Differences in antioxidant, morphological and biochemical responses to drought stress in different cultivars of common bean (*Phaseolus vulgaris* L.)

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Abstract

Drought stress is the most common environmental stress that can significantly influence crop productivity. In this study, morphological and biochemical properties of 17 genotypes of common bean were evaluated under different levels of drought stress and the most sensitive and tolerant genotypes were identified using multivariate analysis. The results indicated that morphological and biochemical characteristics of common bean were significantly influenced by drought stress, genotype, and genotypes ×drought stress interaction. Principal component analysis summarized the 14 indices to four components which explained 85.71%, 84.52%, 85.86% and 84.94% of the total variation at control, moderate, severe and combined data, respectively. The correlation coefficients among most of the quantitative traits were statistically significant at all drought stress levels. A significant and positive correlation between chlorophyll and carotenoids with ascorbate peroxidase activity was observed at both moderate and severe drought stress conditions. These associations suggest that this enzyme plays an important role in ROS scavenging under drought stress. Clustering analysis grouped the genotypes into four divergent groups. The genotype-by-trait biplot analysis indicated that genotypes 1 and 2 were the most drought-tolerant and genotypes 12 and 16 were the most drought-sensitive genotypes under both moderate and severe drought stress conditions.

Key words: Anti-oxidant enzymes, Biplot analysis, Cluster analysis, Drought stress**.**

INTRODUCTION

Crop plants are exposed to several environmental stresses affecting plant growth and development (Seki *et al*., 2003; Farooq *et al*., 2009a, b, 2011). Drought stress is one of the most common environmental stresses which decrease crop productivity more than any other environmental stresses (Lambers *et al*., 2008). Inadequate water availability coupled with increasing air temperature leads to drought stress (Mishra and Cherkauer, 2010). This stress is among the main limiting factors for the production of common bean, *Phaseolus vulgaris* L. (Rosales *et al*., 2012); the second most important commercial legume after soybean (Singh *et al*., 1999). Common bean is considered to be the "perfect food", because of its protein, fiber and mineral content (Beebe *et al*., 2000). According to Martínez *et al*. (2007), drought stress at reproductive stage causes a 58–87% reduction in common bean yield. This stress resulted in a decline of leaf water potential, stomatal conductance, photosynthesis rate and all growth, productivity and quality parameters of common bean (El-Tohamy *et al*., 1999). There are a number of mechanisms by which plants can tolerate drought and recover themselves from its effects. During evolution, plants have

Table 1. Common bean genotypes used in this study.

No.	Genotypes/vareity	Co.	No.	Genotypes/vareity	Co.
1	E3	Wa-8563-13k	10	Goli	Ks31167
2	Naz	Ks31165	11	Colombia	Colombia
3	Kousha	KS21193	12	Cos16	Ks21478
4	Sadri	Ks21481	13	Ks31169	Ks31169
5	Dorsa	Ks41105	14	E8	E8
6	Mahali Khomein	Ks21467	15	Ghafar	KS21191
7	E6	6x-9536-7k	16	D81083	Ks31164
8	Talash	Talash	17	Pak	Ks41128
9	Azna	Azna			

Genotype/ line Code (Co.)

developed morphological and biochemical responses to enhance their survival under various stresses. Loss of leaf area (Acosta-Gallegos, 1988), slower growth in order to divert assimilates and energy into protective molecules to overcome stress (Zhu, 2002), and stomatal control are important mechanisms for adapting to drought stress in common bean (Laffray and Louguet, 1990). Stomatal closure limits photosynthesis rate, which is the main process responsible for dry matter accumulation and thereby affects plant development and growth (McCree, 1986). Drought induces protein expression such as proteins implicated in the biosynthesis of osmolytes (Bohnert *et al*., 1995; Ishitani *et al*., 1995), uptake and compartmentation of ions (Lisse *et al*., 1996; Niu *et al*., 1995), hydroxyl-radical scavenging (Ingram and Bartels, 1996; Bohnert *et al*., 1995; Smirnoff and Cumbes, 1989), protein turnover (Kiyosue *et al*., 1994; Koizumi *et al*., 1993) and different groups of late embryogenesis abundant (LEA) proteins such as dehydrins (Neslihan-Ozturk *et al*., 2002; Colmenero-Flores *et al*., 1997; Lisse *et al*., 1996). Availability and utilization of drought-tolerant bean genotypes would decrease amount of irrigation water and thereby increase farmer's profit margins through allowing more consistent crop production and reducing production cost. So, in this study the relative contribution of various morphological and biochemical traits to drought- tolerance of common bean genotypes were studied using principal component analysis, factor analysis and clustering analysis. Drought-tolerant genotypes and potentially useful traits that may be used in breeding programs were identified according to the result of these analysis.

MATERIALS AND METHODS

Plant material and growth condition

The present study was carried out at the Graduate University of Advanced Technology, Kerman- Iran. Seventeen common bean genotypes and local varieties were studied in this rsearch (Table 1). Seeds of these genotypes and local varieties were obtained from the Agriculture and Natural Resources Research Center of Zanjan, Iran.

The experiment was conducted under controlled conditions in the growth chamber (25±3 ^{o}C; 8 h night, 16 h day and 50-60% relative humidity). Plants were grown in plastic pots (15×25 cm); the experimental pots were arranged in a factorial experiment based on completely randomized design with three replications. Drought stress treatments were applied as follows: control (irrigated every 7 days) and drought (moderate drought stress: irrigated every 10 days and severe stress: irrigated every 14 days). All agronomic activities including fertilizer application were performed in the same way for all pots. Morphological and biochemical traits were measured 45 days after sowing.

Measurement of morphological traits

Stem height, length and width of three leaves (average of upper, middle, and lower leaves), number of nodes, flowers and leaves and internode length were measured at 45 days after sowing.

Measurement of Biochemical traits

Chlorophyll content (total chlorophyll, chlorophyll a and chlorophyll b) and carotenoids in common bean leaves were measured according to the method of Arnon (1949). In brief, the youngest fully expanded leaves were subjected to extraction using 80% acetone and the extracts were stored in a dark room for 2h and then centrifuged (5500×*g*, 10 min, and 25 °C). Chlorophyll a and b, and carotenoids were measured at 645, 663 and 480 nm, respectively. Chlorophyll and carotenoid concentration were expressed in mg g^{-1} fresh weight according to the Arnon's formula (Arnon, 1949):

Chlorophyll 'a' (mg.g^{-1})= $0.0127\times A_{663} - 0.00269\times A_{645}$

Chlorophyll 'b' (mg.g^{-1})= $0.0229\times A_{645} - 0.00468\times A_{663}$

Total chlorophyll (mg.g^{-1})= $0.0202\times A_{645} + 0.00802\times A_{663}$

(Carotenoid (mg.g^{-1})= $A_{480} + (0.114\times A_{663} - 0.638\times A_{645}$

Total protein content was estimated by the Bradford method (1976), using bovine serum albumin as the standard. The results were calculated as µg/ml. Catalase (EC 1.11.1.6) (CAT) and ascorbate peroxidase (EC 1.11.1.11) (APX) activities were estimated using Cakmak and Horst (1991) and Nakano and Asada (1981) methods, respectively. Enzyme activities were expressed as Unit/mg protein.

Data analysis

The experiment was carried out as factorial experiment based on completely randomized design with three replications. Variance analysis (F Test) was used to investigate the effect of drought stress on different parameters. Data from three replications were normalized and subjected to analysis of the variance using SPSS 21.0 software in probability level of 1%. The data were also analyzed by principal component analysis and factor analysis using SPSS software. Correlation analysis and hierarchical clustering were performed using MiniTab (Ver.15.0).

RESULTS AND DISCUSSION

The results of variance analysis (Table 2) showed that there were significant differences among all drought stress levels, genotypes, and the genotypes ×drought stress interaction for Stem height, length and width of the leaves, number of flowers, content of chlorophyll b and activity of APX . According to Table 2, the effect of drought level was not significant for total chlorophyll, chlorophyll a, Carotenoid, protein and activity of CAT. The effect of genotypes×drought stress (A×B) interaction was not significant for internode length, number of nodes and leaves. The significant A×B suggests that some of the genotypes were not stable between treatments (Andrade *et al*., 2016). These differences could be attributed to genetic variation between genotypes, differences in the nature of traits (Darkwa *et al*., 2016), growth stage, severity and duration of stress (Chaves *et al*., 2003), environmental factors (Rizhsky *et al*., 2002; McDonald and Davies, 1996), different patterns of genes expression (Denby and Gehring, 2005), the activity of respiration (Ribas-Carbo *et al*., 2005) and photosynthesis machinery (Flexas *et al*., 2004). For example, Darkwa *et al*., (2016) demonstrated that some traits such as plant height and leaf chlorophyll content were more sensitive

Table 2. Analysis of variance of morphological and biochemical traits in common bean genotypes under drought stress.

Source of variation	df	Mean squares												
		int L	SH	Lwi	Lli	Fn	Nn	Ln	Tchl	ChlA	ChlB	Car	Pr	APX	CAT
A	16	9.78**	1660.36**	1.94**	10.66**	12.99**	14.57**	10.79**	0.000213**	0.0000632**	0.0000844**	0.11**	0.0728*	0.00255**	0.00779**
B	2	30.58**	6864.25**	3.49**	10.79**	19.78**	18.84**	21.26**	0.0000169$^{n.s}$	0.0000253$^{n.s}$	0.0000183*	0.0679$^{n.s}$	0.091$^{n.s}$	0.00133*	0.000898$^{n.s}$
A*B	32	2.71$^{n.s}$	114.87**	0.355**	1.303**	2.083**	0.982$^{n.s}$	0.9$^{n.s}$	0.000104**	0.0000363*	0.0000461**	0.05531**	0.07918**	0.00125**	0.00256**
Error	102	2.026	49.286	0.122	0.347	0.974	0.771	1.007	0.0000374	0.0000196	0.00000548	0.0258	0.033	0.00037	0.00113
Coefficient of variation (%)		14.36	9.78	14.2	12.58	15.34	12	16.17	23.43	25.11	27.93	24.05	15.95	34.12	29.38

A: Genotype; B: Drought stress; Stem height (SH), length (Lli) and width (Lwi) of three leaves, number of nodes (Nn), internode length (int L), number of flowers (Fn), number of leaves (Ln), total chlorophyll content (Tchl), chlorophyll a (chlA), chlorophyll b (chlB), carotenoid (Car), total protein content (Pr), activity of ascorbate peroxidase (APX) and catalase (CAT).

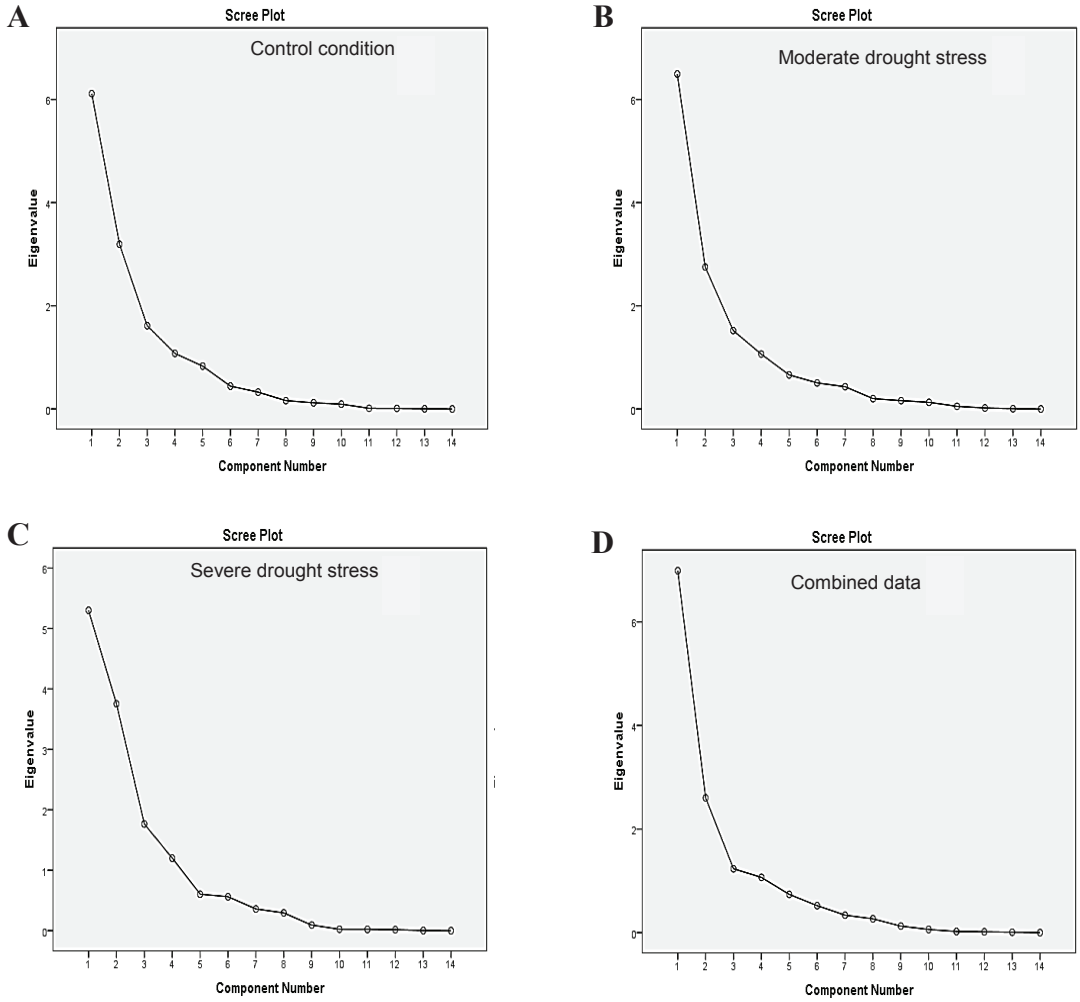

Figure 1. Scree plot and respective eigenvalues using principal component analysis.

to drought stress than others. Drought stress decreases plant growth by reducing cell division and elongation and causes a decline in assimilates transport to the root surface, which leads to a further decrease in plant growth (Farooq *et al*., 2012; Yordanov *et al*., 2003; Pugnaire *et al*., 1999).

In the present study, drought tolerance was assessed using 14 variables in the 17 genotypes under control, moderate and severe stress condition using principal component analysis. Also, this analysis was performed on the combined data obtained from three irrigation regimes (Figure 1A-D). Principal component analysis (PCA) has been widely used to overcome the effect of large number of independent variables and grouping of genotypes (Cirilo *et al*., 2009). Similarly, Greenacre (2010) reported that eigenvalues (in PCA) have primary importance for numerical diagnostics to assess variation attributed by number of large variables on the dependent structure and their data matrix in a graphical display. Based on PCA and after varimax rotation, factor analysis (FA) was performed to determine the latent factors or groups of variables (Table 3).

Principal components analysis grouped the estimated variables into four main components which all together accounted for 85.71%, 84.52% and 85.86% of the total variation at control, moderate and severe drought stress conditions, respectively. Principal components analysis of combined (joint) data grouped the estimated variables into four components which explained 84.94% of variation. Under control condition, PC1 which was negatively correlated with length and width of the leaves (growth factor) and positively correlated with chlorophyll a and number of nodes, leaves and flowers, explained 37.65% of the total variation. Meanwhile, PC2 was positively correlated with total chlorophyll, chlorophyll b content and carotenoid and was found to have 21.3% contribution in the total variation. The third component (PC3) was positively correlated with protein content and CAT activity and explained 13.54% of the total variation. PC4 was positively

correlated with internode length, stem height and activity of APX traits and explained 13.22% of the total variation (Table 3). Under moderate drought stress, the variables included in the first factor were stem height, number of flowers, nodes and leaves (productive factor), APX and CAT activity (antioxidative enzymes factor) which explained 34.45% of the total variation. PC2 negatively correlated with length and width of the leaves, and positively correlated with total chlorophyll, chlorophyll a, carotenoid and protein content and explained 30.77% of the total variation. PC3 related to internode length and stem height traits, and accounted for 10.84% of the variation. Finally, PC4 included chlorophyll b accounting for 8.46% of the variation (Table 3). At severe drought stress, PC1 contained total chlorophyll, chlorophyll b, chlorophyll a, carotenoid (pigment factor) and protein content and activity of APX and CAT enzymes. The PC2 and PC3 were correlated with productive and growth factors and PC4 included internode length trait.

The results presented in Table 3 showed that PC1 accounted for 34.98%; PC2 for 24.98%, PC3 for 14.19% and PC4 for 11.71% of the total variation. Principal component analysis for combined data revealed that the first component (PC1) associated to chlorophyll a, productive and antioxidative enzyme factors accounting for 40.02% of variation. PC2 correlated to total chlorophyll, carotenoid and protein content and APX activity with 19.49.01% of variation. PC3 contained length and width of the leaves and chlorophyll b content with 15.11% of variation. PC4 correlated with internode length and stem height with 10.33% of variation (Table 3). The first principal component showed high importance for selection of common bean genotypes. At control condition growth and productive factors, at moderate and combined data the productive and antioxidative enzyme factors were highlighted as PC1. Comparison of control and moderate conditions indicated that drought stress reduced the growth factors, but activated antioxidative enzymes. Under severe drought stress, the pigment, protein and antioxidative enzyme factors were introduced as the first principal component (PC1). Therefore, with increasing the level of drought stress, the productive factors were also weakened and biochemical parameters showed increase in activity to overcome the effect of water stress (Table 3). Hence, biochemical traits such as total chlorophyll, protein and activity of APX and CAT can be considered as the most important factors in the selection of tolerant bean genotypes at drought stress. These findings are in line with Eslami (2012) and Naseh-ghafoori (2010) results.

The correlation coefficients among most of the quantitative traits were statistically significant at all drought stress levels. The basic statistics of various traits studied under control conditions demonstrated considerable variation among 17 common bean genotypes (Table 4). Simple correlation coefficients revealed 30 significant correlations among morphological and biochemical traits. There is a positive and significant correlation between number of flowers, nodes and leaves traits and stem height. Therefore, stem height positively affected the number of flowers, leaves and nodes. Also, number of flowers was positively correlated with stem height and number of nodes and leaves, chlorophyll a and activity of CAT, and negatively with length and width of the leaves traits at control condition. Negative correlations show that the selection of one trait reduced the expression of another (Ramalho *et al*., 1993). The negative correlation between the number of flowers, length and width of the leaves traits could be due to the competition for light and food (Gautier *et al*., 2001). CAT activity had a positive and significant correlation with number of flowers and leaves, chlorophyll a and protein content. The induction of catalase activity is likely related to biosynthetic pathways of these traits at normal conditions. There was no significant association between investigated traits and ascorbate peroxidase (APX) activity at control condition. It may be due to the low production of this enzyme or the involvement of unexplained factors in the APX activity.

Under moderate environment 36 significant correlations were recognized (Table 5). Positive and significant correlations were observed for stem height with internode length, number of flowers, nodes and leaves, chlorophyll a and activity of APX and CAT. The number of flowers positively correlated with stem height, number of nodes and leaves, chlorophyll a, carotenoid and activity of APX and CAT. APX had a positive correlation with stem height, number of flowers, nodes, and leaves, chlorophyll a, carotenoid, protein and CAT. The activity of CAT enzyme was positively correlated with stem height, number of flowers, nodes and leaves, and activity of APX. Hence, catalase activity influenced productive and enzymatic factors at moderate drought stress condition. Under severe drought stress 24 significant associations among traits were observed (Table 6). Some of these associations are the correlation of the stem height with internode length, number of flowers, nodes, leaves; number of flowers with stem height, number of nodes and leaves; activity of CAT with protein content; activity of APX with total chlorophyll, chlorophyll b, chlorophyll a, carotenoid and protein content.

Table 3. Summary of factors loading for the estimated variables of 17 common bean genotypes.

	Control				Moderate				Severe				Combined			
Variable	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
intL				0.593			0.961					0.941				0.92
SH				0.579	0.712		0.676			0.723			0.806			0.532
Lwi	-0.911					-0.893					0.948				0.834	
Lli	-0.912					-0.847					0.883				0.759	
Fn	0.762				0.936					0.937			0.95			
Nn	0.87				0.943					0.924			0.945			
Ln	0.827				0.92					0.923			0.905			
Tchl		0.909				0.805			0.828					0.76		
ChlB		0.85						0.945	0.922						0.724	
ChlA	0.717					0.633			0.922				0.682			
Car		0.874				0.85			0.877					0.713		
Pr			0.92			0.867			0.711					0.859		
APX				0.776	0.74				0.816				0.64	0.575		
CAT			0.751		0.786				0.663				0.631			
Factor variance (%)	37.65	21.3	13.54	13.22	34.45	30.77	10.84	8.46	34.98	24.98	14.19	11.71	40.02	19.49	15.11	10.33
Cumulative (%)	37.65	58.95	72.49	85.71	34.45	65.22	76.06	84.52	34.98	59.96	74.15	85.86	40.02	59.5	74.61	84.94

Stem height (SH), length (Lli) and width (Lwi) of three leaves, number of nodes (Nn), internode length (intL), number of flowers (Fn), number of leaves (Ln), total chlorophyll content (Tchl), chlorophyll a (chlA), chlorophyll b (chlB), carotenoid (Car), total protein content (Pr), activity of ascorbate peroxidase (APX) and catalase (CAT).

Table 4. Correlation coefficient among quantitative traits of common bean genotypes grown under non-stress condition.

Trait	intL	SH	Lwi	Lli	Fn	Nn	Ln	Tchl	ChlB	ChlA	Car	Pr	APX	CAT
intL	1.000													
SH	.262	1.000												
Lwi	.497*	-.461	1.000											
Lli	.510*	-.426	.943**	1.000										
Fn	-.120	.799**	-.547*	-.542*	1.000									
Nn	-.259	.859**	-.726**	-701**	.873**	1.000								
Ln	-.100	.849**	-.698**	-703**	.869**	.894**	1.000							
Tchl	.127	.416	.050	-.016	.441	.329	.260	1.000						
ChlB	.520*	.479	.246	.294	.210	.182	.082	.696**	1.000					
ChlA	-.220	.633	-.484*	-.508*	.680**	.750**	.615**	.397	.161	1.000				
Car	-.019	.385	-.112	-.214	.450	.397	.229	.884**	.567*	.567*	1.000			
Pr	.628**	.260	.223	.311	.040	-.077	-.050	.240	.341	.250	.208	1.000		
APX	.260	.429	-.119	-.216	.360	.320	.447	.225	-.036	.093	.082	.044	1.000	
CAT	.134	.480	-.383	-.311	.582*	.405	.514*	.300	.068	.542*	.251	.543*	.412	1.000

Stem height (SH), length (Lli) and width (Lwi) of three leaves, number of nodes (Nn), internode length (intL), number of flowers, number of leaves (Ln), total chlorophyll content (Tchl), chlorophyll a (chlA), chlorophyll b (chlB), carotenoid (Car), total protein content (Pr), activity of ascorbate peroxidase (APX) and catalase (CAT).

Table 5. Correlation coefficient among quantitative traits of common bean genotypes grown under moderate condition.

Trait	intL	SH	Lwi	Lli	Fn	Nn	Ln	Tchl	ChlB	ChlA	Car	Pr	APX	CAT
intL	1.000													
SH	.552*	1.000												
Lwi	-.093	-.262	1.000											
Lli	.079	-.238	.894**	1.000										
Fn	-.111	.688**	-.219	-.451	1.000									
Nn	-.167	.719**	-.258	-.379	.901**	1.000								
Ln	-.101	.662**	-.227	-.380	.863**	.894**	1.000							
Tchl	-.001	.047	-.588*	-.538*	.074	.080	-.172	1.000						
ChlB	-.209	.038	-.197	-.179	.101	.169	.051	.283	1.000					
ChlA	.101	.588*	-.550*	-.517*	.536*	.601*	.359	.425	.000	1.000				
Car	.048	.480	-.776	-.775	.568*	.549*	.398	.709**	.108	.753**	1.000			
Pr	-.148	.148	-.744**	-.759**	.286	.284	.167	.538*	.162	.613**	.738**	1.000		
APX	-.072	.539*	-.336	-.481	.732**	.701**	.547*	.235	.065	.551*	.524*	.485*	1.000	
CAT	.008	.526*	-.160	-.356	.706**	.662**	.640**	-.001	-.245	.424	.457	.198	.737**	1.000

Stem height (SH), length (Lli) and width (Lwi) of three leaves, number of nodes (Nn), internode length (intL), number of flowers (Fn), number of leaves (Ln), total chlorophyll content (Tchl), chlorophyll a (chlA), chlorophyll b (chlB), carotenoid (Car), total protein content (Pr), activity of ascorbate peroxidase (APX) and catalase (CAT).

Table 6. Correlation coefficient among quantitative traits of common bean genotypes grown under severe condition.

Trait	intL	SH	Lwi	Lli	Fn	Nn	Ln	Tchl	ChlB	ChlA	Car	Pr	APX	CAT
intL	1.000													
SH	.536*	1.000												
Lwi	.149	-.205	1.000											
Lli	.387	-.155	.880**	1.000										
Fn	-.128	.658**	-.212	-.359	1.000									
Nn	-.135	.757**	-.353	-.501*	.844**	1.000								
Ln	-.226	.617**	-.268	-.447	.829**	.912**	1.000							
Tchl	.380	.474	.048	.080	-.054	.285	.105	1.000						
ChlB	.221	.265	.160	.089	.101	.141	-.020	.702**	1.000					
ChlA	.221	.265	.160	.089	.101	.141	-.020	.702**	1.000**	1.000				
Car	.047	.366	.024	-.077	.121	.414	.240	.896**	.777**	.777**	1.000			
Pr	.362	.121	.026	.213	-.303	-.129	-.232	.603*	.601*	.601*	.457	1.000		
APX	.246	.447	.073	.001	.179	.353	.175	.689**	.737**	.737**	.678**	.543*	1.000	
CAT	-.073	.049	-.102	-.264	-.069	.164	.028	.385	.469	.469	.448	.484*	.467	1.000

Stem height (SH), length (Lli) and width (Lwi) of three leaves, number of nodes (Nn), internode length (intL), number of flowers (Fn), number of leaves (Ln), total chlorophyll content (TChl), chlorophyll a (chlA), chlorophyll b (chlB), carotenoid (Car), total protein content (Pr), activity of ascorbate peroxidase (APX) and catalase (CAT).

On the basis of Table 6, morphological traits did not affect biochemical parameters, and vice versa. Juliana *et al*., (2001) reported that the stem height correlated positively with number of nodes, flowers and leaves. This result is in agreement with our finding at all levels of drought stress conditions (Table 4-6). The positive and significant correlation between stem height and internode length at stress condition indicated the strong effect of drought stress on this relationship (Desclaux *et al*., 2000). The relationship between stem height and antioxidant enzymes activity and chlorophyll content showed that some biochemical reactions strengthened during moderate drought stress. Therefore, biochemical traits are likely to be affected by other factors such as increased activity of antioxidant enzymes and protein content (Table 6). In this study, the number of flower buds exhibited positive and significant correlation with number of nodes and leaves, and stem height at all levels of drought stress (Table 4-6). At control condition, CAT activity and chlorophyll a had a direct relationship with the number of flowers. In addition, APX activity and carotenoid were also involved in this correlation at moderate conditions. These results suggest that drought stress has triggered more biochemical reactions in the common bean genotypes. While in severe stress condition, no significant correlation was found between the number of flowers and biochemical traits. Kholova *et al*., (2011) indicated that the activities of APX and CAT enzymes were closely related to chlorophyll/carotenoids ratio under drought stress. In our experiment a significant positive correlation of chlorophyll and carotenoids with APX activity was observed at both moderate and severe drought stress conditions.

The relationship between the genotypes and variables can be plotted in the same graph. Biplot provides an effective tool for data analysis. Considering the biplot, genotypes with larger first component and lower second component values gave high yields (stable genotypes), and genotypes with lower PCA1 and larger PCA2 values exhibited low yields (unstable genotypes) (Drikvand *et al*., 2012). A high correlation between the first principal component and a variable revealed that the investigated variable is associated with the direction of the maximum amount of variation in the data set (Leilah and Al-khateeb, 2005). At control condition, PCA1 and PCA2 assigned 58.95% of total variation. According to Figure 2A genotypes 1 and 2 showed larger PC1 and lower PC2, also genotypes 12 and 16 exhibited lower PC1 and larger PC2 at non-stress condition. It is likely that genotypes 1 and 2 are the most stable and genotypes 12 and 16 are the most unstable ones in this

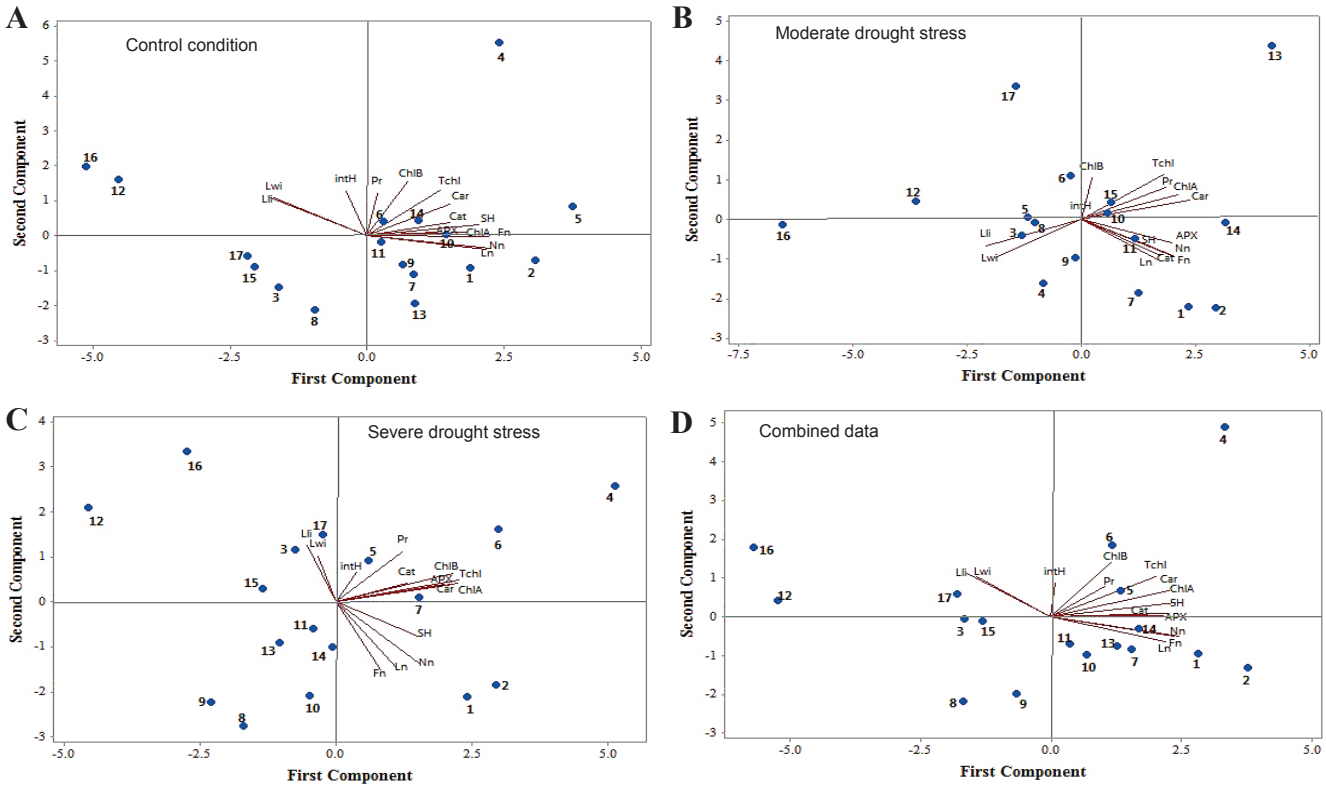

Figure 2. The biplot analysis for morphological and biochemical traits common bean genotypes; 1-E3, 2-Naz, 3-Kousha, 4-Sadri, 5-Dorsa, 6-Mahali Khomein, 7-E6, 8-Talash, 9-Azna, 10-Goli, 11-Colombia, 12-Cos16, 13-Ks31169 (line), 14-E8, 15-Ghafar, 16-D81083 (line), 17-Pak.

condition. However, the results of control environment are not sufficient to make the final decision about the efficiency of the investigated genotypes under water stress conditions. Under moderate drought stress, the PCA1 and PCA2 explained 65.22% of the variations between criteria. Using the biplot diagram (Figure 2B) genotypes 1 and 2 were identified as tolerant and genotype 12 and 16 were recognized as sensitive to drought stress at this condition. Under severe drought condition, PCA1 and PCA2 accounted for 59.96% of the total variance. As shown in Figure 2C, genotypes 1 and 2 are drought tolerant and genotypes 12 and 16 are sensitive genotypes. The first and second components in combined data represented 59.5% of total variation. Figure 2D revealed that genotypes 1 and 2 are drought tolerant and genotypes 12 and 16 are sensitive at drought stress condition. Consequently, we suggest genotypes 1 and 2 as tolerant common bean genotypes and genotypes 12 and 16 as sensitive to drought stress.

Grouping or clustering of genotypes is an efficient tool to minimize the plant pool during selection process (Ali *et al.,* 2015). In order to carry out cluster analysis, squared Pearson distance, agglomerative hierarchical algorithm and ward linkage were used. These methods start with the calculation of the distance of each genotype in relation to other genotypes. Clusters are then formed by the process of agglomeration division. In this process, all genotypes start individually in groups of one. The dendrogram was plotted and the various populations were grouped into four clusters including Cluster I, Cluster II, Cluster III and Cluster IV (Figure 3). According to Figure 3, genotypes are divided into four groups, but the number of genotypes in each group varies in different conditions. Also, some genotypes showed the same reaction at different conditions, which of course, could be expected due to the same genetic background. At control condition and based on Figure 3A, the 17 bean genotypes were grouped into four clusters (I, II, III and IV) which contained 10, 1, 4 and 2 genotypes, respectively. Furthermore, in moderate drought stress based on Figure 3B, the 17 bean genotypes were grouped into four groups including 3, 11, 1 and 2 genotypes for clusters I to IV, respectively. The first cluster consisted of genotypes 1 and 2 that are the most tolerant genotypes to drought stress according to biplot results. The last (IV) cluster contained the most drought sensitive genotypes (12 and 16 genotypes). On the other hands, clusters I and IV represented tolerant and sensitive bean genotypes,

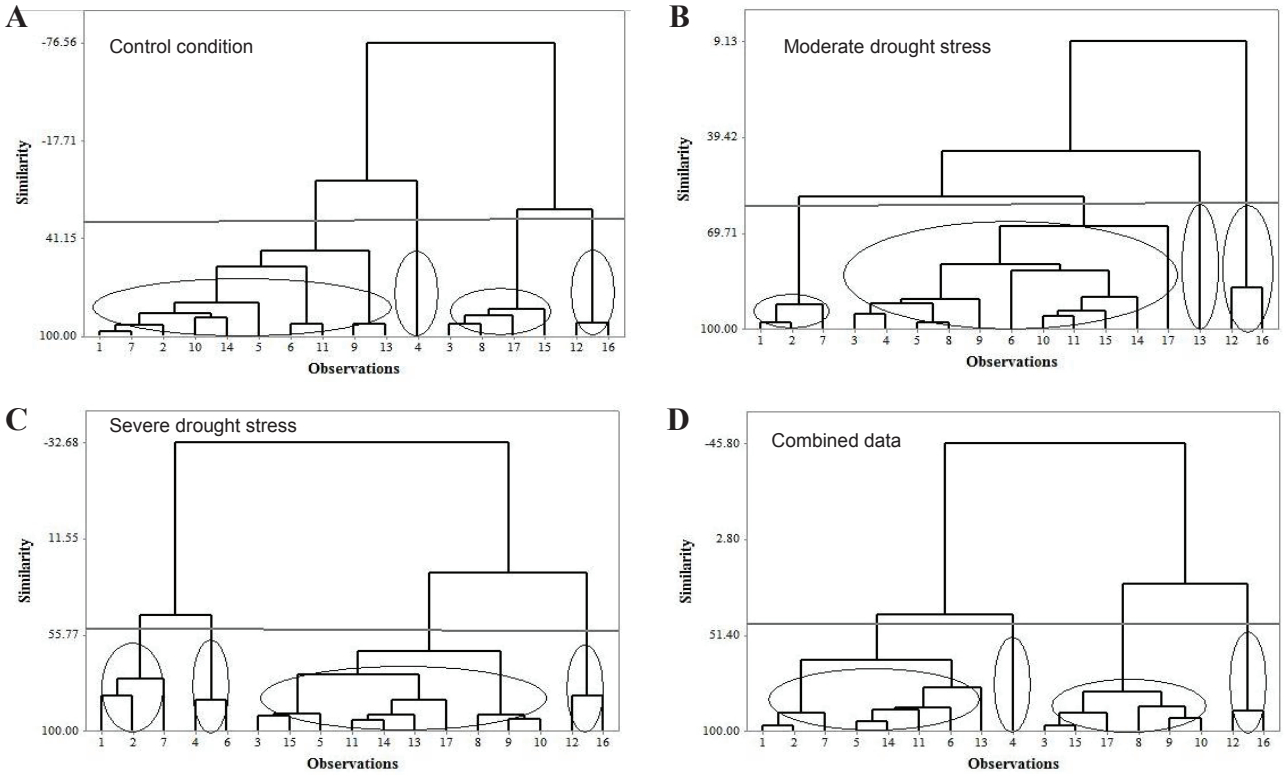

Figure 3. Tree diagram of 17 common bean genotypes based on different physiological traits; 1-E3, 2-Naz, 3-Kousha, 4-Sadri, 5-Dorsa, 6-Mahali Khomein , 7-E6, 8-Talash, 9-Azna, 10-Goli, 11-Colombia, 12-Cos16, 13-Ks31169 (line), 14-E8, 15-Ghafar, 16- D81083 (line), 17-Pak.

respectively (Figure 3B). Also, genotypes in cluster II and III can be introduced as semi-resistant to drought in moderate environment (Figure 3B). Also, at severe drought stress and based on Figure 3C, the genotypes were divided into four main groups. Cluster analysis of severe environment revealed that cluster I comprised of 3 tolerant genotypes. Cluster II consisted of 2 genotypes and cluster III of 10 genotypes, both of which are semi-resistant. Cluster IV contained 2 sensitive genotypes (Figure 3C). At combined data and based on Figure 3D, the investigated genotypes were grouped into four groups. Cluster analysis of combined data grouped 8 genotypes in cluster I as tolerant genotypes, 1 genotype in cluster II, 6 genotypes in cluster III as semi-resistant bean genotypes and finally 2 genotypes in cluster IV as sensitive genotypes to drought stress (Figure 3D).

Consequently, by comparing the above analyses and considering the results obtained from different drought stress conditions, genotypes 1 (E3), 2 (Naz) and 7 (E6) were introduced as tolerant and genotypes 12 (Cos16) and 16 (D81083) were introduced as sensitive common bean genotypes to drought stress. Production of tolerant hybrid cultivars to drought with maximum yield in breeding programs could be achieved by crossing genotypes from clusters I to genotypes from cluster IV because of the maximum genetic distance between these clusters (Shafiei *et al*., 2013).

CONCLUSIONS

The present study provided insights into drought tolerance in common bean genotypes for arid/semiarid regions like Iran. We used a combination of physiological and biochemical characteristics to evaluate common bean genotypes under moderate and severe drought stress. The results showed that, statistical methods including PCA, cluster analysis and biplot results facilitate the classification of genotypes and identification of the subset of core genotypes having tolerance to drought stress. Thus, these statistical methods are useful for the identification of drought tolerant common bean genotypes. Our finding suggested “E3”, “Naz” and “E6” as tolerant and genotype “Cos16” and line “D81083” as sensitive common bean genotypes to drought stress. Also, biochemical factors are suggested for identifying drought tolerant genotypes. However, further research studies are required to be performed in different climatic conditions.

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