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Full Length Research Paper

Effect of nitrogen (N) fertilizer and foliar-applied iron (Fe) fertilizer at various reproductive stages on yield, yield component and chemical composition of soybean (*Glycine max* L. Merr.) seed

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Nutritional management is an important factor in the success of crop production. However, research on the effects of nitrogen (N) and iron (Fe) application on soybean yield is limited. In order to study the effects of N and Fe application at various reproductive stages on grain yield and quality of soybean seed, an experiment was conducted using a factorial arrangement based on randomized complete block design with three replications at the research farm of Kurdistan University in 2009. The experimental treatments consisted of three different levels of N fertilizer application as follows: 0, 50 and 100 kg N ha^{-1}, and two levels of Fe fertilizer (spray with iron and non-sprayed). Results indicate that the maximum seeds yield was obtained at N1 (303 g m^{-2}) and N2 (328 g m^{-2}) treatments and the highest number of seed per plant was obtained at N2 (128) treatment. Potassium concentration in seed and SPAD chlorophyll value responded to fertilizer treatments and the maximum protein percentage (32.5%) was obtained at N1F1 treatment. This treatment had no significant difference with N1F0, N2F0 and N0F1 treatments. The Fe and N fertilization treatments had no significant effect on Fe, Zn, Ca, Na, Cu and P concentration of soybean seed.

Key words: Fe and N fertilizers, seed mineral elements, soybean, yield.

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most important legume crops in the world (Ibrahim and Kandil, 2007). Throughout history, legumes have been used for supply of food, fodder, fuel and traditional medicine (Howieson et al., 2008). Protein of soybean seed contains amino acids required for human nutrition and livestock (Raei et al., 2008). For optimum plant growth, nutrients must be balanced and should be sufficient for plant, or in other words the soil must have nutrients that is needed for plants (Chen, 2006).

Biological N_2 fixation and mineral soil or nitrogen fertilizer are the main source of meeting the nitrogen (N) requirement of high-yielding soybean (Salvagiottiet et al., 2008). The mineral nutrition of crops can be supplemented with fertilizer application to soils or foliage (Mallarino et al., 2001). Fertilization with N, phosphorus (P), potassium (K) and other nutrients can affect yield and many physiological processes, which in turn could influence grain yield and protein concentration (Haq and Mallarion, 2005). The legumes are self-sufficient for nitrogen requirements derived from symbiotic nitrogen, but the high-yielding crops are difficult to sustain solely

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Abbreviations: N1, 50 kg N ha^{-1}; **N2,** 100 kg N ha^{-1}; **N1F1,** intraction effect of 50 kg N ha^{-1} and spray with Fe fertilizer; **N1F0,** intraction effect of 50 kg N ha^{-1} and non spray with Fe fertilizer; **N2F0,** intraction effect of 100 kg N ha^{-1} and non spray with Fe fertilizer; **N0F1,** 0 kgN ha^{-1} and spray with Fe fertilizer.

Table 1. Some of physical and chemical characteristics of experimental field soil.

Parameter	Value
Soil texture	Sandy loam
pH	7.9
EC (dSm^{-1})	0.61
N (%)	0.21
P ($mg\ Kg^{-1}$)	5.14
K ($mg\ Kg^{-1}$)	0.83
Zn ($mg\ Kg^{-1}$)	5.56
Fe ($mg\ Kg^{-1}$)	12.2
Cu ($mg\ Kg^{-1}$)	2.3

on biological N_2 fixation (Cheema and Ahmad, 2000). So soybean requires a large amount of N for seed production and hence its yield may be sensitive to N fertilization after flowering (Kinugasa et al., 2011). However, the active period of N_2 fixation is limited during nodule development because nodule senescence occurs rapidly after flowering and during seed maturation (Vauclare et al., 2010). Nitrogen fertilizer applied during soybean reproductive stage (R1 to R5) might increase the capacity and duration of the inorganic N utilization period while maintaining N_2 fixation (Barker and Sawyer, 2005). Supplying N to the soybean plant during peak seed demand may supplement existing N resources, thus preventing premature senescent and increasing seed yield (Freeborn et al., 2001).

It is well documented that the deficiency of micronutrients in soil of arid and semi arid regions limits yield and can greatly disturb plant yield and quality (Eisa et al., 2011). Iron (Fe) nutrient element is the most limiting to agricultural production throughout the world, and plants require a continuous supply of Fe to maintain proper growth (Wiersma, 2005). Fe deficiency chlorosis is a nutritional disorder characterized by a significant decrease of chlorophyll in the leaves, which is often observed in plants grown on alkaline and calcareous soils (Schenkeveld et al., 2008). Fe deficiency has the negative effect on nitrogenase activity and N_2 fixation by soybean (Caliskan et al., 2008). Synthesis of chlorophyll, thylakoid and many ferrous proteins depends on this element (Kabraee et al., 2011). The effect of folair iron application has been inconsistent, being successful at some location in reducing a sign of chlorosis in soybean, and increasing yield in some cases (Me liesch, 2011).

Field studies measuring soybean response to applied N and Fe have been conducted by several researchers. Gan et al. (2003) showed that application N at 50 kg ha^{-1} at either the V2 or R1 stages, significantly increased N accumulation and yield. However, N at the same rate at either R3 or R5 stages did not show the positive effect in soybean grain yield. Barker and Sawyer (2005) also showed that N application increased N concentration in R4 (full pod) soybean plants. Caliskan et al. (2008) reported that Fe fertilization increased growth parameters and seed yield at R4 and R6 stages. Furthermore, application of nitrogen as a starter fertilizer and top-dressing of N at later stages increased growth parameters and seed yield. They consulted that application of N in combination with Fe fertilization can be beneficial to improve early growth and final yield of soybean. Moreover, Chakerolhosseini et al. (2003) showed that application of Fe up to 2.5 mg Kg^{-1} increased dry matter but decreased it at higher rates, while the concentration and uptake of Fe increased by Fe application.

Considering the importance of soybean as one of the most important legume crops in the world, it seems that the study of the yield and yield components of soybean, and the possible changes of oil and protein percentage by nutritional management so as to improve the yield and quality of product, as well as efforts to provide food is essential. Therefore, the objective of this field experiment was to study the effect of N fertilizer applied to the soil at R4 and foliar spray, and the Fe fertilizer effects at the R1 and R3 stages on soybean grain yield and the quality.

MATERIALS AND METHODS

Field experiments

This experiment was conducted at the Research Farm Kurdistan University of Sanandaj, Iran, to study the effect of N fertilizer and foliar fertilization with Fe fertilizer during the growing season of 2009. The area is located at latitude of 35°15 N and longitude of 47°1 E at an altitude of 1300 m above the mean sea level. Soil samples were taken from the upper 15 cm layer of the soil profile of each plot and were analyzed for physical and chemical characteristics for fertilizers recommendation. Some of the soil physico-chemical properties of experimental field are presented in Table 1. The treatments were arranged in factorial expriment based on a completely randomized block design with three replications. Treatments consisted of three nitrogen fertilizer rates (0, 50 and 100 kg N ha^{-1}) and two Fe fertilizers (spray with Fe fertilizer and non spray with Fe fertilizer). Nitrogen was supplied in the form of urea and was added to plants at R4 stage, while Fe fertilizer Fe-chelate (EDDHA Fe 6% chelated) was used as foliar spray in two periods at R1 (beginning flower) and R3 (beginning pod) stages. Foliar treatments were applied by a backpack sprayer.

After land preparation plowing, disking and ridging the plots were done, soybean (*G. max* L. Merr.) cultivar Williams seeds were sown on the 1st of June in 2009. Before sowing, the seeds were inoculated with *Bradyrhizobium japonicum* to promote N_2 fixation. The size of each plot was 20 m^2 (8 × 2.5 m), consisting of five rows with 60 cm between rows and 8 cm between plant on rows. To avoid the effects of plots adjacent, the distance between plots was considered 1.5 m.

Yield and yield components

Soybean grain was harvested from three central rows of each plot at September in 2009. After harvesting, the grain yield and yield components were determined for all plots. The following data were recorded: weight of 1000 seeds, number of seeds per plant, weight of seeds per plant, weight of pods per plant, seed yield per ha. SPAD chlorophyll value was measured with a Minolta SPAD-502 meter at the early flowering. The chlorophyll meter was shielded from direct sunlight by the operator during measurement. 16 leaves were chosen to measure SPAD chlorophyll value from each plot and averaged to a single SPAD value per plot (Wu et al., 2007).

The nitrogen concentration in seeds was measured with micro-Kjeldahl method as described by Peach and Tracey (1956) and the seed protein content was determined by multiplying the nitrogen percentage and protein factor using the following formula (Breese, 1931):

Protein percentage = Nitrogen percentage × 5.71.

Potassium and phosphorous were determined using a fame photometer and spectrophotometry methods, respectively as described by Skroch et al. (1999). The concentration of micronutrients was determined by an atomic absorption spectrophotometry (Walsh, 1971). The protein yield was calculated by multiplication of seed yield and seed protein percentage (Akbari et al., 2008).

Statistical analysis

The data were analyzed using the Statistical Analysis System (SAS) software package. Comparisons of all means were done at the 5% probability level based on Duncan's method. Graphs were generated using Excel software.

RESULTS

Chlorophyll

The chlorophyll meter values (SPAD values) of soybean leaf was influenced by N fertilizer treatment. Fe foliar application and interaction of Fe and N fertilizers had no significant effect on SPAD values (Table 2). The result in Table 4 shows that the SPAD values in leaves of soybean increased significantly ($p < 0.01$) by nitrogen application. However, no significant difference was found between two treatments of application 50 and 100 kg N ha^{-1}.

Yield and yield components

The results indicate that the N fertilizer treatments had a significant effect on seed yield and number of seeds per plant. However, there were no significant difference in the application of N fertilizer treatments on the 1000 seeds weight and weight of pods per plant. Fe foliar application and interaction between Fe and N fertilizer had no significant effect on yield and yield components (Table 2). The treatments of N2 (328 g m^{-2}) and N1 (303 g m^{-2}) led to maximum seed yield, and the minimum seed yield (192 g m^{-2}) was obtained from control treatment N0. The maximum seed number per plant (128 seeds) obtained from N2 treatment showed that this treatment had no significant differences from N1 treatment (Table 4).

Protein percentage of seed

Analysis of variance showed statically significant differences between N fertilizer application levels and also significant effects for interaction of Fe and N fertilizer usage on the protein percentage (Table 2). The F_1N_1 treatment indicated maximum amount of protein (32.5%) and nitrogen. This treatment had no significant different with F_1N_0, F_0N_1 and F_0N_2 treatments. The lowest protein and nitrogen concentration (25%) was obtained from F_0N_0 treatment (Figure 1).

Protein yield

The protein yield was influenced from N fertilizer treatments. However, Fe foliar application and interaction between Fe and N fertilizer had no significant effect on protein yield (Table 2). The highest protein yield was obtained from the N2 (97 g m^{-2}) and N1 (96 g m^{-2}) treatments, and the least protein yield (53 g m^{-2}) obtained from control treatment (Table 4).

Mineral elements

Results indicate that the treatments of Fe fertilizer had a significant effect on the absorption of potassium by plant (Table 3). The maximum concentration of soybean seed potassium (10 mg/100 g) was obtained from application of foliar Fe treatment (Table 5). Results also indicate that N fertilizer treatments had significant effect on the Mn cocentration of seeds (Table 3). The maximum Mn concentration (85.5 mg/100 g) were recorded at 100 kg urea in ha (Table 5). In addition, there were no significant effect from application of N fertilizer and spraying Fe, and interaction between fertilizers' usage on Zn, Ca, Na, Cu, Fe and P concentration of soybean seed (Table 3).

DISCUSSION

From these results, we can generally conclude that increasing nitrogen fertilizer level significantly increased seed yield and number of seed per plant than that of zero level. Soybean has a relatively high N requirement, particularly during the seed filing period and biological N fixation may not supply sufficient amount of N for the crop requirement. Therefore, N application during reproductive stages can be beneficial to improve yield (Caliskan et al., 2008). The nitrogen shortage accelerated the aging process in vegetative organs such as leaves, which are known as photosynthesizing organs, and this led to soybean yield increased (Gohari and Noorhosseiny Niyaki, 2010). Meanwhile, there was no significant interaction among the studied treatments on soybean yield. Achakzai and Bangulzai (2006) showed that pod yield, number of pods per plant and pod lengths of pea significantly increased with a progressive increase in application N fertilizer. Gohari and Niyaki (2010) reported

Table 2. Analysis of variance of SPAD chlorophyll value, yield and yield components in respons to different levels of N and Fe fertilizers application in soybean plant.

Source of variation	Mean square							
	Df.	Seed yield (g m^{-2})	Weight of pods per plant (g)	1000 seeds weight (g)	Number of seeds per plant	SPAD cholorophyll value	Protein (%)	Protein yield (g m^{-2})
R	2	1660^{ns}	63.8^{ns}	268^{ns}	282^{ns}	27.3**	3.05^{ns}	54.2^{ns}
Fe	1	8520^{ns}	6.64^{ns}	268^{ns}	2020^{ns}	11.4^{ns}	15.8^{ns}	1082.5^{ns}
N	2	31500*	47.8^{ns}	22.1^{ns}	4200*	47.7**	18.5*	3717.33*
Fe*N	2	3250^{ns}	51.4^{ns}	221^{ns}	331^{ns}	0.38^{ns}	36.6*	343^{ns}
Error	10	724	57.3	133	989	3.26	3.4	686.5
CV %		31.0	35.1	8.86	30.0	5.06	6.16	31.95

^{Ns}, * , **Not significant and significant at P < 0.05 and P < 0.01, respectively. R, Reproductive stage; CV, coefficient of variation.

Table 3. Analysis of variance of seed mineral elements in respons to different levels of N and Fe fertilizers application in soybean plant.

Source of variation	Mean square								
	Df.	K	P	Mn	Fe	Zn	Ca	Na	Cu
R	2	1.1^{ns}	0.029^{ns}	882.3*	0.01^{ns}	0.36*	0.22^{ns}	0.002^{ns}	28.4^{ns}
Fe	1	74**	0.0008^{ns}	574.1^{ns}	0.25^{ns}	0.00001^{ns}	0.22^{ns}	0.002^{ns}	1.6^{ns}
N	2	4.7^{ns}	0.077^{ns}	1051*	0.6^{ns}	0.067^{ns}	0.55^{ns}	0.023^{ns}	21.9^{ns}
Fe*N	2	11.8^{ns}	0.12^{ns}	344.6^{ns}	0.05^{ns}	0.21^{ns}	0.02^{ns}	0.012^{ns}	3.003^{ns}
Error	10	4.84.5	0.04	139.7	0.2	0.061	0.3	0.29	8
CV %		26.81	8.97	16.81	21.23	12.91	17.2	17.14	17.05

^{ns}, *, **Not significant and significant at P < 0.05 and P < 0.01, respectively. R, Reproductive stage; CV, coefficient of variation.

Table 4. The effects of Fe and N fertilizers on yield, components yield, protein and the SPAD chlorophyll value in soybean plant.

Treatment	Seed yield (g m^{-2})	Weight of pods per plant (g)	1000 seeds weight (g)	Number of seeds per plant	SPAD cholorophyll value	Protein yield (g m^{-2})
N fertilizer						
N0 (0 kg N ha^{-1})	192^{b}	18.5^{a}	123a	76^{b}	32.5^{b}	53^{b}
N1 (50 kg N $ha^{-1)}$	303^{a}	24a	132a	110^{ab}	37^{a}	96^{a}
N2 (100 kg N ha^{-1})	328^{a}	22^{a}	134.5a	128^{a}	37.5^{a}	97^{a}
Fe fertilizer						
F0 (non spray with Fe fertilizer)	252.3^{a}	22^{a}	131a	94^{a}	35^{a}	74^{a}
F1 (spray with Fe fertilizer)	296^{a}	21^{a}	129a	115^{a}	36.5^{a}	90^{a}

Each value is the mean of three replicates (Duncan's test, P ≤ 0.05).

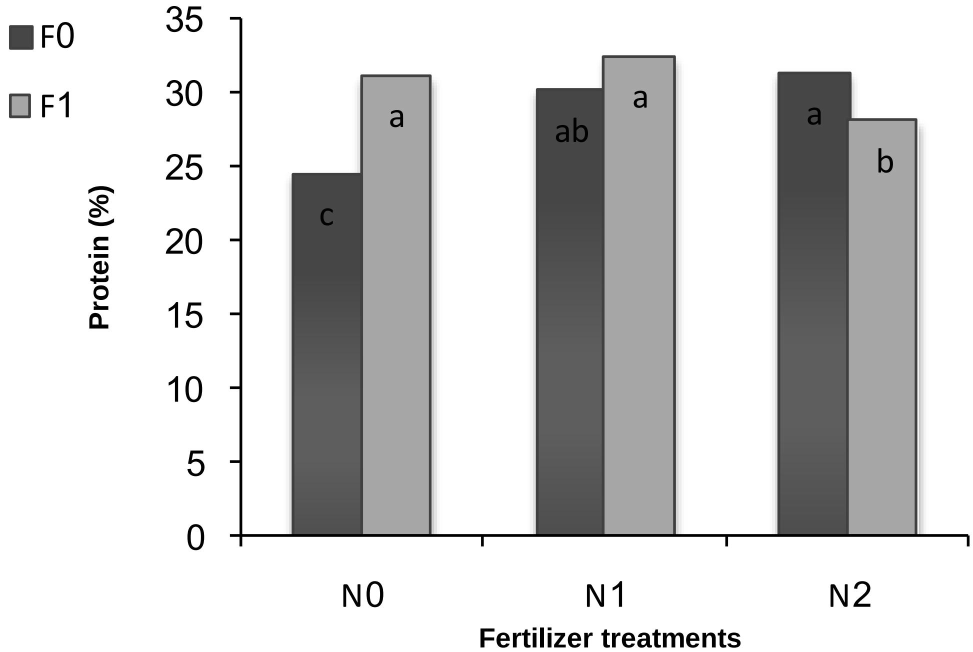

Figure 1. The effects of foliar application of Fe and N on the concentration protein in the seed of soybean plant. Means with one common letter have no significant difference (P ≤ 0.05). F0, Non spray with Fe fertilizer; F1, spray with Fe fertilizer; N0, 0 kg N ha^{-1}; N1, 50 kg N ha^{-1}; N2, 100 kg N ha^{-1}.

Table 5. The effects of Fe and N fertilizers on seed mineral elements in soybean plant.

Treatment	K (mg/100 g)	P (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Ca (mg/100 g)	Na (mg/100 g)	Mn (mg/100 g)
N fertilizer							
N0 (0 kg N ha^{-1})	8.5^a	2.6^a	4^a	142^a	3^a	9^a	62.5^b
N1 (50 kg N $ha^{-1)}$	7^a	2.5^a	5^a	108^a	2.8^a	12.5^a	62.5^b
N2 (100 kg N ha^{-1})	9^a	2.5^a	5.5^a	89.5^a	3.5^a	10^a	85.5^a
Fe fertilizer							
F0 (non spray with Fe fertilizer)	6.8^b	2.4^a	4^a	115^a	3.3^a	10^a	65^a
F1 (spray with Fe fertilizer)	10^a	2.3^a	5^a	111.3^a	3^a	11^a	76^a

Each value is the mean of three replicates (Duncan's test, *P* ≤ 0.05).

that application 4.5 kg ha^{-1} Fe fertilizer and 60 kg ha^{-1} N fertilizer increased seed yield of peanut, but no intereaction was seen between the N and Fe fertilizer and seed yield. Albareda et al. (2009) also reported that application of N fertilizer to inoculated soybean plants did not increase seed yield in comparison with treatments that were only inoculated.

Chlorophyll meter reading values with N application increased in comparison to the zero level. Nitrogen is the major part of the chlorophyll molecule (Silva and Uchida, 2000; Mahmoud, 2010). This result may indicate the role of nitrogen on synthesis of chlorophyll. Rashid et al. (2004) reported that lower chlorophyll meter reading values were observed for N deficient plots and higher chlorophyll meter reading values were observed for N sufficient plots. Aminifard et al. (2010) showed that the lowest leaf chlorophyll content was obtained by control treatment. However, no significant difference was found between three treatments 50, 100 and 150 kg N ha^{-1}. The results of this study indicate that application N alone and the usage with Fe foliar had a significant effect on the protein present in soybean seed. Therefore, there was a positive and significant relationship between the level of nitrogen applied and its mean of concentration in the plant.

The increase in the protein is an expected result to the successive increase in nitrogen level in response to urea fertilizer. Nitrogen is an important macronutrient for plants, and it is essential for the synthesis of protein (Ralf Winter, 2010). Al-shaikh (2004) reported that the application of N fertilizer had a higher significant effect on crude protein of a leguminous herb. Protein yield was obtained by multiplying two factors (seed yield and protein percentage). Protein yield has a direct relation with both factors of N_2 and N_1 treatments, because high seed yield and protein percent had the highest protein yield per unit area, compared to control treatments. Control treatment with a minimum amount of seed yield has produced minimum protein yield per unit area. The best way to achieve high- protein production in plants is increasing their seed yield.

In additon, the results of this investigation indicated that foliar fertilization of soybean with Fe did not affect the yield production and nutrient elements concentration of soybean seed, except for K concentration, which showed a positive relationship and with application of Fe, its concentration increased. Lack of response to Fe applications could originate from sufficient Fe levels in the soil or incorrect timing of Fe application. Kobraee et al. (2011) showed that the effect of iron on leaf zinc concentration of soybean was not significant, but increased iron concentration in grain and leaf. Results of this study indicate that with application of nitrogen fertilizer, Mn concentration increased. Nitrogen fertilizer might promote the Mn absorption of roots and translocation of Mn from root to shoot of plant (Hu-Lin et al., 2007), leading to an increase of Mn in seed of soybean. Other researchers also believe that one of the impacts of nitrogen increase; is the increase of cations absorption, which is due to the increase of the plant metabolic activity, acceleration in most of processes and increase of the plant absorption power. (Adeairan and Bonjorko, 1995).

Our results are in agreement with those previously found by Alizadeh and Nadian (2010)). In their study, they evaluated the effect of water stress and nitrogen rates on the amount of absorption of some macro and micro elements in corn plant mycorrhiza and non-mycorrhiza, and stated that the impact of nitrogen treatment on absorbing micro elements like iron was significant in the level of 1%; while its impact on copper and zinc was not significant. Furthermore, Zeidan et al. (2006) reported that Mn concentration in leaves of maize increased with increasing application rates of nitrogen fertilizer.

Conclusion

Soybean requires a large amount of N for seed production, however, N_2 fixation may not supply sufficient amount of N for the crop requirement. Thus, nitrogen fertilizer application has a great potential to increase yield and protein of soybean seeds. Also foliar fertilization of soybean with iron resulted in very small and infrequent trait increase.

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