co ₀ | 67.57 bc | 1.19 abc | 85.00 cdef | 0.030 c | 166.7 bc | 1415 bcd | 2150 bcde | 3.10 bcde | 36.00 cd |
| Fe ₁ Mo ₀ Co ₁ | 68.10 bc | 1.21 abc | 88.67 bcde | 0.033 bc | 167.3 abc | 1449 bc | 2227 bcde | 3.30 bcde | 36.00 cd |
| Fe ₁ Mo ₁ Co ₀ | 71.27 ab | 1.38 abc | 94.67 abcd | 0.037 bc | 173.3 abc | 1788 a | 2611 bc | 3.70 abc | 37.00 abc |
| Fe ₁ Mo ₁ Co ₁ | 72.40 ab | 1.40 ab | 99.00 abc | 0.050 ab | 174.7 ab | 1801 a | 2688 ab | 3.80 ab | 38.00 ab |
| Fe ₂ Mo ₀ Co ₀ | 69.13 abc | 1.22 abc | 88.67 bcde | 0.033 bc | 169.7 abc | 1542 b | 2483 bcd | 3.40 abcd | 36.33 cd |
| Fe ₂ Mo ₀ Co ₁ | 70.23 ab | 1.28 abc | 89.00 bcde | 0.037 bc | 171.0 abc | 1547 b | 2586 bc | 3.50 abcd | 36.40 bcd |
| Fe ₂ Mo ₁ Co ₀ | 76.70 a | 1.50 a | 106.7 a | 0.067 a | 179.3 a | 1834 a | 3226 a | 4.20 a | 38.40 a |
| Fe ₂ Mo ₁ Co ₁ | 73.10 ab | 1.40 ab | 103.3 ab | 0.050 ab | 179.0 a | 1821 a | 3226 a | 3.90 ab | 38.17 a |
| LSD(P≤0.05) | 8.15 | 0.35 | 16.82 | 0.02 | 12.13 | 230.90 | 545.30 | 0.87 | 1.633 |

Means not sharing same letter with in column differ significantly from each other at 5% level of probability by LSD test

$$\begin{split} &Fe_0Mo_0Co_0 \ (Control), Fe_0Mo_0Co_1 \ (Fe = 0, Mo = 0, Co = 20 g ha^{-1}), Fe_0Mo_1Co_1 \ (Fe = 0, Mo = 20 g ha^{-1}, Co = 20 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 0, Mo = 20 g ha^{-1}, Co = 20 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 0, Mo = 20 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 200 g ha^{-1}, Co = 20 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 200 g ha^{-1}, Mo = 20 g ha^{-1}), Fe_0Mo_1Co_1 \ (Fe = 200 g ha^{-1}, Mo = 20 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 200 g ha^{-1}), Fe_0Mo_1Co_1 \ (Fe = 400 g ha^{-1}), Fe_0Mo_1Co_0 \ (Fe = 400 g ha^{-1$$

The perusal of data given in Table 2 indicated that interaction between various micronutrients viz iron (Fe), molybdenum (Mo) and cobalt (Co) for yield parameters were statistically significant. It revealed that the highest thousand seed weight (179.3) was recorded in Fe₂Mo₁Co₀ and the percent increase over control was 10 % that was at par with Fe₀Mo₁Co₀ and Fe₁Mo₀Co₀ where thousand seed weight was 163.0 and 166.7 respectively. Whereas the lowest thousand seed weight was recorded control. Similarly the lowest seed yield (1213 kg ha⁻¹) was noted in control (Fe₀Mo₀Co₀) whereas the highest seed yield (1834 kg ha⁻¹) was recorded in $Fe_2Mo_1Co_0$ (Table 2). Similarly the significant increase in thousand seed weight (Table 2) can also be attributed to Fe and Mo application as both are the important components of enzyme nitrogenase and nitrate reductase that enhance bacterial growth and symbiotic efficiency resulting in improved plant growth and yield (Solaiman, 1999). These results favors the findings of Rahman et al., (2008) and Heidarian et al., (2011).

The percent increase in seed yield was 42.28 % over control. It was at par with $Fe_2Mo_1Co_1$, $Fe_1Mo_1Co_1$ and $Fe_1Mo_1Co_0$ where seed yield were 1821, 1801 and 1788 kg ha⁻¹, respectively. The seed yield and number of nodules plant⁻¹ of soybean showed a linear correlation (Fig. 1). It revealed that with an increase in number of nodules plant⁻¹ the seed yield can also be increased. The highest response in seed yield (Table 2) can be attributed to Fe and Mo application as Fe plays an important role in plant growth and crop yield (Guerinot and Yie, 1994) and Mo is responsible for increasing process of nitrogen

fixation by nodule tissues formation (Sharma et al., 1988) that ultimately resulted in better plant growth and yield. Caliskan et al., (2008) and Adesoji et al., (2009) reported highest yield with Fe and Mo application.

Also the highest dry matter yield (3226 kg ha⁻¹) was recorded in Fe₂Mo₁Co₀ (Table 2) and the percent increase was 85.29 % over control. It was at par with Fe₂Mo₁Co₁ and Fe1M01C01 where dry matter yield was 3226 and 2688 kg ha⁻¹ respectively. While the lesser dry matter vield (1741 kg ha⁻¹) were observed in control. The significant increase in dry matter yield in Fe₂Mo₁Co₀ might be due to Mo and Fe application for activation of nitrogenase enzymes. Similarly, Fe has a role in formation of nodule protein nitrogenase and leghaemoglobin (Moran et al., 1997) thus reduces iron deficiency chlorosis that results in excellent growth and yield. These results are confirmed by Goos and Johnson (2001); Goos et al., (2004) and Adesoji et al., (2009) who concluded that Fe and Mo application resulted in better dry matter yield of soybean.

Micro-nutrients viz iron (Fe), molybdenum (Mo) and cobalt (Co) produce significant effect on seed nitrogen content. It was highest (4.20 %) in $Fe_2Mo_1Co_0$ where Fe was applied at the rate of 400 g ha⁻¹ and Mo at the rate of 20 g ha⁻¹(Table 2).Whereas the lowest was produced by control. The percent increase over control was 68 %. When correlation between seed nitrogen content and number of nodules plant⁻¹ was developed, a positive correlation was observed between seed nitrogen content and number of nodules plant⁻¹ of soybean (Fig. 2).

Significant effect of micronutrients on seed nitrogen content can be attributed to Fe and Mo application as Fe and Mo are the key components of nitrogenase enzyme and nitrate reductase that enhance bacterial growth and symbiotic efficiency (Solaiman, 1999). Campo et al., (2009) noticed significant effect of applied micronutrients on seed nitrogen content.

Likewise the lowest seed protein content (34 %) was recorded in control (Fe₀Mo₀Co₀) while the highest seed protein content (38.40 %) was noted in Fe₂Mo₁Co₀ where Fe was applied at the rate of 400 g ha⁻¹ while Mo at the rate of 20 g ha⁻¹. The percent increase in seed protein content in this treatment was 12.94 % over control. It was at par with Fe₂Mo₁Co₁, Fe₁Mo₁Co₁ and Fe₁Mo₁Co₀ where seed protein content were 38.17 %, 38 % and 37 % respectively. The significant increase in seed protein content can be attributed to Mo, Co and Fe application, as Mo take a part in increasing photosynthetic rates (Pang et al., 2001; Liu and Yang, 2003; Sun et al., 2006) and Co due to its utilization by micro-organisms to continue the process of atmospheric nitrogen fixation (Evan and Kliwer, 1964) that enhance growth, yield and mineral contents of plant. Along with Co and Mo, Fe significantly improved photosynthesis activity that contributed for overall plant growth, mineral content and yield (Guerinot and Yie, 1994; Caliskan et al., 2008). Campo et al., (2009) and Gad (2010) concluded from their studies that Mo and Co significantly enhance protein contents of seed.

CONCLUSION

From the results it may be inferred that the plots fertilized with Fe, Mo and Co @ 400 g, 20 g and 20 g ha⁻¹ respectively revealed similar results to plots with same level of Fe and Mo but without Co application. Hence keeping in view the cost effectiveness for the farmer, it could be recommended that better growth, nodulation, yield and quality of soybean could be achieved by the application of Fe @ 400 g ha⁻¹ and Mo @ 20 g ha⁻¹.





Fig.02: Relationship between number of nodules plant¹ and seed nitrogen content (%)



Fig. 03 Monthly rainfall (mm) of experimental area during the growing period of soybean

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