Assessment Agro-Physiological Traits Response of Green Bean to Different Level of Nitrogen, Potassium and Zinc Fertilizers

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ABSTRACT

Nutritional management is an important factor in the success of crop production. In order to evaluate different level of nitrogen, potassium and zinc fertilizers on agro physiological characteristics of green bean a field experiment was conducted as split-plot based randomized complete block design in three replications, in Varamin university field research during 2011. The main plot included urea fertilizer at three levels (n_1=250, n_2=300, n_3=350 kg.ha^{-1}). The sub plots consisted b_1 (120 kg K.ha^{-1} from potassium sulfate), b_2 (Zn: foliar application of zinc solution with concentration 6 gr.l^{-1}) and b_3 (K+Zn =120 kg K.ha^{-1} and zinc foliar application with concentration of 6 gr.l^{-1}). The results showed that nitrogen uptake increased green bean yield. The interaction between nitrogen level and K and Zn applications was observed that the highest nitrate uptake was obtained that N_3B_3 treatment. The highest radiation use efficiency (RUE) and chlorophyll were obtained from 350 kg.ha^{-1} urea with application of (K*Zn fertilizers) treatment. The highest pod yield (4306.2 kg.ha^{-1}) was presented by application of 250 kg.ha^{-1} urea with K and Zn fertilizers, however the lowest pod yield (2298 kg.ha^{-1}) was observed at 350 kg.ha^{-1} urea with K. The results of this experiment showed that the Zn-foliar application increased all features in bean and also, reduced nitrate fertilizer rate without reduction in more plant characteristics.

Keywords*: Growth index, Nitrate, Radiation use efficiency.*

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INTRODUCTION

Seed legumes, as a protein-rich food, play an important role in human nutrition, especially in developing countries. Alone, they contribute up to 33% of the dietary protein needs of humans (Vance, 2002). *Phaseolus vulgaris* is a warm season annual legume crop grown primarily for its protein and energy-rich dry seeds. Bean grains are a good source of iron and zinc (Buruchara, 2011) and have a low glycemic index (Widers, 2006). *Phaseolus vulgaris* are composed of 50% of the grain a legume consumed worldwide and as sources of protein in the diet is the most important grain legume in the world for direct human consumption (Broughton *et al.,* 2003). Environmental factors such as low soil nitrogen and phosphorus, drought and acid soil conditions are important limitations for bean production in most of the bean grown areas (Graham *et al.*, 2003). In the developing countries, leguminous crops serve as the major source of protein in the diet. Green bean (*Phaseolus vulgaris* L.), also known as the snake bean, is a warm-season annual legume crop grown for their edible fresh pods. In northern Iran, it is usually grown during the warmer months from spring to early autumn. Worldwide, green beans is produced on 1.5 million ha, with an estimated of total production of 20.7 million tons in 2012 (FAO, 2012). Bean with 20-25% protein has as equal as meat protein alternative to meat (Costa *et al.*, 2006) which is very important for developing countries (France *et al.*, 2000). When legumes are in rotation with other crops, they can improve soil fertility and reduce the spread of weeds, pests and diseases (Lopez-Blido *et al.*, 2005). Foliar fertilization is an effective practice for the application of some micronutrients, since it uses low rates and the micronutrient does not directly contact the soil, avoiding losses through fixation (Volkweiss, 1991). Foliar fertilization has the advantage of low application rates, uniform distribution of fertilizer materials and quick responses to applied nutrients. Moreover, hidden hungers can easily be managed (Umer *et al.*, 1999). Zinc deficient soils have been widely found in India, USA, Canada, New Zealand, Africa, Europe and South America. On the other hand, World Health Organization (WHO) reported that human population of developing countries faced with the deficiencies of zinc. Zn deficiency of human is the fifth major cause of diseases and deaths in these countries (WHO, 2002). The role of micronutrients such as zinc involved from very simple to very complex reactions. Zn plays a very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase and stabilization of ribosomal proteins (Tisdale, 1984). Zinc activates the plant enzymes by carbohydrate metabolism, maintaining the integrity of cellular membranes, protein synthesis and regulation of auxin synthesis (Marschner, 1995). Also, Zn is required for regulation and maintenance of the gene expression to induce tolerance of environmental stresses in plants (Cakmak, 2000). Nitrogen is one of the fundamental compounds in nutrition of the plants. If there are enough water and food for plant, then the light is the only factor that effects on qualitative and quantitative properties of the crop (Zimdahl, 2006). One of the objectives of agricultural management is maximum utilization of solar energy by the canopy. It was evidenced that the application of N-fertilizer affects chlorophyll content and then it cause an increase in RUE (Pilbram *et al.*, 2009). On the other hand, the maximum energy from light absorption and RUE cause the

highest photosynthesis, and therefore biomass production and yield increase by penetrating light into different canopy layers (Haverkort, 2007). Majd Nasiri and Ahmadi (2005) showed that plants produce dry matter by sunlight absorption and store it in themselves during their vegetative stages. Lecocu and Ney (2008) proved that there was a linear relationship between the total dry matter production and photosynthetic active radiation (PAR) that the slope of that line was defined RUE. There are many factors affected RUE including nitrogen uptake, nitrate accumulation in the plant tissues, the amount of chlorophyll, leaf area index (LAI), crop growth rate. The application of plant nutrients specially nitrogen, potassium and zinc affect the plants shoot characteristics such as leaf size, leaves direction and the ageing process of lower leaves that they cause an increase the light absorption by plants (Weiner *et al.*, 2008). High application of N-fertilizer (especially in winter cultivation) increase nitrate accumulation in plant tissues (Mare, 2001). It announced that there was a positive relationship between LAI and RUE. An increase in LAI per area unit causes a decrease in the rate of extinction coefficient but an increase in RUE (Richards, 2004). It reported that the RUE for navy bean is between 0.8-1.05 gr.Mj^{-1} (Morrison *et al.*, 2009) and it is between 0.8-0.96 gr.Mj^{-1} for vetch (Ramberg *et al.*, 2008). Honeycutt *et al.* (2006) showed that yield of wax bean were strongly influenced by the nitrogen application. It is reported that plants accumulate nitrate in their tissues when sulfur deficiency is suffering plants or available nitrogen increase. Nitrate accumulation in tissues of green bean occurs by absorption and transfer of this component. In the young plant, nitrate accumulates in stems and petioles (Caslo, 2003). Ni-

trogen is a major nutrient element, which provides lush green color to the plant because of increase in chlorophyll concentration. It also plays a key role in plant growth and protein synthesis, protoplasm, cell size and photosynthetic activity and thus provides a frame on which more flowers and pods are produced (Yasari and Patwardhan, 2006). Nitrogen (N) fertilizer played a significant role in in-crease of crop yield (Modhej *et al.*, 2008). Nitrogen deficiency generally results in stunted growth and chlorotic leaves caused by poor assimilate formation that leads to premature flowering and shortening of the growth cycle (Lincoln and Edvardo, 2006). Limitation of nitrogen in any phase of the plant growth, causes reduction in yield (Mohammadian, 2002). Mahady (1990) found that foliar application of ZnSO$_4$ for faba bean plants increased number of pods/plant and seed yield. Ali and Mowafy (2003) reported that application of foliar spray with Zn (2%) slightly improved groundnut yield and it's attributed as well as quality. Thalooth *et al.* (2005) indicated that foliar spraying with Zn had a positive effect on yield and yield attributes of sunflower plants. Salehin and Rahman (2012) by evaluated effects of zinc and nitrogen fertilizer and their application method on yield and yield components of *Phaseolus vulgaris* L. reported the highest seed yield (1996 kg.ha^{-1}) was obtained by zinc spray application in amount 1 g.L^{-1}, also use of 90 kg.ha^{-1} pure nitrogen produced highest seed yield. This study has been conducted to increase radiation use efficiency (RUE) with optimization the application of nitrogen fertilizer in green bean and decrease nitrate accumulation in crop. Therefore the results of this study can provide a safe agricultural yield and prevent environment from nitrate pollution.

MATERIALS AND METHODS
Field and Treatment Information

In order to evaluate different level of nitrogen, potassium and zinc fertilizers on agro physiological traits of green bean a field experiment was conducted as split-plot based randomized complete block design in three replications, in Varamin university field research during 2011. The main plot included urea fertilizer at three levels (n_1=250, n_2=300, n_3=350 kg.ha^{-1}). The sub plots consisted b_1 (120 kg K.ha^{-1} from potassium sulfate), b_2 (Zn: foliar application of zinc solution with concentration 6 gr.l^{-1}) and b_3 (K+Zn =120 kg K.ha^{-1} and zinc foliar application with concentration of 6 gr.l^{-1}). The place of experiment is located in geographic coordinates of 39 and 51 degree along east and 19 and 35 degree north latitude at an altitude of 898 meters above sea level. The plot area was 15 m^2 including 6 ridges, each with 5 meters long and 3 meters width and plants were 20 × 50 cm^2 apart. All the potassium and phosphor fertilizers and 1/3 nitrogen fertilizer was applied during planting and the remaining nitrogen fertilizer was applied two times as excess based on the phonological stages of the plant. The distribution time of excessive fertilizer was after observing fifth leaf on the stem. According to the results of soil analysis, foliar application of micro nutrients was done at vegetative growth and early flowering stages.

Measured Traits

Shoot height from soil surface was measured. Five samples of green leaves from each treatment were collected for Chlorophyll measurement. The extinction coefficient (k) was calculated by the received daily light by canopy surface (I_0) and latitude according to Nassiri and Kropft (1997). The extinction coefficient (K) was calculated by the following equation Based on Floyed et al. (2007): **Equ. 1.** $I_i/I_0=e^{-kl}$

Where (I_0): active photosynthetic radiation in upper part of the plant, (I_i): active photosynthetic radiation in "i" layer of leaves, K: extinction coefficient or reduction of radiation, e: base of natural logarithm that is equal to 2.71827 and L: leaf area index in "i" layer. Dry matter (DM) and the accumulative radiation (PAR$_{absorbed}$) were used to measure the radiation use efficiency (RUE) by following formula (Monteith, 1997):

Equ. 2. DM = RUE* PAR$_{adsorbed}$

The leaf area was measured immediately in the samples by using the portable LAI meter, model IM-300.

Statistical Analysis

The data was analyzed by MSTATC software. The differences between averages of treatments were compared by Duncan test at 5% probability level.

RESULTS AND DISCUSSION
Fresh pod yield

Result of ANOVA revealed effect of main factor and sub factor on fresh pod yield was significant at 5% probability level, also interaction effect of treatments was significant at 1% probability level (Table 1). According result of mean comparison maximum of fresh pod yield (4306.20 kg.ha^{-1}) was obtained for N_1B_3 and minimum of that (2298.60 kg.ha^{-1}) was for N_3B_1 treatment (Table 2). The first condition for achieving a high yield per unit area is high dry matter production because about 90 percent of the dry weight of plants results from CO_2 assimilation during photosynthesis. The results showed Nitrogen treatments had the lowest fresh pod yield because the number of flowers formed in the treatments of high nitrogen and without zinc

or potassium application had significant decrease therefore the process of flowering and pod was reduced in these treatments. N_1B_3 treatment with suitable environmental conditions lead to increase fresh pod yield by sugar production which resulted from photosynthesis and transferring them to seeds and pod, although the treated plants by N_2B_3 had the suitable environmental conditions at flowering and graining stage. Therefore the produced assimilates was used for vegetative growth and thus the production of assimilation and fresh pod yield was reduced, so it was classified in to the second group.

Table 1. Analysis of variance of measured traits

S.O.V	df	Fresh pod yield	Radiation use efficiency	Extinction coefficient	Chlorophyll	Plant height	Nitrate concentration of pod
Block	2	3499.864^*	0.00026^{ns}	0.00001^{ns}	0.0008^{ns}	825.86^*	0.00007^{ns}
Factor A	2	23452.845^*	0.00121^{ns}	0.00031^*	0.0092^{ns}	449.92^*	0.00281^*
Error I	6	1085.266	0.00078	0.00002^{ns}	0.0041	332.87	0.00047
Factor B	2	11489.206^*	0.00334^{ns}	0.00303^*	0.0122^{ns}	3070.18^*	0.00321^*
A*B	4	86029.146^{**}	0.0245^*	0.00048^*	0.0687^*	3842.2^*	0.00993^{**}
Error II	18	1459071	0.00103	0.00025	0.0097	555.45	0.00098
CV (%)	-	15.56	9.87	12.07	10.87	14.49	9.83

ns, * and **: non-significant, significant at 5% and 1% probability level, respectively.

Table 2. Means comparison interaction effect of treatments on measured traits

Treatment		Fresh pod yield (kg.ha^{-1})	Radiation use efficiency (gr.mj^{-1}.m^{-2})	Extinction coefficient	Chlorophyll content (µg.gr^{-1})	Plant height (cm)	Nitrate concentration of pod (mg.kg^{-1})
N_1	B_1	3716.70^{*bc}	1.9269^c	0.5501^a	42.2^b	40^c	104.3^c
	B_2	4027.60^{ab}	1.9311^b	0.5489^b	43.2^b	46^b	99.7^{cd}
	B_3	4306.20^a	1.9468^b	0.5368^{bc}	43.6^{ab}	52^{ab}	76.4^d
N_2	B_1	2796.30^d	1.9342^b	0.5462^b	43.2^b	42^b	139.7^{bc}
	B_2	3461.80^c	1.9582^b	0.5396^{bc}	43.7^{ab}	53^{ab}	121.3^c
	B_3	3794.20^b	2.0521^{ab}	0.5301^{bc}	44.1^{ab}	54^{ab}	100.8^{cd}
N_3	B_1	2298.60^f	1.9642^b	0.5411^b	43.9^{ab}	38^b	185.4^a
	B_2	2438.30^e	2.0967^{ab}	0.5389^{bc}	44.6^{ab}	54^{ab}	157.9^b
	B_3	2827.11^d	2.2111^a	0.5296^c	45.3^a	55^a	135.6^{bc}

*Means in each column by a similar letter are not significantly different at 5% probability level via Duncan test.
N_1=250, N_2=300, N_3=350 kg.ha^{-1}.
B_1=120 kg K.ha^{-1} from potassium sulfate), B_2= Zn: foliar application of zinc solution with concentration 6 gr.l^{-1}), B_3= K+Zn =120 kg K.ha^{-1} and zinc foliar application with concentration of 6 gr.l^{-1}.

Radiation Use Efficiency (RUE)

RUE is the slope of the curve line of dry matter and accumulative radiation. According result of analysis of variance effect of main factor and sub factor on RUE was not significant but interaction effect of treatments was significant at 5% probability level (Table 1). The results showed that the highest and the lowest RUE (2.2111 and 1.9269 g.MJ^{-1}.m^{-2}, respectively) was obtained by $N_{3*}B_3$ and N_1B_1 treatments, respectively (Table 2). One of the objectives of agricultural management is maximum utilization of solar energy by the canopy (Haverkort, 2007). The results showed that, LAI increased in N_1B_3 and this led to increase in the light absorption and then an increase was obtained in dry matter production per unit area (Fig.1). The positive effect of nitrogen on chlorophyll content was recorded by Pilbram *et al.* (2009).
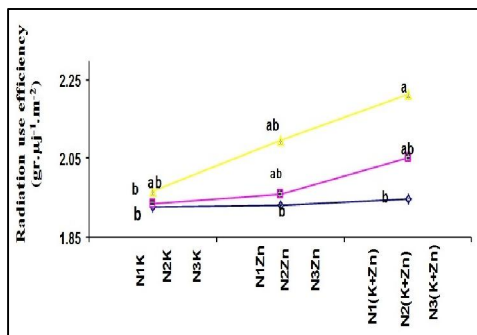
Fig. 1. Interaction effects of N, K and Zn application on Radiation use efficiency via Duncan test at 5% probability level.

The effect of nitrogen and micronutrients on the canopy structure has been proved by changing the shoot arrangement such as leaf size, leaf orientation and aging of the lower leaves of biomass and then they caused an increase of the canopy light absorption (Weiner *et al.*, 2008; Bange and Sinclair, 2007). Leaves shadow limits the optical processes therefore the available carbon dioxide is decreased and electron transfer is reduced as a result of carbon dioxide limitations and it is limited power of assimilation, in this case RUE is reduced as is evident in some treatments of this study.

Extinction coefficient
Result of analysis of variance revealed effect of main factor, sub factor and interaction effect of treatments on extinction coefficient was significant at 5% probability level (Table 1). According mean comparison result of treatments the maximum extinction coefficient (0.5501) was observed in N_1B_1 and the lowest one (0.5296) was found in N_3B_3 treatments (Table 2). Plants can reach their maximum photosynthetic capacity with the whole environmental light. If plants are in the shadows, their growth rate decreases (Radosevich, 1997). The upper leaves of canopy receive the light more than utilization capacity.

On the other hand, the light is prevented to the lower strata of canopy. Thus the rate of extinction coefficient increases (Floyed *et al.*, 2007). It showed that extinction coefficient decreased when LAI and the use efficiency of N-fertilizer increased. The biomass increased at N_3 treatment because the light within the canopy was distributed more than another treatment and then it caused a reduction of extinction coefficient in this treatment. It announced a negative relationship between two factors of LAI and extinction coefficient (Majd nasiri and Ahmadi, 2005). This is consistent with the results of this study.

Chlorophyll content
The results of variance analysis showed that the simple effects of treatments on chlorophyll content were not significant but interaction effect of treatments was significant at 5% probability level (Table 1). That result showed chlorophyll content decreased from 45.3 mg.g^{-1} ($N_{3*}B_3$ treatment) to 42.2 mg.g^{-1} (N_1B_1 treatment) for fresh leaves (Table 2). The changes in chlorophyll concentration are due to ability of plants to maintain the source of power in environmental conditions; it was observed that Chlorophyll content is one of the key factors in determining the rate of photosynthesis and production of dry matter (Ghosh *et al.*, 2004). The results of researches of Honeycutt and Trusty (2006) indicated that wax bean yield is strongly affected by nitrogen nutrition. Also the application of nitrogen associated with potassium and zinc foliar use cause an increase in chlorophyll content. Main factors which affected chlorophyll content are nitrogen and iron concentrations; chlorophyll is being made in enough nitrogen concentration. Plant photosynthesis is increased by an increase in chlorophyll and it will lead to increase in yield.

The highest level of chlorophyll was obtained by $N_{3*}B_3$ treatment because of the important role of nitrogen in production and activity of vegetative pigments, especially chlorophyll and direct effects of zinc and potassium on production of chlorophyll.

Plant height

Result of analysis of variance revealed effect of main factor, sub factor and interaction effect of treatments on plant height was significant at 5% probability level (Table 1). According mean comparison result of treatments maximum plant height (55 cm) was observed in N_3B_3 and the lowest one (40 cm) was found in N_1B_1 treatments (Table 2).

Nitrate concentration of pod

Result of analysis of variance revealed effect of main factor and sub factor on nitrate concentration of pod was significant at 5% probability level, also interaction effect of treatments was significant at 1% probability level (Table 1). According mean comparison result of treatments the maximum nitrate concentration of pod (185.4 mg.kg^{-1}) was observed in N_3B_1 and the lowest one (76.4 mg.kg^{-1}) was found in N_1B_3 treatments (Table 2). Many scientists reported the effect of sources and amounts of nitrogen fertilizer and micronutrients in the reduction of nitrate accumulation in the plant (Krauss, 1999; Wei Hong, 2003; Martin and Anac, 2006). The reduction of nitrate to nitrite and ultimately hydroxyl amine is affected nitrate and nitrite reeducates enzymes when these processes are activated effectively by micronutrients such as zinc, iron, sulfur and copper. The effect of these components on decrease of nitrate accumulation in vegetations has been reported by Kheir *et al.* (1999). Also the period of vegetative growth has become longer in N_3K_1 treatment with higher nitrogen application, and the plant has entered its reproductive phase later and thus its flowering and pod has coincided with hot season, function of enzymes was disrupted by environmental temperature and thus nitrate in the treatment above-mentioned increased significantly.

LAI

LAI indicates the amount of photosynthesis. When LAI decreases according to various factors, assimilate rate is reduced, so the amount of yield decreases and in this study it was evident that it corresponds to result of researches Chaillou *et al.* (2003). Fig. 2 showed a trend of changes in LAI at different levels of nitrogen application. The maximum LAI was at N_3 treatment. The treated plant root by 350 kg N.ha^{-1} could be developed better than other treatments, therefore the LAI showed an increase at this treatment. Of course, there was a slightly decrease in RUE; it might be due to the leaves shadow on lower one. Fig. 3 showed a trend of changes in LAI when the plants were treated by potassium and zinc application. The maximum LAI was found in the flowering stage therefore it caused to increase photosynthetic materials. Furthermore the leave development was stopped at flowering stage and the old leaves gradually became yellow and then LAI was decreased. In the beginning of the growth stage, LAI of green beans was approximately measured up to the third leaflet of plant (V_4 section of growth stage) and after that the plant were exposed to potassium and zinc treatment. These treatments were applied at reproductive stage of green bean plants. Therefore these treatments affected the photosynthetic materials and then it increased filling the grains in pod.
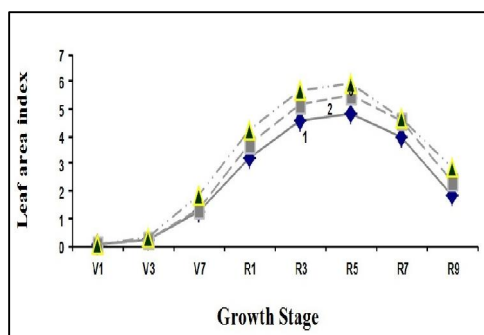
Fig. 2. Response of LAI (Leaf area index) to different level of nitrogen.
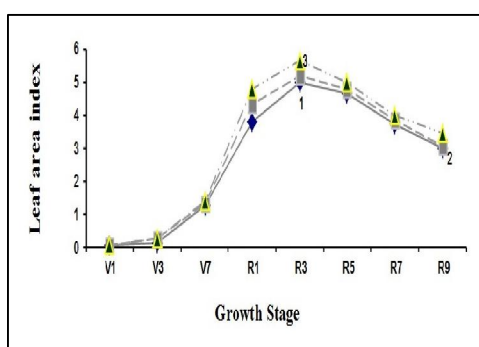N_1=250, N_2=300, N_3=350 kg.ha^{-1}.

Fig. 3. Response of LAI (Leaf area index) to different level of N, K and Zn.
1= K, 2= Zn, 3= K+Zn.

It can be concluded that green bean was treated by potassium and zinc had the maximum LAI and the highest yield compared to the untreated plant by potassium and zinc.

CONCLUSION

A significant interaction between the application of nitrogen, potassium and zinc with together was observed on measured parameters except extinction coefficient at 5% level. The highest plant height was recorded 55 cm at N_3B_3. It had 30.9 percent more than that the lowest plant height by 38 cm at N_3B_1 treatment. The plants which were treated by N_3B_3 had more new leaves on the top of stem than other treatments. It caused an increase in plant height and light efficiency. The results showed that the radiation absorption decreased when

there were coating agents of nitrogen and potassium and zinc. The photosynthetic efficiency coefficient of these treatments showed a strong reduction and the vegetative growth and then rates of plant height decreased. On the other hand, Leaf Area Index (LAI) increased at N_3B_3 treatment and then the receiving of the light source caused etiolating of the stem. It means that the solar radiation cannot be adsorbed enough because of the leaves shadow on older leaves then it causes a decrease in the photosynthesis efficiency. Finally these caused a decrease in yield and RUE.

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