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Use of Physiological Parameters for Screening Drought Tolerant Barley Genotypes

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Abstract
With the aim of understanding and identifying the traits which can be used as the suitable criteria for quick screening of the water deficit tolerant barley genotypes, an experiment based on randomized complete blocks design with three replications was conducted during two years to evaluate the biochemical responses of 20 barley genotypes to full irrigation and terminal water stress in the field condition. Results showed large genetic differences among barley genotypes in response to water deficit, which could be utilized in breeding programs. Proline, sucrose, glucose, fructose, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), auxin, gibberellin and abscisic acid (ABA) content were significantly affected by different irrigation conditions and genotypes. Water deficit significantly increased proline, carbohydrates accumulation and activities of CAT, SOD, GPX and ABA concentration in the barley genotypes. However, indole acetic acid (IAA) and gibberellic acid (GA3) contents decreased under the terminal water stress. Cluster analysis showed that genotypes 11, 18 and 19 had higher values of proline, fructose, glucose, IAA, GA3, GPX, CAT and SOD. These genotypes could be considered as drought tolerant genotypes which can tolerate unfavorable environmental conditions as compared to other genotypes through overproduction of some osmolytes, effective phyto-hormone signaling and better antioxidant enzymes activity for scavenging reactive oxygen species and consequently enhanced potential for production of higher grain yield. Thus, it seems that biochemical and phyto-hormonal responses could be introduced as desirable and suitable indicators for screening genotypes with better potential under water deficit stress condition.

Keywords: Antioxidant enzymes; Barley; Phyto-hormones; Proline; Soluble sugars

Introduction

Abiotic stresses are the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray *et al.* 2000). Drought stress is one of the most important environmental stresses that has adverse effects on plant productivity (Araus *et al.* 2002; Reddy *et al.* 2004).

In general, all stress conditions can increase reactive oxygen species (ROS). ROS are recognized as detrimental to biological systems because they cause the oxidation of lipids, proteins, deoxyribonucleic acid and carbohydrates. Ultimately, toxic levels of ROS causes a chain reaction of cellular oxidation, which results in unhealthy situations and lethality. In addition to the oxidative stress related products, ROS are an inevitable outcome of normal physiological processes, such as glycolysis and photosynthesis (Mittler *et al.* 2004). Plant antioxidant systems, both enzymatic and non-enzymatic, play an important role in balancing and preventing oxidative damage (Foyer *et al.* 1994; Baysal Furtana and Tıpırdamaz 2010). Enzymatic antioxidants consist of superoxide dismutase (SOD), (EC 1.15.1.1), ascorbate peroxidase (APX) (EC 1.11.1.11), catalase (CAT) (EC 1.11.1.6), peroxidase (POX) (EC 1.11.1.7), and glutathione reductase (GR) (EC 1.8.1.7) (Celik and Atak 2012).

In addition to the enzymatic scavenging systems, accumulation of proline is one of the important adaptive strategies of plants to cope with environmental stresses, particularly low water stress. Proline is also closely related to the plant drought stress as free proline can significantly accumulate in crops and other plants (Kim *et al.* 2004; Lee *et al.* 2009). Proline can accumulate to high concentrations in plant cells without disrupting cellular structure or metabolism. Therefore, proline accumulation plays an important role in osmotic adjustment, detoxification of ROS and membrane integrity in plants under stress conditions (Matysik *et al.* 2002; Demiralay *et al.* 2013).

Rapid changes in hormonal levels are commonly observed in response to stress in some plant species. Endogenous content of auxins, gibberellins and cytokinin usually decrease under drought, while those of abscisic acid (ABA) and ethylene increase (Nilsen and Orcutte 1996). Nevertheless, phytohormones play vital roles in drought tolerance of plants (Farooq *et al.* 2009). Thus, this research was designed to study the response of barley genotypes to limited water availability in terms of changes in biochemical compounds.

Materials and Methods

The experiment was conducted during 2011-2013 growing seasons at the Agricultural Research Station of Miyandoab (Latitude 36˚58' N, Longitude 46˚6' E, Altitude 1314 m), West Azarbaijan Province, Iran. Soil texture at the 30 cm depth was clay loam with pH=7.5-8 and EC= 2 ds/m^2. The experiment was laid out as a randomized complete block design with three replications in each growing season. Twenty barley genotypes were evaluated under full irrigation and terminal water stress conditions (withholding irrigation at anthesis). Seedbed preparation, weed control and use of fertilizer for the two experiments were similar. Each plot consisted of 6 rows with 5 m length, spaced 20 cm apart. Seeds were sown with a density of 400 seeds/m^2.

Proline analysis was performed according to Bates (1973). Glucose, fructose and sucrose were measured in the extracts as described by Morcuende *et al.* (2004). Extraction and purification of ABA, indole acetic acid (IAA) and gibberellic acid (GA3) were according to Yurekli *et al.* (2004). Catalase activity was measured using the method of Paglia and Valentine (1987). Glutathione peroxidase (GPX) activity was measured using the Paglia and Valentine (1987) method. SOD activity was assayed by a spectrophotometer at 560 nm based on the method used by Dhindsa *et al.* (1980). Analysis of variance and comparison of means by using Duncan's multiple range test at the 5% probability level were performed using the MSTATC software. Furthermore, the SPSS software was used for the cluster analysis of barley genotypes based on Ward's minimum variance method.

Results

Analysis of variance of data showed significant effect of water treatment on barley proline, sucrose, fructose, glucose, IAA, ABA, GPX, CAT and SOD. There were significant differences among

Table 1. Code and name of barley genotypes under study

Code	Genotype
1	(EDB82-9)Rhn-03//L.527/NK1272
2	Manitou//Alanda/Zafraa
3	Pamir-149/ Victoria
4	AcuarioT75/Azaf
5	Pamir-146//EA389-3/EA475-4
6	Alpha/Durra/Pamir-160
7	Pamir-013/Sonata
8	Robur/WA2196-68//Wysor
9	Bugar/DZ48-232
10	Rhn-03//Lignee527/NK1272/5/Lignee527/Chn-01/4/Lignee527/
11	Mnitou//Alanda/Zafraa
12	Kny/K-273
13	Pamir-065
14	Pamir-168
15	Prodcutiv/3/Rono//Alger/Ceres362-1-1
16	Belt67-1608/Slr/3/Dicktoo/Cascade//Hip/4/CWB117-77-9-7
17	Belt67-1608/Slr/3/Dicktoo/Cas
18	U.Sask.1766/Api//Cel/3Weeah/4/Lignee527/NK1272/5/Express
19	(EC82-6)TWWd85-37/Kavir
20	(Bahman)WA196-68,F1//Scotia I

genotypes for all of the characters under study. Interaction of genotype × irrigation was also significant for all traits (data not shown).

Proline content

Proline content was significantly increased by decreasing water availability. The highest proline content was obtained for genotypes 20, 18 and 2 under water deficit stress. The highest and the lowest increase in proline content due to water deficit rather than well watering were recorded for genotype 20 and 7, respectively (Figure 1).

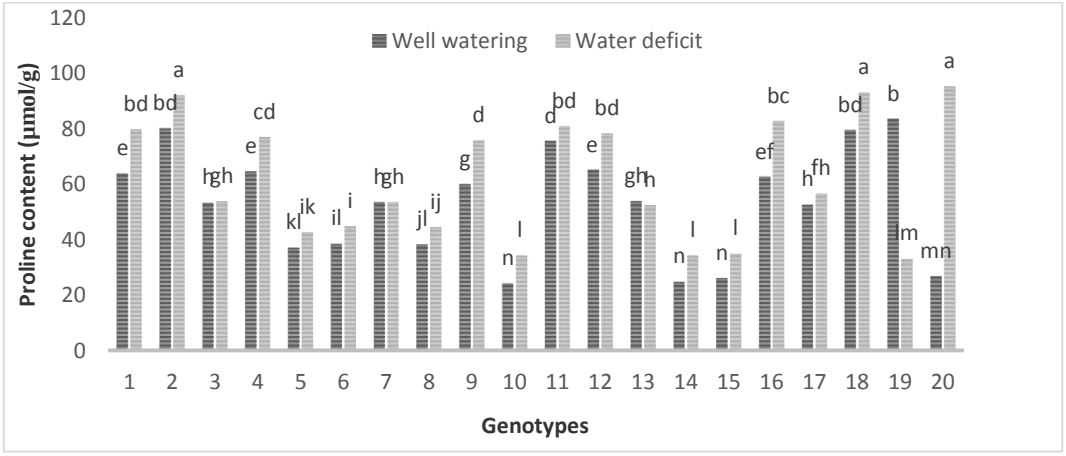

Figure 1. Proline content of barley genotypes affected by different water treatments. Different letters indicate significant difference at p≤ 0.05.

Sucrose, Fructose and Glucose contents

Barley genotypes showed different responses to water treatments in terms of sucrose content. Water deficit stress reduced the sucrose content in most of the genotypes under study. The highest reduction of sucrose content as a result of limited watering was observed for genotype 15. However, the lowest and the highest sucrose content under different irrigation treatments were obtained by the genotypes 11 and 20, respectively (Figure 2). Fructose content of barley leaves significantly increased by increasing the water deficit severity (data not shown). However, changes in fructose content to watering condition was different among barley genotypes. Genotypes 1 and 9 did not show any changes of fructose content in leaves by changing the water status. The highest increase in fructose content as a result of water deficit stress was recorded for the genotype 17 (Figure 3). Water deficit stress caused an increase in glucose content of barley genotypes except for the genotypes 9 and 19. However, some of these increases were not significant. The lowest and highest changes in glucose content due to water deficit stress were observed for the genotypes 15 and 7, respectively (Figure 4).

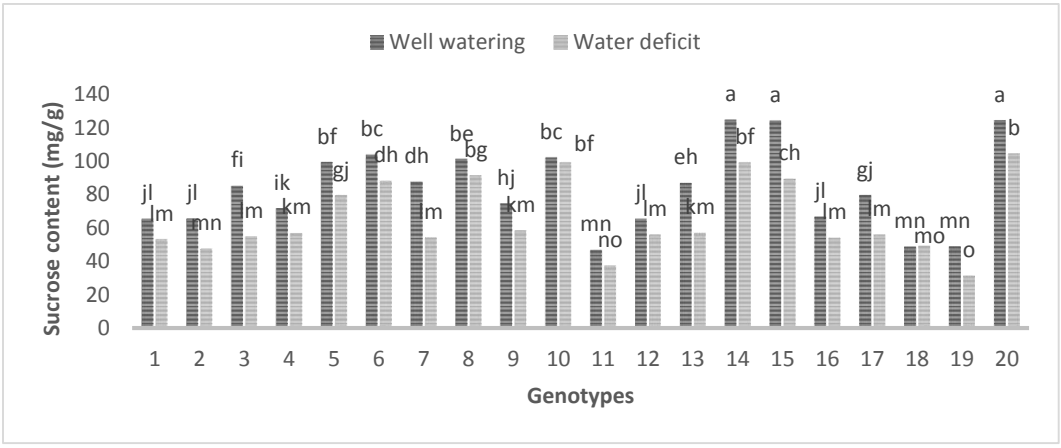

Figure 2. Sucrose content of barley genotypes affected by different water treatments Different letters indicate significant difference at p≤ 0.05.

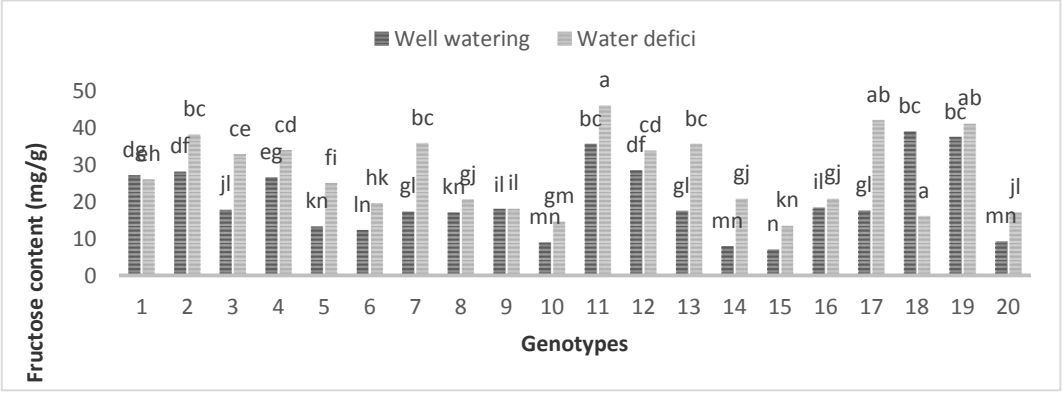

Figure 3. Fructose content of barley genotypes affected by different water treatments. Different letters indicate significant difference at p≤ 0.05.

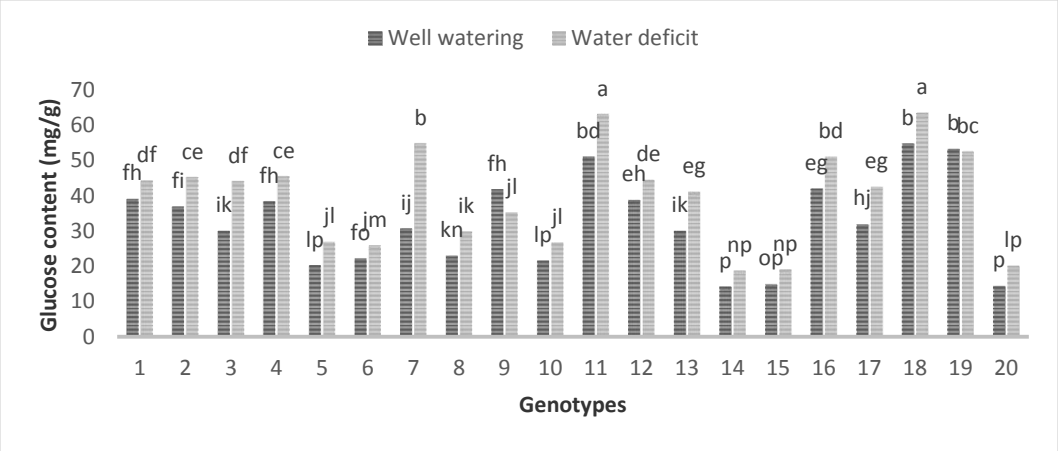

Figure 4. Glucose content of barley genotypes affected by different water treatments Different letters indicate significant difference at p≤ 0.05.

Hormone content

The highest and the lowest content of auxin under favorable water condition were obtained for the genotypes 19 and 5 with 361.3 and 171 ng/g, respectively. Under water deficit stress, the maximum and minimum auxin concentration were recorded for the genotypes 18 and 5, respectively. The auxin concentration in leaves of barley genotypes considerably diminished with declining in water availability during the experiment. The highest decline in auxin content as a result of water deficit stress was observed in genotype 4 (Table 2). Water deficit stress caused a reduction in GA content of barley genotypes. Changes in GA content due to limited irrigation was different among genotypes. Barley genotypes 19 and 20 responded to water deficit as an increase in GA content (Table 2), while the GA content of other genotypes decreased due to water stress. Our results indicated that the ABA concentration in leaves of barley genotypes increased with diminishing water availability. The highest change in ABA content of leaves due to water deficit was recorded for genotype 10 (Table 2).

Antioxidant enzyme activity

Barley genotypes differently responded to water deficit stress by changing in GPX content. In general, water deficit stress caused a significant rise in GPX activity, but increasing the GPX activity for some genotypes was more evident than others. Remarkable increase in GPX activity due to water stress was detected for the genotypes 6 and 16 (Table 3). At the water deficit stress condition, the activity of CAT was markedly increased in all 20 genotypes. The highest increase in CAT activity as a result of water deficit stress was obtained for the genotype 13 (Table 3). Water stress had different effect on SOD activities of studied genotypes. Under water deficit stress condition, the greatest SOD content was recorded for genotype 11 with 4242.5 nmol.mg-1 proteins. With reduction of water availability, genotypes 11, 19 and 18 showed the highest increase in SOD content (Table 3).

Table 2. Endogens hormone accumulation in barley genotypes affected by different water treatments

Genotype	Auxin (ng/g)		GA (ng/g)		ABA (ng/g)	
	Well watering	Limited irrigation	Well watering	Limited irrigation	Well watering	Limited irrigation
1	298.67d-g	282.00d-h	363.17a-f	326.83f-j	168.50h	183.17d-h
2	300.67c-f	259.67e-j	332.33e-i	310.00h-k	172.83gh	190.67d
3	206.83j-n	180.50m-p	288.17j-m	257.67l-n	137.33i-m	184.83d-g
4	304.83b-e	234.00h-m	373.50a-e	305.50h-k	172.67gh	190.50d
5	171.00no	137.67p	299.83no	184.50p	125.67l-n	140.50i-l
6	181.33m-p	177.17op	294.67i-l	231.33no	128.83k-n	145.83ij
7	263.83d-i	230.67h-m	317.83g-j	273.33k-n	137.33i-m	193.67d
8	203.33k-n	168.33n-p	266.00k-n	244.17m-o	127.00k-n	140.83i-l
9	294.33d-g	289.50d-g	402.33ab	365.67a-f	173.33f-h	151.00i
10	205.83j-n	188.33m-p	258.50l-n	208.50op	130.33j-n	215.17ab
11	249.67f-l	247.33f-l	331.00f-j	242.67no	193.67i-l	215.17 ab
12	358.67ab	313.67a-d	385.33a-c	342.50c-h	174.5e-h	186.00d-g
13	255.00e-k	215.67i-n	338.17d-i	295.33i-l	140.67i-l	189.17de
14	227.33i-m	186.50m-p	269.00k-n	204.00op	103.83op	122.00mn
15	195.17l-o	167.33n-p	253.67l-n	168.33p	100.33op	115.00no
16	316.17a-d	245.50g-l	359.33a-g	305.00h-k	173.00gh	198.83cd
17	256.50e-k	202.00k-n	344.50d-h	332.83e-i	142.67i-k	210.33bc
18	353.67a-c	316.00a-d	401.17ab	318.00g-j	198.83cd	229.50a
19	361.33a	300.50c-f	232.00no	377.83a-d	191.50d	215.00ab
20	172.67n-p	146.00op	310.00h-k	407.00a	94.33d-h	125.67a

Different letters within each hormone indicate significant difference at p≤0.05.

Table 3. Antioxidant enzymes activities in barley genotypes affected by different water treatments

Genotype	GPX (nmol/mg)		CAT (nmol/mg)		SOD (nmol/mg)	
	Well watering	Limited irrigation	Well watering	Limited irrigation	Well watering	Limited irrigation
1	255.5b	222.1cd	358.0fg	409.3bc	2711.0ef	2911.5de
2	192.0e-g	126.6h-j	325.0gh	352.3fg	2640.2fg	2931.7de
3	108.6i-k	211.5de	297.8hi	348.6fg	2126.8ij	2352.7hi
4	183.0fg	104.0j-l	291.6hi	306.6e-g	2579.8f-h	3036.2b-d
5	86.6k-m	135.6h	236.1k-m	273.1i-k	1118.3lm	1661.7k
6	10.35j-l	174.8g	230.3lm	273.0i-l	994.3m	1284.2l
7	127.0h-j	124.0h-j	299.0hi	353.0fg	2110.8ij	2536.2f-h
8	104.8j-l	206.3d-f	244.5j-m	264.6i-k	1137.0lm	1337.7l
9	187.1e-g	102.6j-l	345.0fg	369.0d-f	2549.5f-h	2927.0de
10	84l.0n	289.5a	236.1k-m	278.0ij	962.5mn	1620.5k
11	243.0bc	102.6j-l	398.0b-e	422.3ab	3260.8b	4242.5a
12	176.8g	206.1d-f	325.0gh	351.5fg	2550.5f-h	2985.8cd
13	129.6hi	174.5g	290.0hi	365.0d-f	2123.7ij	2478.8fg
14	61.8no	74.3m-o	185.1n	215.6mn	695.0no	871.8m-o
15	54.5o	74.1m-o	205.5mn	239.5j-m	650.7o	908.2m-o
16	17.3g	204.5d-f	344.5fg	374.1c-f	2461.5f-h	2448.0f-h
17	131.0hi	177.3g	292.8hi	362.3e-g	2000.8j	2430gh
18	255.1b	294.6a	402.1b-d	449.8k-m	3127.0b-d	4037.3a
19	249.8b	288.3a	411.3a-c	448.6a	3229.7bc	4146.8a
20	58.3o	76.6m-o	214.8mn	236.1k-m	655.5o	880.3m-o

Different letters within each hormone indicate significant difference at p≤0.05.

Cluster analysis

The cluster analysis under well watering condition based on standardized data grouped genotypes into three main clusters including the genotypes 1, 2, 3, 4, 7, 9, 12, 13, 16 and 17 in the first cluster; 11, 18 and 19 in the second cluster and 5, 6, 8, 10, 14, 15, and 20 in the third cluster (Figure 5). The first group had the highest ABA. The second group had the highest proline, fructose, glucose, IAA, GA, GPX and SOD. In the third group sucrose had the highest content (Table 4).

Table 4. Cluster means of barley traits under well watering condition

Cluster	Proline	Sucrose	Fructose	Glucose	Auxin	GA	ABA	GPX	CAT	SOD
1	60.96b	74.93b	21.75b	36.06b	285.55a	206.6a	156.43a	166.32b	316.97b	2385.5b
2	79.50a	48.22c	37.38a	53.11a	321.22a	251.33a	110.39b	249.33a	40.38a	3205.8a
3	30.85c	111.43a	10.33c	18.76c	193.81b	117.81b	155.95a	79.09c	221.6c	887.62c

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

The highest similarity was found among the genotypes 1, 2, 3, 4, 7, 9, 12, 13, 16 and 17 under water deficit stress condition (Figure 6). Genotypes fallen in the cluster 1 generally had higher values of sucrose, ABA and IAA. The second cluster consisted of genotypes 11, 18 and 19 which had the highest content of proline, fructose, glucose, IAA, GA, GPX, CAT and SOD. The third cluster comprised of the genotypes 5, 6, 8, 10, 14, 15 and 20. Its ABA content was not significantly different from the first cluster but was significantly higher than the second cluster (Table 5).

Table 5. Cluster means of barley traits under limited water condition

Cluster	Proline	Sucrose	Fructose	Glucose	Auxin	GA	ABA	GPX	CAT	SOD
1	70.25b	89.88a	31.73b	44.86b	245.40a	176.47b	189.68a	200.03b	364.62b	2703.8b
2	89.83a	55.03b	44.44a	59.72a	287.94a	233.83a	125.94b	290.83a	440.28a	4142.2a
3	38.45c	39.66c	17.85c	24.04c	162.62b	84.76c	177.40a	98.78c	254.31c	1223.5c

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

Discussion

The accumulation of free proline under stress conditions has been correlated with stress tolerance in many plant species, and concentrations are generally higher in stress-tolerant as opposed to stress-sensitive plants (Ashraf and Foolad 2007). Proline is considered as a potent antioxidant and potential inhibitor of plant death. Therefore, proline can now be regarded as non-enzymatic antioxidants that microbes, animals and plants require to mitigate the adverse effects of ROS (Chen and Dickman 2005). The increase in leaf proline content of barley genotypes (Figure 1) could be related with OH scavenging capacity, redox signaling and effective quenching of ROS (Alia and Pardha 1991). The higher accumulation of proline in the genotypes 18 and 20 as a result of water deficit stress confers advantages by protecting membranes and proteins (Reddy *et al.* 2004). Osmoregulation via proline molecules appears to be an essential part of the protection mechanism against drought stress in barley stress-tolerant genotypes.

Overproduction of different types of soluble sugars such as fructose and glucose in barley genotypes as a result of limited irrigation is one of the most common stress tolerance strategies in plants (Serraj and Sinclair 2002) which protect plants from stress through different ways such as contribution towards osmotic adjustment, detoxification of ROS, stabilization of membranes, and native structures of enzymes and proteins (Farooq *et al.* 2009). Remarkable accumulation of fructose and glucose in the genotypes 7 and 11 under water deficit stress could have been resulted through the reduction of osmotic potential of the cells, which increases water uptake and helps with the maintenance of turgor (Subbarao *et al.* 2000). Proline and soluble sugar accumulations were highly enriched in the drought-up-regulated genes, suggesting that those metabolic pathways are important mechanisms operating in drought tolerant genotypes (Umezava *et al.* 2006). Drought stress limits the production of endogenous auxins, usually when contents of abscisic acid and ethylene increase (Nilsen and Orcutte 1996). Lack of reduction or small decrease in auxin content of barley genotypes 6, 9, 11 and GA content of genotypes 8 and 17 could be related with auxin participation in signaling mechanisms of drought-induced proline accumulation (Sadiqov *et al.* 2002) and gibberrelic acids capacity in withstanding a prolonged drought period which give rise to a new functional root system (Vartanian *et al.* 1994). Abscisic acid is a growth inhibitor and is produced under a wide variety of environmental stresses, including drought. All plants respond to drought and many other stresses by accumulating abscisic acid (Farooq *et al.* 2009). An increase in ABA concentration in the leaves of barley genotypes (Table 2) under stress is thought to occur due to de novo synthesis or transport from roots (Zhang *et al.* 2004). Therefore, when plants are subjected to water deficit stress, ABA is produced in the roots

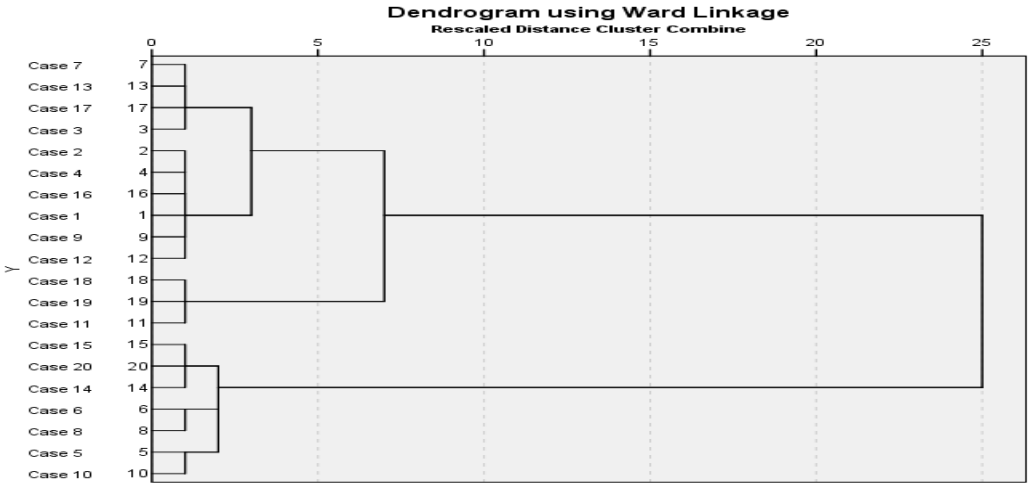

Figure 5. Dendrogram of 20 genotypes of barley constructed for traits under study using Ward method based on Euclidean distance under well watering condition.

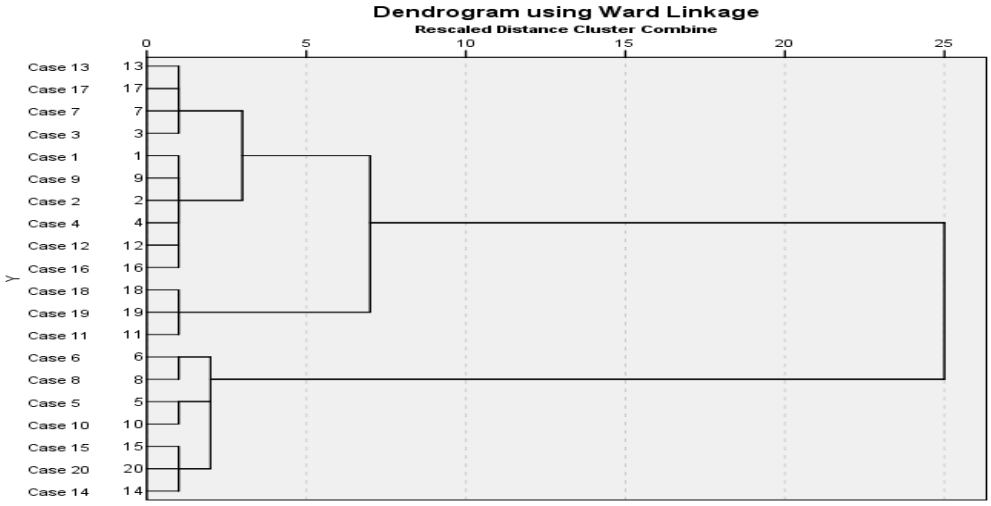

Figure 6. Dendrogram of 20 genotypes of barley constructed for traits under study using Ward method based on Euclidean distance under limited water condition.

and transports to leaves to close stomata and decrease water loss. Leaf ABA concentration have been found to be significantly correlated with leaf water status (Kannangara *et al.* 1982) or the osmotic potential of the root medium (Ribaut and Pilet 1991). Lower concentration of ABA in some genotypes (Table 2) could be related with greater leaf RWC and tolerance mechanism of drought-tolerant species (Yurekli *et al.* 2004).

Water dificit stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O2– and H2O2 in chloroplasts, mitochondria and peroxisomes. Therefore, plant cells need different mechanisms that will enable the detoxification of excess ROS and keep the formation and removal of ROS in balance (Pereira *et al*. 2002). Higher concentrations of antioxidant enzyme activities induced by drought stress (Table 3) may have removed the $O2^{\cdot}$ radicals and their product H2O2 (Sairam *et al*. 2000). Hydroxyl radicals attack bio molecules within cells and lead to strong metabolic disruptions (Mittler 2004). In the present experiment, the increase in CAT, GPX and SOD activities in barley genotypes caused by water stress (Table 3) appeared to be derived from effective capacity of these genotypes in handling the scavenge of ROS and therefore could be attributed to general adaptation strategy of plants to overcome oxidative stresses (Foyer and Noctor 2003).

Water deficit stress is responsible for the induction of oxidative stress and thus related damage is occurred by increase in the MDA content (i.e., lipid peroxidation), dityrosine accumulation, degradation of protein (Mohammadi *et al*. 2015). Therefore, enhanced antioxidative responses, as evident in the differential or varied levels of antioxidative enzyme activities (Table 3), such as accumulation of soluble sugars (Figures 2-4) and proline (Figure 1) could have been the protective mechanism of barley genotypes against oxidative damage. However, genotypes which were less tolerant accumulated lower amount of proline (Figure 1) and carbohydrates (Figures 2-4). In general, genotypes 11, 18 and 19 showed a much more pronounced antioxidative mechanisms after the induction of water stress and hence, they seem to be protected from the detrimental effects or

damage caused by oxidative stress as a result of water stress.

The results of cluster analysis showed that the genotypes fallen in the cluster 2, including genotypes 18, 19 and 11, generally had higher values of tolerance indices. It appeared that this cluster composed of a group of tolerant genotypes (Tables 4, 5).

Conclusion

The investigation of various biochemical characteristics showed a wide variation in water deficit tolerance of 20 barley genotypes. Thus, these genetic differences among barley genotypes can be used in breeding programs to produce appropriate genotypes at normal and water deficit stress conditions. They differed significantly in their osmolytes accumulation (proline and sugar), endogens hormone concentration and antioxidant enzymes (CAT, SOD, GPX) contents under water deficit stress condition. Considering all aspects of our study, it was revealed that the genotypes 11, 18 and 19 were more tolerant, whereas genotypes 5, 8, 10, 14, 15 and 20 were less tolerant or, in other words, more susceptible to water deficit stress. Our findings showed that among barley genotypes, genotype 11, due to better hormonal signaling to reduction in water availability and higher accumulation of osmolytes as a defensive mechanism, have been able to reduce cell water potential and enhance antioxidant enzymes activity. Consequently, this genotype could have tolerated the limited irrigation condition better than others which resulted in higher grain yield potential under unfavorable environmental condition (Sorkhilalehloo *et al.* 2014). According to our results and pervious findings (Sorkhilalehloo *et al*. 2014) the genotype 11 could be recommended as a tolerant genotype to water deficit stress and could be utilized as a genetic source in breeding programs. Finally, we may suggest the assessment of some morphological or any fast and cheaper measurable traits in the future experiments to find secondary traits for indirect selection in different barley breeding programs.

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