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Nitrogen changes in the leaves and accumulation of some minerals in the seeds of red, white and Chitti beans (*Phaseolus vulgaris*) under water deficit conditions

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Abstract

Field studies were carried out using split-plot experiments in randomized complete block design with four replications. The objective of the studies was to determine the nitrogen (N) contents in the leaves and seeds in addition with some other mineral nutrients in the seeds of common bean (*Phaseolus vulgaris* L.) genotypes grown under contrasting water regimes. Two levels of irrigation (irrigation after 55-60 and 100-110 mm evaporation from class A pan as normal and water stress conditions, respectively) and eight genotypes of beans including Chitti (COS16, KS21486, and MCD4011), red (Akhtar, AND1007, and D81083) and white (WA4502-1 and WA4531-17) were investigated in main- and sub-plots, respectively. Total N content of leaves at two growth stages (pre-flowering and seed filling period) and total contents of seed iron (Fe), zinc (Zn), phosphorous (P), nitrogen and protein were measured at harvesting. Analysis of variance indicated significant differences between genotypes in the studied traits. Irrigation regimes had significant effect on all traits except to seed N and protein contents. The results indicated that white beans had lower leaf N and seed protein contents than red and Chitti beans under both irrigation regimes. Under drought condition, AND1007 and COS16 showed significantly ($p \leq 0.05$) higher levels of leaf N (1.88 and 1.83 in vegetative stage and 0.72 and 0.73 in R8 stage, respectively). Also, seed protein in water stressed plants was higher in Chitti beans. Water deficit reduced the mean leaf N by 19% and mean grain yield by 52.7% in all genotypes. Water deficiency significantly reduced seed Fe, Zn, and P contents, but the impact of drought on Fe and P contents was more than on Zn and N contents. Under stress condition, Chitti beans showed the lowest decrease (16.5%) in their seeds iron contents. Genotypes AND1007, COS16, MCD4011 and WA4502-1 were classified as efficient water users based on grain yield efficiency index (GYEI). Overall, genotypes that produce high grain yield under stress conditions and respond well to irrigation are the most desirable because they are able to express their high yield potential in a wide range of water availability.

Keywords: bean, drought, iron, nitrogen, phosphorous, yield, zinc.

Abbreviations: BNF- biological nitrogen fixation; E- efficient water user; ENR- efficient and nonresponsive; ER- efficient and responsive; GHI- grain yield harvest index; GYEI- grain yield efficiency index; IE- inefficient water user; IENR- inefficient and nonresponsive; IER- inefficient and responsive; ME- moderately efficient water user.

Introduction

In many developing countries, beans provide high amount of available protein. For example, beans consumption per capita in Eastern and Southern Africa is 40-50 kg year^{-1} (Blair et al., 2010; Mwale et al., 2009). These crops are good sources of proteins, vitamins, and minerals such as Fe, Zn, P, Ca, Cu, K, and Mg, and are excellent sources of complex carbohydrates (Camacho Barron and Gonzalez de Mejia, 1998). Approximately two-thirds of common bean production in the world occurs under drought conditions (Beebe et al., 2008; Serraj and Sinclair, 1998). It was reported (Frahm et al., 2004) that mild to high drought stress reduces plant growth, seed yield and quality of dry bean. Furthermore, it was reported that drought stress reduced accumulation of seed reserves between 8% and 12%. For example, there was a general decreasing trend in total soluble proteins in all plant tissues due to water deficit (Castaneda-Saucedo et al., 2009). Minerals accumulation in the seeds have essential role in crop production. Nitrogen (N), a key element, is essential component of proteins and nucleic acids in plants (Sanchez et al., 2005). Nitrogen fixation activity of common bean is generally low and sensitive to soil drying, consequently drought stress has major negative effect on its N accumulation and grain yield. Furthermore, drought reduces N partitioning and fixation (Serraj and Sinclair, 1998) and plant and seed uptake and utilization of nutrients (Dos Santos et al., 2004; Munoz-Perea et al., 2005). De Souza et al. (1997) studied the effect of water deficit on leaf characteristics and concluded that severe drought stress accelerated leaf senescence by reducing leaf nitrogen (N) and chlorophyll contents. As Rosado et al. (1992) mentioned about 40% of iron (Fe) intake in developing countries is derived from cereals and legumes. Fe

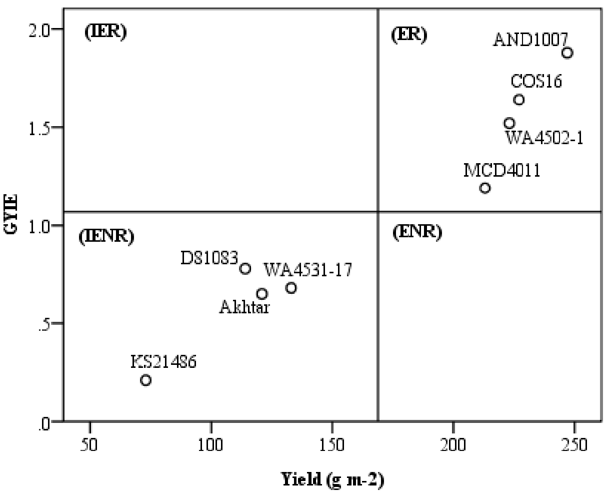

Fig 1. Classification of bean genotypes for irrigation efficiency (ENR- efficient and nonresponsive; ER- efficient and responsive; IENR- inefficient and nonresponsive; IER- inefficient and responsive).

plays important roles in the metabolism of chlorophylls (Sultana et al., 2001). Moreover, it enters many enzymes that play important roles in oxidoredox reactions of photosynthesis and respiration (Ghasemian et al., 2010), therefore high content of Fe increases photosynthesis, net assimilation and relative growth in the stressed plants (Sultana et al., 2001). Zinc (Zn) has a major role as a metal component of the enzymes or as a functional, structural or regulatory cofactor of many enzymes (Ghasemian et al., 2010; Grotz and Guerinot, 2006), plays an important role in biomass production (Cakmak, 2008) and in protein and carbohydrate synthesis and takes part in metabolism regulation of saccharides, nucleic acids and lipids (Ghasemian et al., 2010). High seed-Zn plays major physiological roles during seed germination and seedling growth (Cakmak, 2008). Cakmak (2008) and Sultana et al. (2001) stated that zinc and iron nutrition can affect the susceptibility of crops to water deficit. Phosphorus (P) is another essential element for crop growth and development. It is a component of many cell ingredients and plays an important role in several physiological processes such as photosynthesis, respiration, photosynthetic energy production and carbohydrate transport, and cell division and enlargement (Dos Santos et al., 2004; Gutierrez-Rodriguez et al., 2006). Also, P is necessary for seed formation and is a fundamental element for nodule metabolism in legumes (Gutierrez-Rodriguez et al., 2006) and is required to regulate the activity of several proteins through phosphorylation reactions (Martinez-Ballesta et al., 2010). Drought stress has a long-term effect on P uptake and accumulation by crops (Dos Santos et al., 2004), particularly for a poor Pi extractor such as common bean (Fageria et al., 1997). Grain yield harvest index (GHI) and grain yield efficiency index (GYEI) in crop genotypes are influenced by nature of genotype and environmental factors and appears to be a good index in differentiating dry bean genotypes for their ability to produce grain yield and indicators of their efficiency for P use at differing soil P levels. The GYEI has been successfully used to differentiate genotypes into efficient and inefficient nutrients (N, P, K, micronutrients) utilizations in rice (Fageria et al., 2010). For importance of mineral nutrients gained by grain crops such as beans in human health, the objective of present study was the evaluation of nitrogen changes in the leaves and accumulation of Zn, Fe, P, and N in the seeds of three common bean groups (Chitti, red, and white) under contrasting moisture regimes, and the classification of genotypes based on grain yield and irrigation efficiency.

Results

Leaf nitrogen contents

According to the analysis of variance (data not shown), leaf N content at the vegetative (V4-R5) and reproductive (R8) growth stages were significantly influenced by water regimes. At both growth stages, white beans had lower leaf N contents under both water regimes (Table 1). Reduction of leaf N content due to water deficit was greater in the R8 stage (Table 3).

Seed mineral nutrient contents

Water deficiency significantly decreased Fe, Zn, and P, but had not significant effect on N and consequently seed protein was not significantly affected. Also, seed Fe, Zn, P, and N contents of the evaluated genotypes showed significant differences under both irrigation regimes. Water deficit caused a significant reduction in all the seed elements contents. According to the data in Table 1, the Fe content of the seeds was severely affected under drought stress, while the seed nitrogen contents were affected the least under this condition. On the other hand, the impact of drought on iron and phosphorus contents was more than on zinc and nitrogen. As shown in Table 3, the amounts of Fe and Zn in the grains of studied genotypes were 61-80.5 mg kg^{-1} dry matter and 21.8-33.5 mg kg^{-1} dry matter in the well watered plants, respectively. Also, the amounts of seed phosphorus and nitrogen contents varied from 3.30-4.55 g kg^{-1} dry matter and 2.98-3.30 percentages, respectively. The highest decrease in the iron content of the plants in the stressed plots was found in the Akhtar genotype. Under stress condition, Chitti beans showed the lowest decrease in their seeds iron contents. White beans had lower seed N and protein contents than red and Chitti beans under both water regimes but their seeds phosphorus concentrations were higher under drought condition. Seed N and protein contents were not that much affected by drought stress. Although the greatest decrease in the iron content was found in the Akhtar seeds, the changes in its other reserves were insignificant. Under drought stress, KS21486 genotype with a minimum decrease in its seed iron content showed substantial changes in its other elements, especially phosphorus.

Grain yield and GYEI

Significant genotypic differences were observed under both normal and stress conditions on grain yield per plant and per area (m^2). One of the Chitti bean genotypes, KS21486 showed the lowest grain yield under both irrigation conditions (Table 2). In this study, grain yield per plant showed lower decreases due to drought stress than yield per m^2. Water deficit resulted

Table 1. The mean values of different traits of genotypes under normal (N) and water stress (S) conditions.

Treatment	Leaf N		Seed					Yield (g plant^{-1})	Yield (g m^{-2})
	pre-flowering (%)	R8 (%)	N (%)	protein (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	P (g kg^{-1})		
N	2.08 a	0.84 a	3.12 a	19.5 a	70.56 a	28.06 a	3.98 a	12.9 a	318 a
S	1.69 b	0.60 b	2.95 b	18.1 b	56.94 b	23.63 b	3.28 b	7.8 b	152 b
Change (%)	18.8	25.6	5.4	6.9	19.3	15.8	17.6	39.8	52.7

Table 2. Leaf nitrogen content at two growth stages, seed protein, and yield per plant and per m^2 under normal (N) and water stress (S) conditions and grain yield efficient index (GYEI) of genotypes.

Genotype	Leaf N at pre-flowering (%)			Leaf N at R8 (%)			Seed protein (%)			Grain yield (g plant^{-1})			Grain yield (g m^{-2})			GYEI
	N	S	PR	N	S	PR	N	S	PR	N	S	PR	N	S	PR	
COS16	2.25 a	1.88 a	16.4	0.90 a	0.72 a	20.0	20.6 a	19.1 a	7.6	13.1 cd	9.7 bc	25.5	389 a	227 a	41.6	1.64
KS21486	2.00 bc	1.60 de	20.0	0.83 bc	0.54 c	34.9	19.9 ab	18.6 ab	6.3	5.8 f	3.2 f	43.9	151 c	73 c	51.7	0.21
MCD4011	2.15 ab	1.70 bc	20.9	0.85 ab	0.63 b	25.9	19.7 bc	18.8 ab	4.8	9.1 e	7.0 d	22.3	299 b	213 b	28.8	1.19
Akhtar	2.15 ab	1.73 b	19.5	0.87 ab	0.66 b	24.1	19.1 cd	18.4 bc	3.3	12.3 d	5.3 e	56.6	290 b	121 b	58.3	0.65
AND1007	2.23 a	1.83 a	17.9	0.88 ab	0.73 a	17.0	19.4 bc	18.6 ab	3.8	23.1 a	10.7 ab	53.7	408 a	247 a	39.5	1.88
D81083	2.10 ab	1.65 cd	21.4	0.83 bc	0.54 c	34.9	19.9 ab	18.3 bc	7.2	8.0 e	5.7 e	28.1	368 a	114 bc	69.0	0.78
WA4502-1	1.85 d	1.60 de	13.5	0.78 d	0.51 c	34.6	18.9 cd	17.7 d	6.6	17.1 b	11.7 a	31.8	365 b	223 b	38.9	1.52
WA4531-17	1.95 cd	1.55 e	20.5	0.80 cd	0.51 c	36.3	18.6 d	18.0 cd	3.4	15.0 c	8.8 c	41.3	273 b	133 b	51.3	0.68

Different letters within each column indicate significant difference at $p \leq 0.05$.

Table 3. Seed mineral nutrient contents under normal (N) and water stress (S) conditions.

Genotype	Seed N (%)			Seed Fe (mg kg^{-1})			Seed Zn (mg kg^{-1})			Seed P (g kg^{-1})		
	N	S	PR	N	S	PR	N	S	PR	N	S	PR
COS16	3.30 a	3.05 a	7.6	79.0 a	65.0 a	17.7	33.5 a	29.5 a	11.9	3.70 bc	3.00 de	18.9
KS21486	3.18 ab	2.98 abc	6.3	61.0 c	51.5 c	15.6	22.0 c	17.8 d	19.1	4.45 a	3.40 bc	23.6
MCD4011	3.15 bc	3.00 ab	4.8	71.5 ab	60.0 ab	16.1	31.0 a	26.5 b	14.5	3.75 bc	3.15 cd	16.0
Akhtar	3.05 bcd	2.95 abc	3.3	80.5 a	61.0 a	24.2	31.8 a	27.0 ab	15.1	3.90 b	3.35 bcd	14.1
AND1007	3.10 bcd	2.98 abc	3.9	80.0 a	64.0 a	20.0	33.0 a	28.5 ab	13.6	3.30 c	2.75 e	16.7
D81083	3.18 ab	2.93 bcd	7.9	65.5 bc	54.0 bc	18.6	25.5 b	20.5 cd	19.6	3.75 bc	3.10 cde	17.3
WA4502-1	3.03 cd	2.83 d	6.6	61.0 c	49.5 c	18.9	21.8 c	18.5 cd	15.1	4.45 a	3. 58 b	19.6
WA4531-17	2.98 d	2.88 cd	3.4	66.0 bc	50.5 c	23.5	26.0 b	20.8 c	20.0	4.55 a	3.95 a	13.2

Different letters within each column indicate significant difference at $p \leq 0.05$.

39.8% and 52.7% reductions in grain yield per plant and per m^2, respectively. The yield reductions of the genotypes due to the water deficit were between 39 to 69%. The greatest effect of drought on yield reductions per plant was observed with one of the red beans (Akhtar) while yield reduction per m^2 was highest with another red bean (D81083). MCD4011 showed greater level of drought resistance with low value of percentage reduction in grain yield. According to results of the present study (Table 2), AND1007 and COS16 genotypes indicated the highest GYEI, respectively. AND1007 and COS16 as well as MCD4011 and WA4502-1 were classified as efficient water users. D81083, Akhtar and WA4531-17 were classified as moderately efficient water users and KS21486 was solely classified as inefficient water user.

Discussion

Improving genetic resistance of crops to drought stress has been a major challenge for plant breeders. Crop resistance to drought has been attributed to different mechanisms leading to different response types (Chaves et al., 2003). According to the results of this study, WA4502-1 showed the lowest amount of leaf nitrogen content at both the vegetative and reproductive stages, while the highest values of leaf N at these two growth stages was observed with COS16, indicating better ability of this genotype in acquiring N either from soil or from biological nitrogen fixation (BNF) and in remobilizing N under favorable water regime (normal) conditions. In general, white beans had lower contents of leaf N than the other two groups indicating their poor potential for BNF and N metabolism. For many plant species, a strong correlation has been reported between leaf N and CO_2 assimilation (Baker and Rosenqvist, 2004). In the present study, drought decreased the N accumulation in all genotypes, but this reduction was larger in drought sensitive genotypes. Also, reduction in the leaf N content was greater at R8 stage than at the vegetative stage. Our results indicated that under water deficit conditions, white beans had lower leaf N contents at seed filling period (R8) than the other two bean groups. Our results indicated that there was a general decreasing trend in total seed protein in all genotypes due to water deficit which was in agreement with the findings of Ashraf and Iram (2005). According to Fresneau et al. (2007), drought induces changes in a number of physiological and biochemical processes including inhibition of protein synthesis. It seems that reduction in seed protein contents is related to proline synthesis. Beebe et al. (2008) believed that proline accumulation may be associated with osmotic adjustment, resulting in inhibition of protein synthesis. Of the several biochemical indices of water deficit injury, proline accumulation and decline in protein synthesis have been reported in many plants (Ashraf and Iram, 2005). In fact, proline synthesis has been shown to be associated with protein hydrolysis caused by water deficit. According to our results, one of the most adaptable genotypes to drought stress is AND1007 which showed greater leaf N content at the seed filling period than the other genotypes. This genotype had the lowest reductions in leaf N content at reproductive stage (17%). So, it could be classified as drought resistant compared with other genotypes. COS16 with its highest values of leaf N at both growth stages (V4-R5 and R8) is also considered as drought resistant. This genotype also has a high capacity to acquire and remobilize N under both water regimes. Evaluation of leaf N changes between the vegetative stage (V4-R5) and the seed filling period (R8) revealed that the greatest reduction in the leaf N in normal (well watered) condition was observed with MCD4011, while under stress condition, the greatest and the lowest reduction in leaf N were observed with COS16 and WA4531-17, respectively. These results indicate that N remobilization from leaves was greater in these two genotypes (MCD4011 and COS16) under normal irrigation conditions, while under stress condition; it was greater with COS16 than the other genotypes. These results also suggest the high capability of COS16 for N remobilization to other sinks such as pods. Ramirez-Vallejo and Kelly (1998) found that under moderate water stress N partitioning was not impaired, but under severe stress N remobilization was reduced in common bean. It is well known that drought sensitive genotypes accumulate less N than drought resistant genotypes. Rosales-Serna et al. (2004) believed that capability of cultivar(s) in redistribution of stored assimilates to the seeds is an important trait for adaptation to drought stress. So, drought resistant cultivars may be more efficient in photo-assimilate production and translocation to the seeds. Remobilization of nutrients such as N and P from the vegetative tissues to the reproductive organs have fundamental role in legume grain yield. Common bean pods and seeds are major sinks for N and its allocation to seeds dominates the reproductive N budget (Araujo and Teixeira, 2008). As reported by Schiltz et al. (2005), the contribution of N remobilization for seed N demand is about 84% in common bean. Previous studies indicated that high performance of common bean genotypes under drought was associated with their ability to mobilize photosynthates toward developing seed and to utilize the acquired N more efficiently for seed production (Beebe et al., 2008; Polania et al., 2008). Under drought conditions, WA4502-1 showed the lowest reductions in leaf N content at the vegetative stage (13.5%). The greatest reductions in leaf N content at the vegetative stage (21.4%), and the lowest reduction in leaf N content at R8 stage (17%) were observed with D81083 and AND1007, respectively. Similar to the observations made by Singh (2007), we also found that drought reduced N partitioning and fixation. Our results showed that seed N and protein contents had the lowest reductions under drought stress. COS16 and D81083 showed the highest reductions in seed N under water deficit, indicating high sensitivity of N accumulation in the seeds of these genotypes to drought stress. The results of several studies showed that the seed compound concentrations in the legumes varied in response to genetic and environmental factors (Grusak, 2002). In most of these studies, significant interaction effects of genotype × location or genotype × year (Kigel, 1999) have been reported. Moreover, diversities exist between phenotypes regarding seed size, color and composition, reflecting high genetic diversity within the species (Coelho and Benedito, 2008). Seed quality in beans, including protein and mineral contents, are also significantly influenced by environmental factors. Our results showed that drought stress reduced the accumulation of iron, zinc, and phosphorous in bean plants. Researchers believed that drought reduces N partitioning and fixation (Singh, 2007), resulting in reductions in the rate of protein accumulation in the seeds. According to Serraj and Sinclair (1998), since common bean is generally sown in the soils subject to drought, high sensitivity to soil dehydration can have major limitations on N accumulation and potential yield. Teran and Singh (2002) also reported that drought stress reduced N harvest index and consequently seed N and protein contents in common bean. Fresneau et al. (2007) reported that drought effects are associated with changes in physiological and biochemical processes, including inhibition of protein synthesis. Riccardi et al. (1998) believed that induction of changes in proteins responding to drought plays a pivotal role in the adaptive mechanism of crops to stress. However, some studies have reported no effect of drought stress on protein levels (Kigel, 1999), and even increase in protein content based on seed dry weight under drought stress has been reported (Singh, 2007). In general, the mineral nutrient contents change when environmental limitations affect the crop growth and reduce biomass at harvest, accompanied by less dilution of nutrients on a dry mass basis (Martinez-Ballesta et al., 2010). Results of the present study showed that phosphorus, as a fundamental element in crops, is another mineral that is severely affected by water deficit. Under drought conditions, white beans had the highest amount of seed P, while the amount of their seed N and Fe contents were the lowest (Table 3). It was reported that water deficit has a direct effect on the stomatal and enzymatic apparatus as well as a long-term influence on uptake and accumulation of phosphorus by crops (Dos Santos et al., 2004), especially for common bean as a poor inorganic phosphorus (Pi) extractor (Fageria et al., 1997). In general, several studies (Graham et al., 1999; Blair et al., 2005) indicated genetic differences in seed mineral concentrations among the bean genotypes and landraces for micronutrients such as Zn and Fe and macronutrients such as P and Ca. Reduction of seed reserves by drought stress indicated that the iron content of seeds was the most affected (19.3%) mineral by water deficit. Among the genotypes, Akhtar seeds had the greatest reduction in iron, but its iron content was not differed significantly from those of COS16 and AND1007 genotypes. Grain yield as the most important trait was genetically differed under water stress in common bean (Teran and Singh, 2002). In the present experiment, average grain yield reduction due to water deficit was higher than 52%. Reductions in yield varied among the genotypes, indicating their different responses to drought conditions and their susceptibility to water deficit. According to the yield loss, AND1007, COS16, and MCD4011 were more drought tolerant than the other genotypes. Other researchers have reported yield reductions ranging from 47 to 69% under drought conditions (Munoz-Perea et al., 2006; Singh, 2007; Urrea et al., 2009). In our study, AND1007 had the highest plant yield in well watered treatments, but in the stressed plots, WA4502-1 showed higher yield per plant than the others. Water deficit reduced mean grain yield of all genotypes by 52.7% which varied between 69% (D81083) and 28.8% (MCD4011). Similarly, Singh (2007) and Teran and Singh (2002) found average yield reductions of 52% to 62% in the dry bean varieties under drought stress. Classification of genotypes for irrigation efficiency, based on GYEI and grain yield under stress conditions, indicated that genotypes were classified into four groups (Figure 1). This type of classification has been suggested for the nutrient-use efficiency of crop genotypes (Fageria et al., 2008). The first group was the efficient and responsive (ER) genotype. The genotypes that produced above average yield at stress level and higher than average grain yield efficiency index were classified in this group. Genotypes AND1007, COS16, WA4502-1 and MCD4011 fall into this group. The second group of genotypes is those that produce less than average yield at stress level and less than average response to irrigation. This type of genotype is classified as inefficient and nonresponsive (IENR). The genotypes that fall into this group are WA4531-17, D81083, Akhtar, and KS21486. Other two groups that had no members in the present study were efficient and nonresponsive (ENR) and inefficient and responsive (IER) genotypes. Genotype belonging to ENR group produce more than average yield of all genotypes at stress level, but response to irrigation is lower than the average. The genotypes that produce less than average grain yield of all genotypes at stress level but respond to irrigation above the average are classified in this group. Overall, the genotypes that fall into the ER group are the most desirable because these genotypes can produce more yields at stress level and also respond well to irrigation. The second most desirable group is ENR genotypes. Genotypes of this type can be sown under low irrigation level and produce more than average yield. The NER genotypes sometimes can be used in a breeding program for their irrigation-responsive characteristics. The most undesirable genotypes are the IENR type.

Materials and methods

Plant materials

Genotypes of three groups of common bean (*Phaseolus vulgaris* L.) (Chitti: COS16, KS21486, and MCD4011; red: Akhtar, AND1007, and D81083; white: WA4502-1 and WA4531-17) were evaluated under normal irrigation and water deficit conditions at the research farm of Seed and Plant Improvement Institute (SPII), Karaj, Iran. These genotypes belong to bush bean types and all of them are promising lines except to Akhtar as a traditional cultivar and KS21486 which is a very early mature line.

Irrigation regimes (normal and water stress)

Normal irrigation was based on 55-60 mm evaporation from class A pan, while water stress was induced after seedling establishment (from emergence of 3rd trifoliate leaf) to maturity, so plants watered same as normal conditions until the third trifoliate leaf was fully expanded and from then watered based on 100-110 mm evaporation from class A pan.

Experimental design and data collections

The study was designed as split-plot experiments in randomized complete block (RCB) with four replications in 2009 and 2010. Irrigation conditions and genotypes were in the main- and sub-plots, respectively. The experimental plots consisted of six rows of 5 m long and 0.5 m apart. The density of planting was 40 plants m^{-2} in each genotype. Fertilization, pest control, and common cultural practices were consistent with bean production in Karaj area. The following data were collected as described:

Grain yield efficiency index (GYEI)

Grain yield efficiency index (GYEI) was calculated to classify genotypes for their water use efficiency based on Fageria et al. (2010) procedure, applied for P-use efficiency, as follows:

$$\mathbf{GYEI} = \frac{\mathbf{Y_S}}{\overline{\overline{\mathbf{Y_S}}}} \times \frac{\mathbf{Y_N}}{\overline{\overline{\mathbf{Y_N}}}}$$

Where Y_S and Y_N are grain yield at stress and normal conditions; $\overline{Y}_S$ and $\overline{Y}_N$ are average grain yield of 8 genotypes at stress and normal conditions, respectively. Genotypes having GYEI values >1 were classified as efficient (E) water user, genotypes having GYEI values between 0.5 and 1 were classified as moderately efficient water user (ME) and those with GYEI values < 0.5 were classified as inefficient (IE) water users.

Measurement of mineral nutrients in the leaves and seeds

At two growth stages, pre-flowering (between V4 and R5 stages) and seed filling period (R8), five plants of each treatment and in each plant, three central leaflets were randomly collected. The leaves owen-dried at a temperature of 75^o C for 48 h and then total nitrogen content of samples was determined using the Kjeldahl method. At harvesting, 30 pods were collected from each treatment and separated seeds were rinsed in distilled water and then samples were dried at 75^o C for 48 h. Nitrogen content of the samples was also determined based on Kjeldahl method. Seed protein contents were determined using nitrogen values multiply by 6.25. Zinc and iron contents of samples were determined based on mg kg^{-1} dry matter using atomic absorption spectrophotometer and seed phosphorus level was determined based on g kg^{-1} dry matter using spectrophotometer according to Gelin et al. (2007) and Moraghan and Grafton (1999). Finally, the grain yield of each treatment was determined based on g per plant and per square meter.

Statistical analysis

Data were analyzed using a two-way analysis of variance (ANOVA) at a significance level of $P \leq 0.05$. Mean comparisons were done using the Duncan's multiple range test. Calculations were performed using SAS and SPSS software.

Conclusion

Comparisons among the genotypes revealed that white beans were more drought-susceptible than red and Chitti groups. According to our results, the highest mean grain yields per m^2 under both conditions were observed with AND1007 and COS16. According to results, increasing N contents in the leaves resulted in grain yield increases under drought stress. Besides, lower values of seed N and protein are associated with higher yields of genotypes. The responses of the studied genotypes to water stress were different in the uptake and accumulation of seed reserves. For enhancing quality value of common bean grains in regards to Fe and Zn concentrations, selection based on these minerals can lead to selection of genotypes containing low P levels. Those genotypes that had high levels of Fe and Zn can be used as gene sources in future breeding programs. Selecting genotypes capable of higher accumulation of Fe and Zn in the seeds could contribute to the improvement of micronutrient status of common bean, leading to improved people's health that is dependents on beans as the major portions of their diet. In general, AND1007 (red bean) and COS16 and MCD4011 (Chitti beans) were the best genotypes based on their grain productions and seed reserves under drought conditions. Also, these genotypes were classified as efficient water users based on GYEI.

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