

Full Length Research Paper

Effect of drought stress on yield and yield components, relative leaf water content, proline and potassium ion accumulation in different white bean (*Phaseolus vulgaris* L.) genotype

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Genetic increase in bean yields in dry areas has not been as great as in more favorable environments. Plants with their ability to change morphologically and physiologically are able to continue their existence in regions where there is not enough rain and soil humidity is low. In order to assess some of these changes, the relationship between proline content, potassium ion and the relative water content with the yield and yield components of bean genotypes under drought stress, an experiment in formed split plot design in a randomized complete block design with three replicates was performed in the research field of the Azad Islamic university of Shiraz. The main plot consisted of irrigation surfaces (well-watered and drought stress) and the sub plot consisted of three white bean genotypes. Data were statistically analyzed with the help of computer facilities and Statistical Analysis System (SAS) program. The results revealed that the effect of irrigation was significant in most characteristics (except the weight of 100 grain and harvest index). A significant difference was seen between the lines regarding the total character (except biomass). The interaction of irrigation surfaces × genotype also showed a significant difference for characteristics such as number of pods in the plant, weight of 100 grain, harvest index, the seed yield of single plant and the potassium ion content. The obtained results showed that the accumulation of more potassium and proline in beans under drought can be a kind of adaptation for tolerating dryness, which can in turn help the plant to survive and reproduce under drought conditions.

Key words: Drought stress, white bean, relative water content, proline content, potassium ion.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food crop grown under rain-fed conditions in Iran where drought is a major limiting factor for production. When water loss through transpiration is more than the water absorption through soil, water stress occurs. Long-term

tension affects all metabolic processes and often leads to a decrease in production. The plant's survival in inappropriate environmental conditions depends on its ability to resist intense osmotic conditions caused by drought. Plants create two main mechanisms to confront drought stress; avoidance and water tension tolerance. Avoidance depends on the existence of specific adaptations in the root and stem structure and the plant's anatomy (Aspinal and Paleg, 1981). However, osmotic regulation is considered as a main component of tolerance to drought stress mechanism in plants (Zhang

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et al., 1999). According to Blam (1996), osmotic regulation is defined as the reduction of cellular sap potential as a result of increase in cellular solutions and not through the reduction of the amount of water in the cell. In different environmental conditions, plants accumulate some soluble material with low cellular weight, amino acids, sugars and some soluble mineral substances named adaptable solutions (Bajji et al., 2001). Adaptable solutions do not interfere with the cell's normal biochemical reactions and act as osmotic protectors during osmotic stress. These adaptable solutions do not only have the initial role of maintaining the tissues turgor, but can also help protein sustainability and cellular structure (Bartolz and Sanker, 2005). Among the known adaptable soluble substances, proline is possibly the most disseminated and its accumulation seems to help the plant to survive just after undergoing drought stress and re-establish growth after tension resolution. Hence, proline accumulation will probably have a positive effect on yield. In long-term tensions, the positive and advantageous effects of proline will not be activated and its accumulation will even have a negative effect on yield because photosynthesis resources will guide the plant towards processes other than seed filling (Sanchez et al., 1998).

The changes that occur in proline biosynthesis during tension include the hydrolysis of protein and the processes involved in their oxidative destruction which lead to proline accumulation in plants under tension (Sarker et al., 1999). The role of proline and its positive effects depends on the plant's structure and the intensity and duration of the stress. Also, the ultimate proline output regarding its stress tolerance depends on the plant's ability to rapidly stimulate proline accumulation in reaction to stress, its ability to rapidly produce high amounts of proline in the cell and the presence of an efficient system for controlling the stimulated and accumulated proline produced by stress. Mineral salts (such as potassium ion), are adaptable and osmotic which are accumulated under drought and can act as osmotic factors or osmotic protectors (Afkari et al., 2009). The positive effect of potassium on drought stress could be a result of root growth resulting in higher absorption of elements and water and also the reduction of water guttation (Umar, 2006). Moreover, potassium ion creates the cells' osmotic potential and turgor (Lindhower, 1985) and the regulations of activating stomas in drought stress conditions (Cant and Cafkafi, 2002), and these regulations are important in enhancing the products yield under drought stress. The positive effect of potassium in plants suffering from drought stress is attributed to high pH maintenance in the stoma and protection against photo-oxidative damage to the chloroplast. The water shortage of the plant can be determined by frequently measuring the water of the leaves or other parts of the plant. The water content at any determined time can be defined as the relative water content which is a percentage of the water content when saturated (Larcher,

1995). The relative water content is a better factor for the plant's water condition, compared to water potential (Sinclair and Ladlow, 1985). Since the relative water content is related to cell mass, it can reflect the balance between the amount of perspiration and the leaf's water more efficiently (Schonfeld et al., 1988).

Studying the aforementioned reactions in agronomic plants like beans in drought stress conditions, can be helpful in recognizing influential mechanisms in resistance to dryness. This study was performed in order to evaluate the effect of drought stress on changes in potassium ion content, proline content and relative water content and the effect of these osmolyte on the yield of white bean genotypes.

MATERIALS AND METHODS

Plant material and multiplication

This study was performed in the agronomic year 2008 and 2009 in the research farm of the Azad Islamic University of Shiraz using split plots in the form of complete random blocks and in three repetitions. The main factor consisted of different irrigation surfaces (well-watered and drought stress). In order to exert stress, irrigation was terminated in the 50% flowering period and sampling was performed in 30% of the field's capacity which was measured by a tensiometer. After taking samples, the plots were irrigated. Minor plot consisted of 3 genotypes including the Daneshkadeh sensitive genotype, the semi tolerant and Shekofa genotype and the tolerant genotype G11867, which were obtained from the national bean research center of Khomein. Each plot consisted of 3 cropping rows with a length of 2.5 m and the distance between each plant was 8 cm for all three genotypes. Between both minor plots, one non-cropping row and between both main plots 3 m distance was considered. The seeds were planted in June. All maintaining procedures (fertilization, irrigation and protecting the plant from weeds, pests and diseases) were undertaken when necessary. After harvest, characteristics related to yield and single plant yield were measured.

Free proline content determination

Proline was determined following Bates et al. (1973). Fresh plant material (1 to 0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate filtered. The filtrate (2 ml) was treated with 2 ml acid ninhydrin and 2 ml of glacial acetic acid, then with 4 ml of toluene. Absorbance of the colored solutions was read at 520 nm, with a spectrophotometer (UVD-2960 model, LaBoMeD, INC.).

Potassium content determination

The concentration of potassium ion was measured based on the Khosh Kholgh Sima method (1999) using by flame photometry (Jenway.pfp7 model) using KCl as the standard.

Water content measurement

The relative water content was estimated based on Barz and Wardley method (1962) and the relative water content (RWC) of leaves was calculated as: $RWC = 100 \times [(fresh\ mass - dry\ mass) / (saturated\ mass - dry\ mass)]$. Saturated mass was determined after

Table 1. Variance analysis of the characteristics.

S.O.V	Degree of freedom	Mean square					
		Number of pods/plant	Number of grain/pod	Pod length	Biomass	Yield of single plant grain	Harvest index
Replication	2	2.77	0.025	0.137	1299.51	40.16	77.28*
irrigation surfaces (A)	1	751.94**	2.39**	1.662*	38367.2**	3449.48**	2.51
Experimental error (Ea)	2	19.50	0.115	0.056	1340.91	15.63	8.52
Genotype	2	3733.80**	2.98**	6.284**	4737.29	3603.24**	121.66**
Irrigation surfaces × genotype	2	1052.60**	0.191	0.226	1.56	1321.18**	166.99**
Experimental error (Eb)	8	16.09	0.060	0.207	1345.79	49.33	9.99
Coefficient of variation	...	8.58	5.19	4.67	16.45	10.65	10.72

S.O.V	Degree of freedom	Mean square			
		Weight 100 grain	Relative water content	Potassium ion content	Proline content
Replication	2	1.72	0.525	126.59	0.020
irrigation surfaces (A)	1	19.71	1065.83**	112860.0**	0.436**
Experimental error (Ea)	2	0.760	3.79	293.54	0.008
Genotype	2	333.14*	43.88**	23833.19**	0.089**
Irrigation surfaces × genotype	2	84.00**	23.96	4960.07**	0.014
Experimental error (Eb)	8	6.72	8.71	187.85	0.008
Coefficient of variation	...	6.45	3.99	1.91	10.91

*, ** are significant at 0.05 and 0.01, respectively.

incubation of the leaf in water for 24 h at room temperature. Dry mass was measured following oven-drying at 75 °C to a constant mass.

Statistical analysis

Analysis of variance (ANOVA), using SAS software for windows and mean comparison procedures, was performed to Duncan's new multiple range test. (DNMRT) ($P < 0.05$). Excel software was used to draw graphs.

RESULTS AND DISCUSSION

Table 1 shows the variance analysis results of the different characteristics. Among agronomic

characteristics, except for weight of 100 grain and harvest index, all were influenced by drought stress. Since the studied bean genotypes had unlimited growth and the plots undergoing stress were irrigated after sampling, these two characteristics were less influenced by drought stress and do not have a significant difference as compared with the well-watered. Considering the difference between the genotypes regarding the assessed characteristics, although, irrigation surfaces influenced biomass, there was no significant difference between the genotypes. However, in other characteristics, a significant difference was seen among the different genotypes. The interaction irrigation surfaces and genotype

was not significant in other characteristics except the number of pods in the plant, weight of 100 grain, harvest index, seed yield and potassium ion content.

Grain yield and yield components

Drought stress resulted in the reduction of the number of grain in the pod and the length of the plant. The Daneshkadeh sensitive genotype, although, having the most pods in the plant, had a small number of grains in the pod (Figures 1 and 2). Water shortage in the flowering phase increases pollen abortion which is consistent with

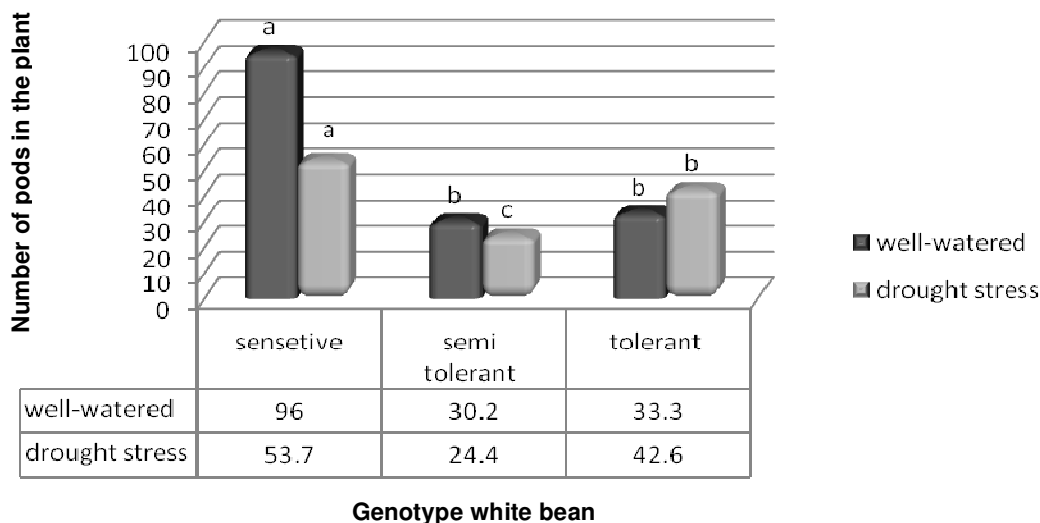


Figure 1. Comparing white bean genotypes regarding the number of pods in the plant. The numbers above the column are for comparing different genotypes in a specific condition.

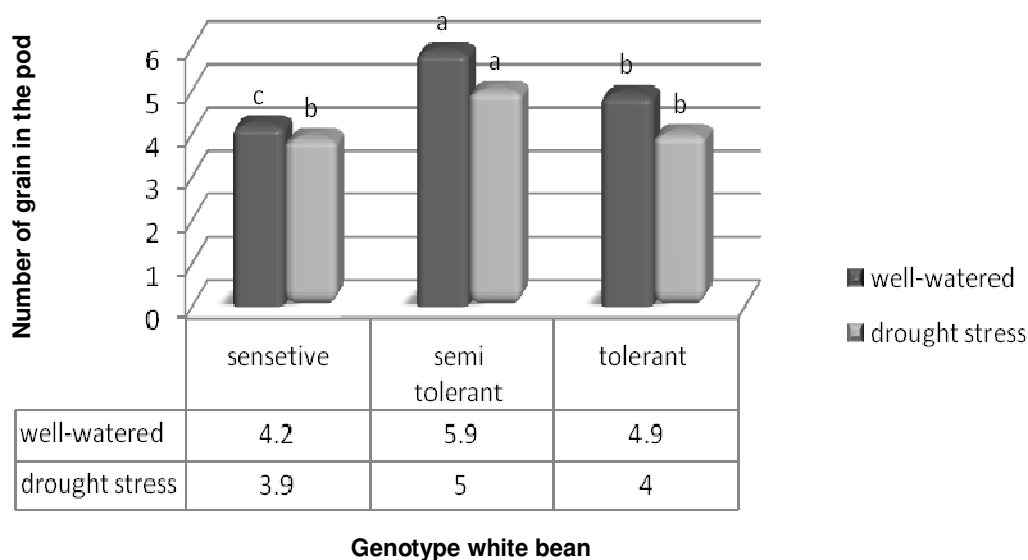


Figure 2. Comparing white bean genotypes regarding the number of grain in the pod. The numbers above the column are for comparing different genotypes in a specific condition.

the report of Teran and Singh (2002). Also, the semi tolerant and Shekofa genotype had the smallest amount of pods in the plant but the largest amount of grain in the pod (Figures 1 and 2). It seems that shortage of water in the reproductive phase leads to the reduction of photosynthesis intensity, ABA increase and loading assimilation (Clavel et al., 2005) and ultimately the abscission of flowers and pods.

According to Figure 1, the G11867 tolerant genotype had more pods under stress than in well-watered. The survival of the pod is an important characteristic in determining yield. Therefore, it is a suitable feature for

current genotypes. In this experiment, the weight of 100 grain increased under stress as compared to normal conditions which can result in pollen abortion, decrease in the number of grain in the pod and the number of pods in the plant and it also reduces the number of physiologic destinations and ultimately photosynthesis substances move towards several limited destinations and lead to the increase of weight of 100 grain under drought stress. The Daneshkadeh genotype had the highest weight of 100 grain and the G11867 genotype had the lowest weight of 100 grain. The weight of the seed is determined by 3 sources namely: (1) Current photosynthesis after

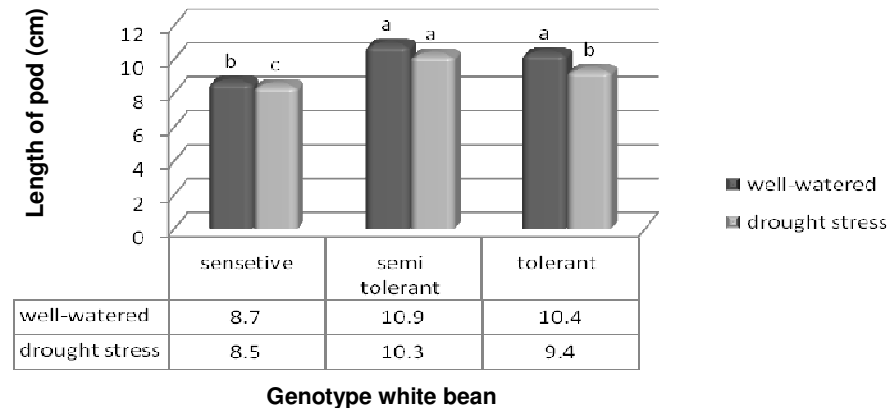


Figure 3. Comparing white bean genotypes regarding pod length (cm). The numbers above the column are for comparing different genotypes in a specific condition.

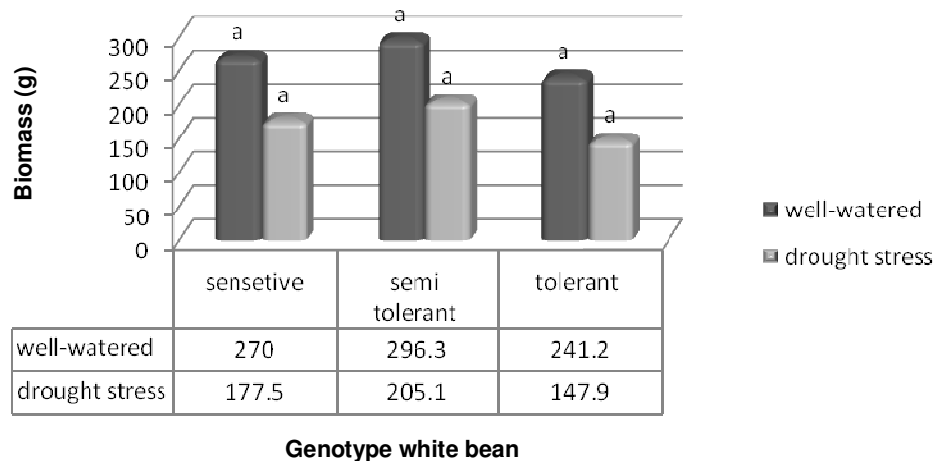


Figure 4. Comparing white bean genotypes regarding biomass (g). The numbers above the column are for comparing different genotypes in a specific condition.

pollination, (2) The transfer of carbohydrates which are produced before pollination in the plant and saved and transferred to the seed after pollination (this process is called remobilization) and (3) The transfer of carbohydrates produce after pollination and in the seeds' slows down the growth period in which the assimilates caused by the plant's current photosynthesis are created and are more than the seeds' need and due to limitations in accepting new grain, they are saved temporarily in the plant (this process is called retranslocation). The combination of remobilization and retranslocation is called redistribution (Ehdaie and Waines, 1996).

According to Figure 5, it is observed that the Daneshkadeh sensitive genotype had the highest amount of grain yield under well-watered as compared to other genotypes but this genotype had a considerable reduction in the single-plant grain yield under stress. However, the G11867 tolerant genotype, although, having the lowest grain yield under well-watered, did not

have a significant reduction under stress. This genotype was able to relatively maintain the characteristics of well-watered under stress. This is also true for the semi tolerant and Shekofa genotype. According to Fernandez (1992), the most suitable selection criteria for stress has to be able to separate genotypes that have a similar and desirable manifestation in both environments from other groups. These two genotypes were able to resist dry material distribution under drought stress. The number of pod and grain in the plant in these genotypes was also less influenced by stress. The pod length in the assessed genotypes in this experiment decreased under stress. The semi tolerant Shekofa genotype had the longest pod and the Daneshkadeh sensitive genotype had the shortest pod (Figure 3). Although, the studied genotypes did not show any biomass difference, the semi tolerant and Shekofa genotype had the most and the G11867 tolerant genotype showed the least amount under drought stress (Figure 4). This similarity between the

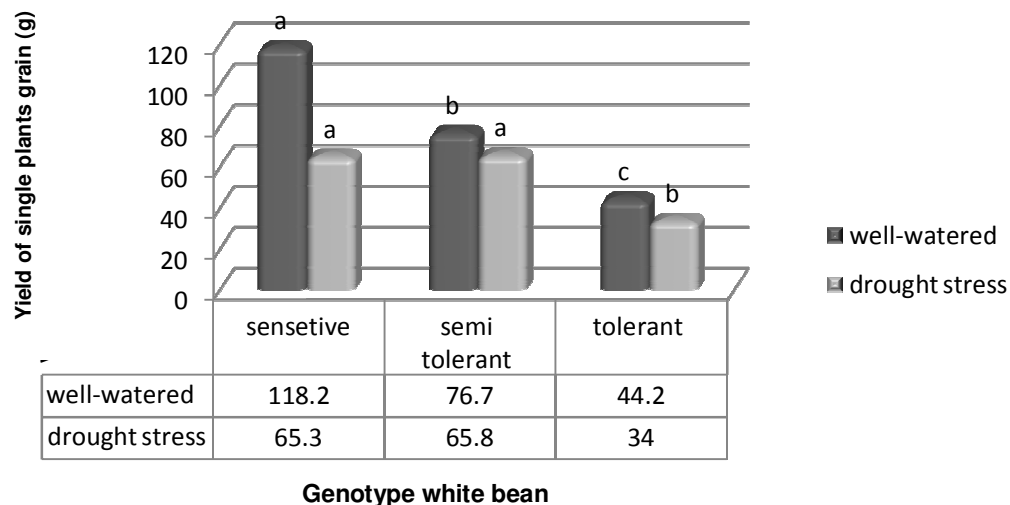


Figure 5. Comparing white bean genotypes regarding the yield of single plant grain (g). The numbers above the column are for comparing different genotypes in a specific condition.

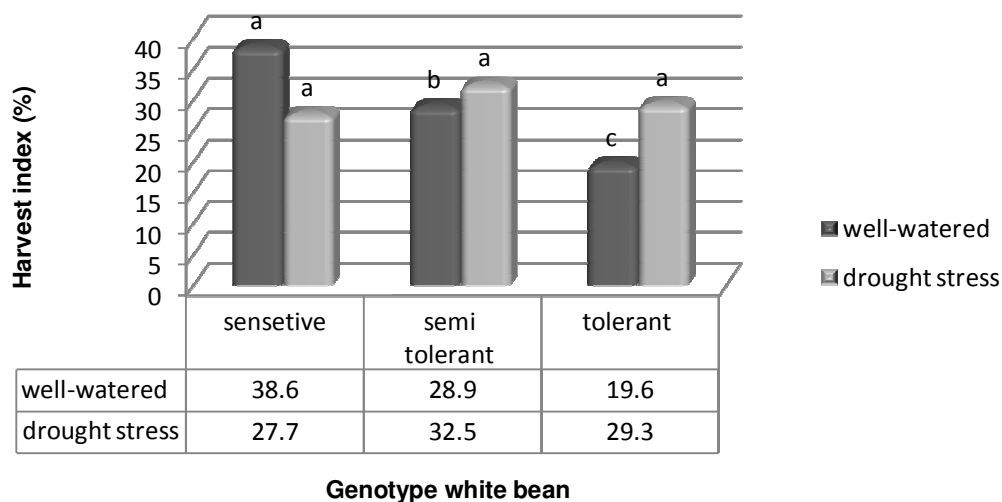


Figure 6. Comparing white bean genotypes regarding harvest index percentage. The numbers above the column are for comparing different genotypes in a specific condition.

genotypes could be related to the time and method of stress because the production of a large portion of biomass is related to vegetative growth and since the studied bean genotypes were indeterminate and the plots under stress were irrigated after sampling, there was no significant difference between the studied genotypes in this regard.

From the aforementioned results, it can be concluded that the effect of drought stress in the reproductive phase (the number and length of the pod and the number of grain in the pod) is more on the vegetative section than the reproductive section which indicates the importance of current photosynthesis of beans in yield determination. As shown in Table 1, the interaction irrigation surfaces \times genotype was significant considering the harvest index.

The considerable difference in the harvest index among different genotypes shows that they are competing for water absorption and gaining acceptable yield and by selecting these conditions, a better result will be obtained, especially considering the fact that the Daneshkadeh sensitive genotype had a higher harvest index under normal conditions and its harvest decreased under drought stress while in the G11867 tolerant genotype and the semi tolerant and Shekofa genotype, the harvest index increased under drought stress (Figure 6). The high harvest index of these two genotypes under stress shows the appropriate and increasing distribution of photosynthesis material towards economical yield and this can be a desirable characteristic in producing high yield plants. Reynolds et al. (2005) reported that high

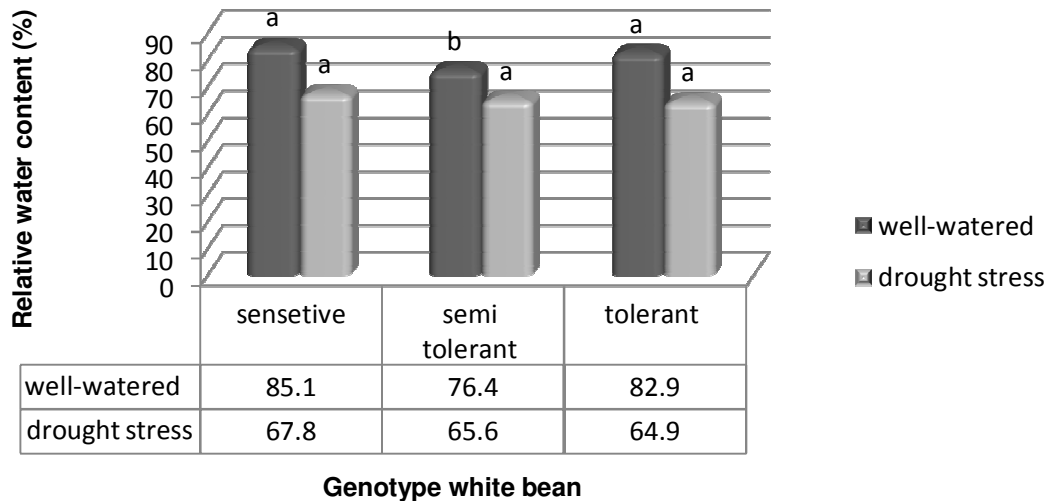


Figure 7. Comparing white bean genotypes regarding relative water content (RWC). The numbers above the column are for comparing different genotypes in a specific condition.

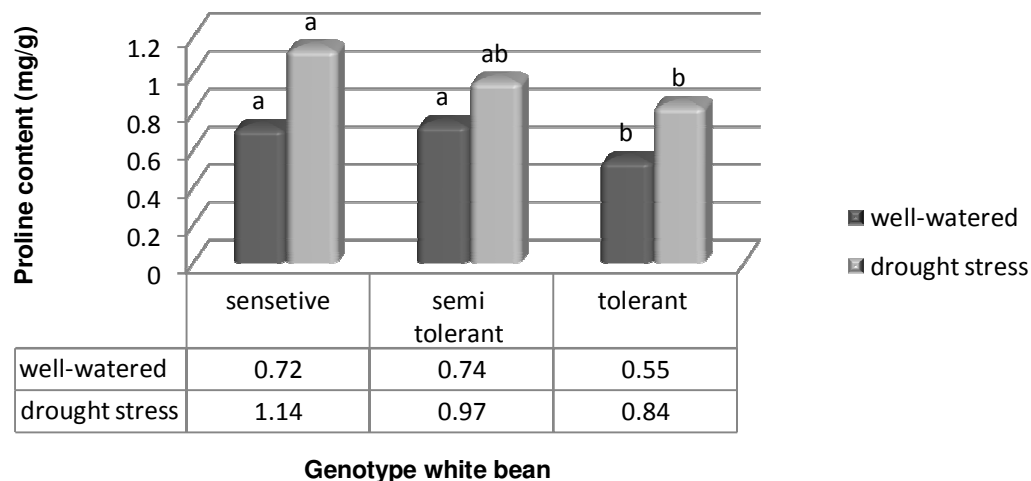


Figure 8. Comparing white bean genotypes regarding proline content (mg/g). The numbers above the column are for comparing different genotypes in a specific condition.

harvest index in water shortage conditions could be related to adaptation to stress and results in optimized yield in the seed filling phase due to the movement of the stem reserve and an increased capability in water accessibility.

Relative water content

In this study, drought stress leads to the reduction of relative water content in the three genotypes of white beans (Figure 7). RWC reduction of the semi tolerant and Shekofa genotype is less than the other genotypes under stress as compared to well-watered. Difference in the amount of this characteristic may indicate the difference

between the genotypes for absorbing water from soil or the ability to reduce water through the stomas or the difference in their ability for osmotic accumulation and regulation for maintaining tissue turgor and increasing physiologic activities.

Changes in proline content

According to Figure 8 in this study, drought stress increased the amount of proline and among the genotypes; the Daneshkadeh sensitive genotype had the highest amount of proline under stress. The considerable increase of proline in this genotype under stress as compared to the other genotypes could be the result of

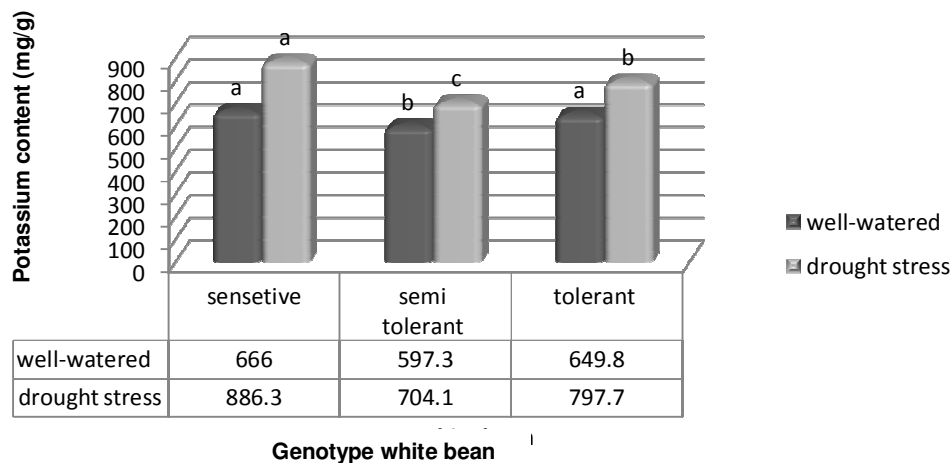


Figure 9. Comparing white bean genotypes regarding potassium ion content (mg/g). The numbers above the column are for comparing different genotypes in a specific condition.

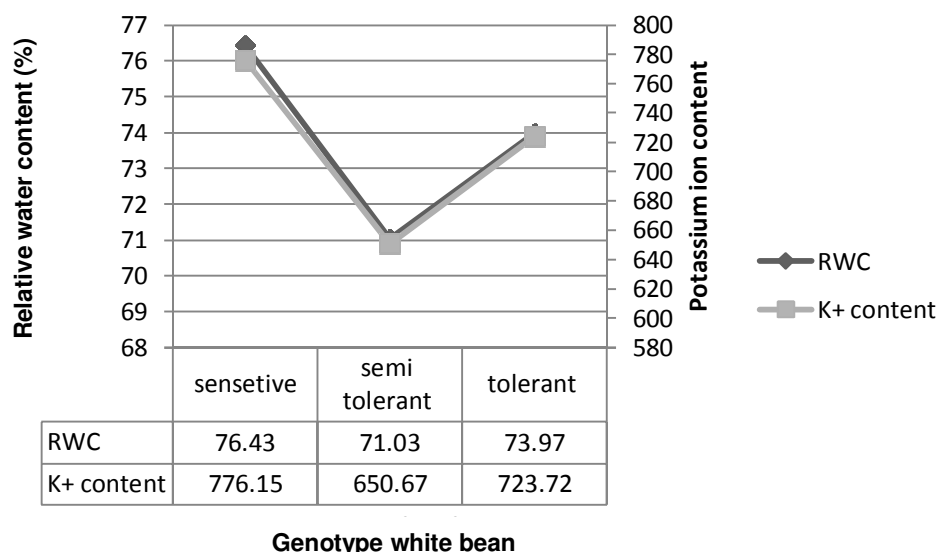


Figure 10. White bean genotypes regarding the two characteristics of relative water content (%) and potassium ion content (mg/g).

higher protein analysis. More carbon in the organic material structure influential in osmotic regulation, including proline, can decrease growth (Natalie et al., 1991). Changes which occur in proline biosynthesis are as follows: hydrolysis of proteins and their oxidative destruction processes which lead to proline accumulation in plants undergoing stress. The proline oxidation amount in plants in well-watered is so little that it cannot be a convincing reason for high amounts of proline in stressful conditions. Therefore, the increase of proline density under stress in plants is often due to their spontaneous synthesis. As shown in Figure 11, the semi tolerant and Shekofa genotype has high proline content when its relative water content is less than the other genotypes.

Regarding proline accumulation, it can be stated that when relative water content decreases, proline protects proteins and membranes (Kameli and Losel, 1993).

Changes in potassium concentration

As shown in Figure 9, the applied drought stress in this experiment increased the potassium ion content, and in this regard, the Daneshkadeh sensitive genotype had higher potassium content. By increasing the potassium ion content, this genotype was able to survive through the maintenance of photosynthesis and chloroplast protection. Moreover, according to Figure 10, the semi tolerant

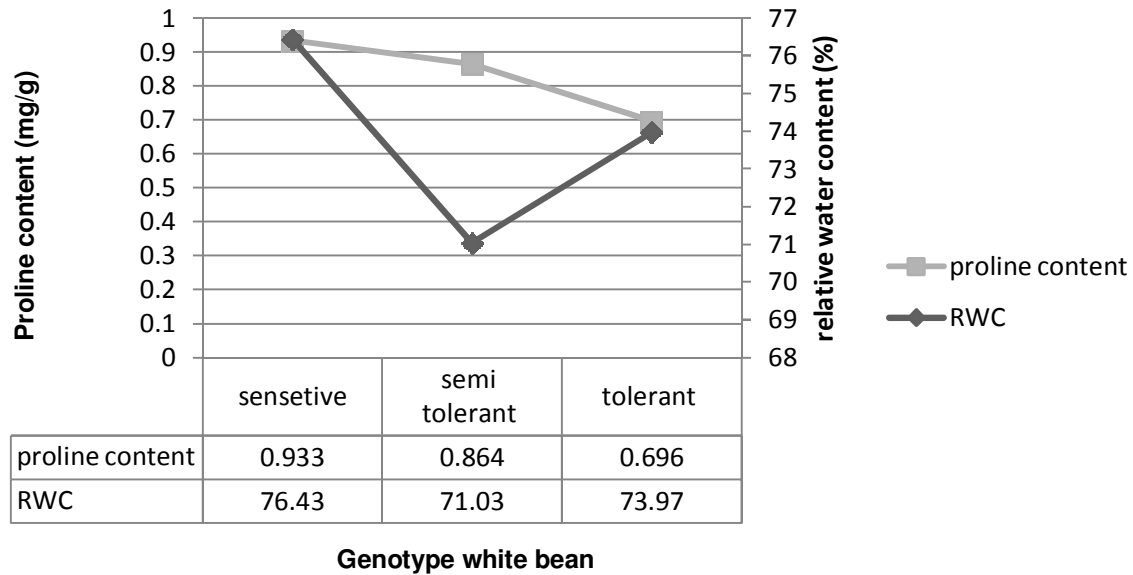


Figure 11. White bean genotypes regarding the two characteristics of relative water content and proline content.

and Shekofa genotype had the lowest relative water content and also the lowest potassium ion content. Also, the G11867 tolerant genotype and the Daneshkadeh sensitive genotype which had higher relative water content had higher potassium ion content as well.

According to Omar et al. (2006), in plants that are faced with potassium ion shortage under drought stress conditions, ROS (reactive oxygen species) productions increase progressively, and as a result interfere with opening of stoma, water relations and photosynthesis. Under drought stress, the chloroplast loses a large amount of potassium which leads to sudden decrease in photosynthesis and stimulates the ROS system. These results inevitably support the hypothesis that the increase of drought stress intensity, increases K requirements for maintaining photosynthesis and protecting the chloroplast from oxidative damages (Marshner, 1995; Mengel and Kirkby, 2001). Environmental stress factors increase the need for potassium ion and also lead to oxidative damage of the cells through ROS system stimulation, especially, during photosynthesis (Kakmak, 2005). It seems that the reason for requiring more potassium ion in the Daneshkadeh sensitive genotype under drought stress is related to the fact that potassium ion is necessary for stabilizing photosynthesis CO_2 . For instance, it helps the plant by closing stomas and hence reducing CO_2 stabilization. The reduction of the damaging effects of drought stress is related to potassium ion, especially in the photosynthesis of legumes that has also been reported by Sangakara et al. (2000). According to Hambel and Hisao (1969), potassium increases osmotic potential and has a positive effect on closing stomas.

Generally, in the present study, it is observed that drought stress has a deterrent effect on relative water

content, but potassium ion density and proline content were considerably increased. High potassium and proline accumulation in bean genotypes under drought stress can be an adaptation for tolerating drought stress and is dependent on osmotic moderation and therefore, it helps the plant's survival and productivity under drought stress.

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