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EFFECTS OF WATER STRESS ON LEAVES AND SEEDS OF BEAN (*Phaseolus vulgaris* L.)

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ABSTRACT

To determine the changes of nitrogen contents in the leaves and seeds of common bean (*Phaseolus vulgaris* L.) genotypes under contrasting moisture regimes, two field experiments were conducted as split-plot in a randomized complete block design with four replications. Two levels of irrigation (irrigation after 55-60 and 100-110 mm evaporation from class A pan, respectively) and eight genotypes including white beans (WA4502-1 and WA4531-17), red beans (Akhtar, D81083 and AND1007) and Chitti beans (KS21486, MCD4011 and COS16) were studied in the main- and sub-plots, respectively. Leaf nitrogen (N) and proline contents were measured at two growth stages (pre-flowering and pod filling period). Grain yield, seed N and seed protein contents were measured at harvest. 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 conditions, AND1007 and COS16 showed markedly higher levels of accumulation of leaf N and proline. Seed protein was higher in Chitti beans. Water deficit reduced the leaf N by 19% and 28% at two growth stages and grain yield by 39.8%. By contrast, proline content of all genotypes was increased by 105%. Seed N and protein contents had the lowest reductions under drought, while increasing N and proline contents in the leaves increased grain yield under this condition. Besides, lower values of seed N and protein is associated with higher yields of genotypes. Totally, based on the grain yield, red beans were more drought-susceptible than white and Chitti groups.

Keywords: Bean, drought, minerals, nitrogen, yield

INTRODUCTION

Beans are food legumes that are consumed by many people worldwide (Broughton et al., 2003). About 60% of the bean growing area in the tropics is affected by terminal or intermittent drought stress (Beebe et al., 2008). New common bean cultivars have been developed through selection and incorporation of various physiological, phenological and morphological characteristics that improve yield under drought conditions (Beaver et al., 2003). De Souza et al. (1997) studied the effect of water deficit on leaf characteristics and concluded that severe drought saccelerated leaf senescence by reducing leaf nitrogen (N) and chlorophyll contents.

Nitrogen is an essential nutrient in the plant production. For many plant species, a strong correlation was observed between leaf N and CO_2 assimilation (Baker and Rosenqvist, 2004). A large part of N in the plant is allocated to leaves and a large amount of leaf N is allocated to photosynthetic system. Photosynthetic activity is related to leaf N and the net photosynthetic rate increases with higher levels of leaf N. Generally, drought decreases leaf N content leading to a decrease in photosynthesis (Nakayama et al., 2007). Excessive production of different types of compatible solutes is a response of plants to drought and other stresses. Proline, as a solute, is widely distributed in plants which accumulate greater than the other amino acids in the stressed plants (Cardenas-Avila et al., 2006). Beebe et al. (2008) believed that proline accumulation may associate with osmotic adjustment resulting 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). According to Sanchez et al. (2007), there is a positive relationship between N availability and proline accumulation.

For importance of N to increase protein in the seeds of common bean, the main objective of the present study was to determine the relationships between grain yield and nitrogenous compounds in the leaves and the seeds of eight common bean genotypes, belonging to three groups: red, white and Chitti, under two contrasting moisture regimes.

MATERIALS AND METHODS

Three groups of common bean (*Phaseolus vulgaris* L.) genotypes consisted of red (Akhtar, AND1007, and D81083), white (WA4502-1 and WA4531-17), and Chitti (COS16, KS21486, and MCD4011) were evaluated under control (irrigation after 55-60 mm evaporation from class A pan) and drought (irrigation after 100-110 mm evaporation from class A pan) conditions at the research farm of Seed and Plant Improvement Institute (SPII), Karaj, Iran. Drought conditions were induced after seedling establishment (from emergence of 3rd trifoliate leaf) to maturity. Split-plot experiments were performed in a randomized complete block design (RCBD). Each year, four replications were used for each treatment and subsequently for each trait. Replications were combined doubles (1 with 3 and 2 with 4), so the number of replications was reduced to two occurrences per year. Replications of the first year and second year were analyzed together. The seeds were sown on 28 June 2009 and 13 June 2010. Irrigation treatments and genotypes were placed into the main- and sub-plots, respectively. Seeds of each genotype were sown at 6 rows of 5 m length with plant spacing 5 cm, separated by 50 cm rows. At two growth stages during crop development, pre-flowering (between V4 and R5 stages) and pod filling duration (R8), five plants of each treatment and in each plant three central leaflets were randomly selected for sampling for leaf N and protein. These leaves were dried at a temperature of 75°C for 48 h and then total N content of samples was determined using the Kjeldahl method. At the flowering (R6) stage, central leaflets were collected from top and middle parts of the plants in each treatment. These samples were transported to the laboratory in the liquid nitrogen and maintained at -80°C. The free proline content in leaf tissue was determined using spectrophotometer according to the method described by Bates et al. (1973). At the harvesting time, seeds were rinsed in distilled water and then N content of samples was determined using the Kjeldahl method. Seed protein content was determined using seed N value multiplied by 6.25. Finally, grain yield from each treatment was determined based on g per plant. Data were analyzed based on experimental design model. Means comparison was performed based on Duncan's multiple range test ($P \leq 0.05$). All statistical analyses were performed using SAS (version 9.1) and SPSS (version 16) software.

RESULTS

Leaf N in vegetative (pre-flowering) and reproductive (pod filling) growth stages was significantly influenced by water regimes. At the both growth stages, white beans had lower leaf N contents under both water regimes (Table 1).

Table 1. Leaf nitrogen and proline contents under control (N) and drought (S) conditions.

Genotypes	Leaf N at pre-flowering (%)		Leaf N at pod filling (%)		Leaf proline (µmol g^{-1} FW)	
	N	S	N	S	N	S
Akhtar	2.15 ab	1.73 b	0.87 ab	0.66 b	0.80 ab	1.60 ab
AND1007	2.23 a	1.83 a	0.88 ab	0.73 a	0.73 bc	1.73 a
D81083	2.10 abc	1.65 cd	0.83 bcd	0.54 c	0.63 c	1.55 ab
WA4502-1	1.85 d	1.60 de	0.78 d	0.51 c	0.88 a	1.25 c
WA4531-17	1.95 cd	1.55 e	0.80 cd	0.51 c	0.88 a	1.40 bc
COS16	2.25 a	1.88 a	0.90 a	0.72 a	0.65 c	1.78 a
KS21486	2.00 bcd	1.60 de	0.83 bcd	0.54 c	0.73 bc	1.45 bc
MCD4011	2.15 ab	1.70 bc	0.85 abc	0.63 b	0.73 bc	1.60 ab
Mean	2.08 a	1.69 b	0.84 a	0.60 b	0.75 b	1.54 a
Mean squares (MS)	Irrigation: 1.24 Error $_a$: 0.003 Genotype: 0.06 Irrigation×Genotype: 0.004 Error $_b$: 0.002		Irrigation: 0.44 Error $_a$: 0.003 Genotype: 0.01 Irrigation×Genotype: 0.003 Error $_b$: 0.001		Irrigation: 0.08 Error $_a$: 0.02 Genotype: 0.01 Irrigation×Genotype: 0.06 Error $_b$: 0.005	

Different letters within each column indicate significant difference at $p \leq 0.05$.
FW: fresh weight of leaves.

Reduction of leaf N content due to water deficit was greater in pod filling duration. Both water regimes significantly influenced the leaf free proline content. This amino acid increased greater than two fold in the stressed plants. In this study, under control conditions white beans had higher levels of leaf proline while these genotypes showed lower proline contents than red and Chitti groups under drought conditions (Table 1).

Seed N and protein contents were significantly affected by both water regimes. White beans had lower seed N and protein contents than red and Chitti beans under both water regimes. Seed N and protein contents were not that much affected by drought compared with the other traits. Significant genotypic differences were observed for grain yield under both control and drought conditions. One of the Chitti bean genotypes, KS21486 showed the lowest grain yield under both growing conditions. In this study, grain yield reduction due to water deficit was 39.8% (Table 2).

The greatest effect of drought on yield reductions per plant was observed with one of the red beans (Akhtar). One of the Chitti beans, MCD4011 showed greater level of drought resistance with low value of % reduction in grain yield (Table 3).

Table 2. Seed nitrogen and protein contents and grain yield under control (N) and drought (S) conditions.

Genotype	Seed N (%)		Seed protein (%)		Grain yield (g plant^{-1})	
	N	S	N	S	N	S
Akhtar	3.05 bcd	2.95 abc	19.1 bcd	18.4 abc	12.34 d	5.35 e
AND1007	3.10 bcd	2.98 abc	19.4 bcd	18.6 abc	23.15 a	10.71 ab
D81083	3.18 ab	2.93 bcd	19.9 ab	18.3 bcd	8.01 e	5.76 e
WA4502-1	3.03 cd	2.83 d	18.9 cd	17.7 d	17.14 b	11.69 a
WA4531-17	2.98 d	2.88 cd	18.6 d	18.0 cd	15.00 c	8.81 c
COS16	3.30 a	3.05 a	20.6 a	19.1 a	13.11 cd	9.77 bc
KS21486	3.18 ab	2.98 abc	19.9 ab	18.6 abc	5.80 f	3.25 f
MCD4011	3.15 bc	3.00 ab	19.7 bc	18.8 ab	9.12 e	7.09 d
Mean	3.12 a	2.95 b	19.49 a	18.14 b	12.96 a	7.80 b
Mean squares (MS)	Irrigation: 0.23 Error $_a$: 0.03 Genotype: 0.02 Irrigation×Genotype: 0.003 Error $_b$: 0.002		Irrigation: 0.23 Error $_a$: 0.14 Genotype: 1.08 Irrigation×Genotype: 0.14 Error $_b$: 0.10		Irrigation: 229.05 Error $_a$: 1.12 Genotype: 9.36 Irrigation×Genotype: 1.61 Error $_b$: 0.22	

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

Table 3. Reduction (%) of leaf and seed N, grain yield and increase (%) of leaf proline of genotypes induced by drought when the means of control treatment for the same traits were took into consideration 100%.

Genotype	Leaf N (pre-flowering)	Leaf N (pod filling)	Leaf proline	Seed protein	Yield per plant
Akhtar	19.5	24.1	100.0	3.3	56.6
AND1007	17.9	17.0	137.0	3.8	53.7
D81083	21.4	34.9	146.0	7.2	28.1
WA4502-1	13.5	34.6	42.0	6.6	31.8
WA4531-17	20.5	36.3	59.1	3.4	41.3
COS16	16.4	20.0	173.8	7.6	25.5
KS21486	20.0	34.9	98.6	6.3	43.9
MCD4011	20.9	25.9	119.2	4.8	22.3
Mean	18.8	28.5	105.3	6.9	39.8

DISCUSSION

Crops respond differently to environmental stresses such as drought. Improving genetic resistance of crops to drought 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 our results, WA4502-1 showed the lowest amount of leaf nitrogen content in both vegetative and reproductive stages while the highest values of leaf N in 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 (control) 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. A large amount of N in the plant is allocated to leaves and a large part of leaf N is invested in the photosynthetic system. Photosynthetic activity is related to leaf N and the photosynthetic rate increases with higher levels of leaf N (Nakayama et al., 2007). In the present study, drought decreased the N accumulation in all genotypes so that this reduction was larger in drought sensitive genotypes. Our results indicated that white beans had lower leaf N contents at pod filling period (R8) and low leaf proline contents than the other two bean groups under water deficit conditions. Sanchez et al. (2007) reported that the relationship between N availability and proline accumulation is usually positive. Given that proline accumulation is one of the mechanisms of crop resistance to stress conditions such as drought (Cardenas-Avila et al., 2006; Chaves et al., 2003), white bean genotypes are considered as drought-susceptible. Our results indicated that there was a general decreasing trend in total seed protein in all genotypes due to water deficit which is in agreement with 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 has been observed that increased amounts of free proline in wheat cultivars could be associated with more effective mechanisms of dehydration tolerance and drought avoidance. It was reported that in chickpea (*Cicer* *arietinum*) amino acid content increased under drought conditions apparently due to hydrolysis of proteins (Ashraf and Iram, 2005). Aranjuelo et al. (2011) found that water stressed plants could invest a large quantity of carbon and N resources into the synthesis of osmoregulants in the leaves such as proline for maintaining cell turgor. According to our results, one of the least susceptible genotypes to drought in refer to N content of leaves is AND1007 which showed greater leaf N content at the pod filling period than the other genotypes. This genotype had the lowest reductions in leaf N content at reproductive stage (17%). COS16 with its highest values of leaf N in both growth stages (V4-R5 and R8) and leaf proline content could be considered as drought resistant. This genotype has also high capacity to acquire and remobilize N under both water regimes. Evaluation of leaf N changes between vegetative stage (pre-flowering) and pod filling duration revealed that the greatest reduction in the leaf N in control conditions was observed with MCD4011 while under drought conditions, 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 MCD4011 and COS16 under control conditions while it was greater with COS16 than the other genotypes under drought conditions. Also, these results 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. Drought resistant cultivars may be more efficient in assimilate production and translocation to the seeds (Rosales-Serna et al., 2004). Nakayama et al. (2007) found decreased N accumulation in the leaves of studied cultivars under drought. It is well known that drought impairs the uptake of N in the plants. Also, drought sensitive genotypes accumulate less N than drought resistant genotypes. Previous studies indicated that high performance of common bean genotypes under drought was associated with their ability to mobilize photosynthates toward developing grain and to utilize the acquired N more efficiently for seed production (Beebe et al., 2008; Polania et al., 2008). According to Araujo and Teixeira (2008), remobilization of nutrients such as N from vegetative to reproductive organs plays a fundamental role in the legume grain yield. As shown by Schiltz et al. (2005), the contribution of N remobilization to seeds varies from 70% in peas, 43 to 94% in lentil, 80% in faba bean, and 84% in common bean. Common bean pods and seeds are major sinks for N and its allocation to seeds dominates the reproductive N budget (Araujo and Teixeira, 2008). Under drought conditions, WA4502-1 showed the lowest reductions in leaf N content at vegetative stage (13.5%). This genotype had also the lowest increase in leaf proline accumulation (42%). The greatest reductions in leaf N content at 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 conditions. 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. Grain yield is the most important trait in many studies. Genotypic differences based on grain yield have been reported for drought resistance in common bean (Teran and Singh, 2002). In our research, AND1007 had the highest grain yield in control treatments, but in the stressed plots WA4502-1 showed higher yield than the others. Water deficit reduced mean grain yield of all genotypes by 39.8% which varied between 56.6% (in Akhtar) and 22.3% (in MCD4011). Singh (2007) and Teran and Singh (2002) found average yield reductions of 52% to 62% in the dry bean varieties under drought conditions. According to results, increasing N and proline contents in the leaves resulted in grain yield increases under drought conditions. Besides, lower values of seed N and protein is associated with higher yields of genotypes. Our results indicated also that based on leaf proline, seed protein and grain yield, AND1007 and COS16 were identified as superior genotypes under drought (Fig. 1).

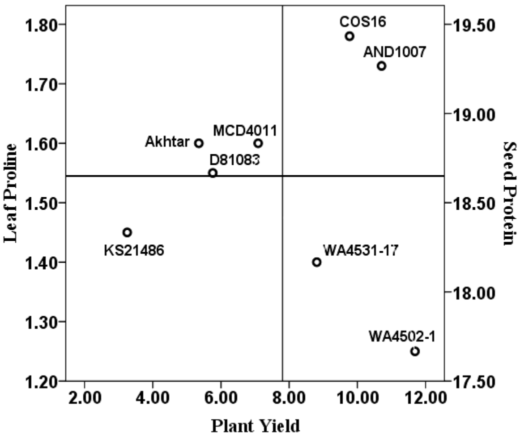

Figure 1. Trilateral relations (plant yield-leaf proline-seed protein) for bean genotypes under drought conditions.

In conclusion, comparisons among the genotypes revealed that white beans were more drought-susceptible than red and Chitti groups in the studied traits except to grain yield. According to our results, the highest mean grain yields under both conditions were observed with AND1007 and WA4502-1. Intra-grouping evaluations showed that WA4502-1 has a relatively better performance under drought when compared with the other white bean genotype. In red beans, AND1007 was superior to others and due to its other desirable attributes it could be a good candidate to introduce to drought-prone areas. In Chitti group, KS21486 is less preferable due to its small seed size and poor market potential while MCD4011 is considered as a promising genotype due to its good market potential for grain and its greater level of drought resistance based on small changes in grain yield under water deficit.

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