Qualitative and Physical Properties of Barley Grains under Terminal Drought Stress Conditions

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ABSTRACT

Drought stress is the major limitation for crop yield, which depending on the time of occurrence, could decrease the number of grain as well as their weight. Barley (*Hordeum vulgare* L.) is one of the tolerant cereals that its grain components have an important role in human and animal nutrition; however, physical and biochemical properties of grains affected through drought stress are still poorly understood. In this study, barley genotypes (n= 6) with different levels of drought tolerance were studied in a 2-year field experiment under well-watered and terminal drought stress conditions. In order to measure physical properties of grains, digital images were taken and some morphological features were obtained by using Image Analysis Toolbox of MATLAB software. Biochemical properties of grains were also measured. Results proved that size, weight and also quality of the grains were significantly affected by drought stress (P< 0.01). Grain starch content decreased and protein content increased under drought stress at anthesis stage in all genotypes, but drought-sensitive genotypes interestingly had more percentage increase in protein content. Furthermore, genotypes varied in total sugar, sucrose, glucose and fructose content. Drought stress affected grain size and finally 1,000-grain weight of barley genotypes by reducing area and minor axis length of grains. Correlations between 1,000-grain weight and minor axis length, grain area, starch and sucrose content were significant (P< 0.01). These results emphasized in both conditions that size-dependent features of grain particularly minor axis length and area may be serving as useful traits for estimation of 1,000-grain weight and biochemical properties in barley.

Keywords: Grain size, *Hordeum vulgare* L., Image analysis, Protein, Starch.

INTRODUCTION

Drought stress is one of the most serious abiotic stresses which has affected crop growth and yield worldwide (Alqudah *et al.,* 2011). In arid and semi arid areas such as Iran where an average annual precipitation is less than one third of global average precipitation (≈ 250 mm) (Bannayan *et al.,* 2010), grain yield of small grain cereals which are grown in these areas can be largely influenced by terminal drought stress (González *et al.,* 2008).

Grain yield in small grain cereals depends on two main components, fertile spike and grain number per m^2 and grain weight (Ugarte *et al.,* 2007). In literatures, it is well documented that grain number is a more

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important factor than grain size in increasing yield (Dolferus *et al.,* 2011). While in some areas with the history of drought and high temperature during grain filling stage, grain weight becomes a more effective factor than grain number per unit area (Cossani *et al.,* 2009). Yield components are affected by the time in which environmental stress occurres. In fact, because of determining grain number before anthesis stage, terminal abiotic stress affects grain size from anthesis onwards (Dolferus *et al.,* 2011). Among all crops, barley is later surpassed by maize, wheat, rice and soybean (Baik and Ullrich, 2008).

Barley (*Hordeum vulgare* L.) is one of the major cereals in the world, and its essential and useful nutrients made it a favorable food for humans and livestock (You and Izydorczyk, 2007). Barley is a principal crop in local cropping systems of Iran, too (Bannayan *et al.,* 2010). Also high tolerance to extreme environmental conditions made barley a suitable plant for cultivation in higher latitudes and altitudes and/or even in deserts (Schulman *et al.,* 2000; Baik and Ullrich, 2008). Nevertheless, drought stress reduces the number of grains per spike, grain weight and grain size in barley (Fox *et al.,* 2006; Samarah *et al.,* 2009). Several attempts have been made to indicate that grain size, in addition to grain quality, is a key factor in various international grain handling, i.e. transportation, marketing, storage, malting and also baking quality (CGC, 2006). Grain size which explains the morphological properties, can affect some nutrition values of grains. In previous studies it has been considered that larger grains usually have a higher level of starch and a lower level of protein (Burger and LaBerge, 1985). Starch is the most fundamental and final product of cereal grain which makes up about 70% of the barley grain weight (Jung *et al.,* 2008). It serves as the primary carbohydrate components in humans diet (Clarke *et al.,* 2008). Since, barley yield has been correlated with starch level in grains (Schulman *et al.,* 2000), therefore drought stress affects yield by reducing starch

content in barley grains (Thitisaksakul *et al.,* 2012). Contrary to starch, grain protein content can increase in water stress at grain filling stage (Zhao *et al.,* 2005).

For evaluating grain quality, there is an effort to develop a rapid, precise and non-destructive technique based on the identification of specific attributes such as grain size (Walker and Panozzo, 2012). Digital image analysis is an application of computer science in which a large amount of data including morphological features are extracted from a digital image (Shouche *et al.,* 2001). Area, perimeter, length and width are the most common used features for size measurements of an object. Shouche *et al*. (2001) measured geometric features such as area, perimeter and length of major and minor axes for shape feature analysis of Indian wheat varieties. Although geometric features/studies may especially be useful in the field of grains discrimination (Majumdar and Jayas, 2000), questions have been raised about the efficacy of image processing by computer-based programming in estimation of quality-based properties of grains.

There are many research studies in which the effects of drought stress on grain yield of crops during seed filling stage were investigated. But, there has been little agreement on effects of drought stress at anthesis stage on seed quality. Also according to authors, to date, no studies have been done using image processing to distinguish reactions of barley grains physical properties to terminal drought stress and finding the relations between the physical and biochemical properties of barley grains in normal and drought stress conditions. Since the physical and biochemical characteristics play a pivotal role in grain yield, we aimed to investigate the effect of drought stress at anthesis stage on some metabolites (protein, starch, total water-soluble carbohydrates, sucrose, glucose and fructose) of barley grains. We were also interested in studying two following objectives: to determine the physical properties of grains *via* image processing technique, and to examine the

correlation between biochemical and physical properties of grain in normal and drought stress conditions. Finally the response of different barley grain genotypes to terminal drought stress can be discussed.

MATERIALS AND METHODS

Plant Material and Condition

For the present study, six barley lines and Iranian cultivars (Table 1) with different levels of drought tolerance, namely Yousof, PBYT 46 and PBYT 97 drought tolerant, Morocco semi-drought tolerant, Fajr 30 and Nosrat drought-sensitive (all of the genotypes are six-rowed genotypes except Morocco, which is a two-rowed barley cultivar) were evaluated in two separated field experiments under well-watered (normal irrigation according to plant requirements) and drought stress (stop irrigation at anthesis stage until the end of the growth period) conditions in Yazd Province (31° 54' North latitude, 54° 17' East longitude, and 1,237 m altitude) of Iran during two growing seasons: 2010-2011 and 2011-2012. Maximum and minimum air temperatures and total monthly precipitation were recorded at the meteorological station located near the experimental fields (Table 2).

Plants were grown in the same conditions until anthesis at Zadoks stage 50 (Zadoks *et al*., 1974), under well-watered conditions. The drought treatment was started by withholding water at the anthesis. The experiment was carried out on RCBD (Randomized Complete Block Design) with three replications. Each experimental unit consisted of a 12 m^2 plot (12 rows 5 m long and 0.2 m between row distances). Seed density was 350 seeds m^{-2}. Spikes were harvested after ripening, in May 2011 and 2012. Grains were separated from the spikes and 1,000-grain weight of each plot was measured by scales (Sartorius ed124s, max 120, d= 0.1 mg, USA). Also, the grain yield of each plot was obtained.

Image Analysis

The grains (n= 240 per plot and in total 17,280) were scanned by a scanner (HP Laser Jet M1132 MFP, Idaho, USA) (Figure 1-a). Image analysis started with transferring RGB image to grayscale one (Figure 1-b). Then the contrast of the gray-scale image was adjusted using *imadjust* formula (Figure 1-c). The threshold value of the gray-scale image was determined using *graythresh* mode and finally the binary image got based on it (Figure 1-d). Actually, threshold is a value of pixel which separates grains from the background. The typical histogram of the gray-scale image of the grain is shown in Figure 2. The grains in binary image were labeled, before extracting some morphological properties using *regionprops* mode. These properties included area, perimeter, major axis length and minor axis length. All steps were performed using Image Analysis Toolbox of MATLAB ver. 7.1 software.

Measurements of Grain Quality

After image processing, grains from each plot of growing season 2011-2012 were finely ground in a mill (Poly mix, PX-MFC 90 D, dispersing and mixing technology by Kinematica AG, CH-6014 Luzern, Switzerland) fitted with a 0.5 mm screen to produce barley flour and analyze the flour for protein, starch and sugar content.

Grain Protein

Total protein was measured according to the Bradford method (1976). In the first step 1.5 ml extraction buffer (potassium phosphate 50 mmol, pH= 7) was added to 0.03 g barley grain flour, then the mixture was shaken and centrifuged (Eppendorf 5417 R) for 20 min at 8,000 rpm at 4°C. In the next step 40 µl of extraction was mixed to 1 ml Bradford 20% (v/v), the mixture was shaken for 5 min in temperature room. The

Table 1. List of barley genotypes analyzed in this study during two growing seasons (2010-2011 and 2011-2012), Yazd-Iran.

Genotype	Origin	Spike type	Pedigree
Yousof	Iran	Six-rowed	L.527/Chn–01//Gostoe/4/Rhn–08/3/Deir Alla106//DL.71/Strain205
Fajr 30	Iran	Six-rowed	Lignee131/Gerbel//Alger-Ceres/3/Jonoob
Nosrat	Iran	Six-rowed	Karoon/Kavir
PBYT 46	Iran	Six-rowed	PETUNIA 2/3/GLORIA- BAR/COME//ESPERANZA/4/CABUYA
PBYT 97	Iran	Six-rowed	Lignee527/NK1272//JLB70-063/3/IPA99
Morocco	International Center for Agricultural Research in the Dry Areas (ICARDA)	Two-rowed	

Table 2. Minimum and maximum air temperatures and total monthly precipitation during two growing seasons (2010-2011 and 2011-2012), Yazd-Iran.

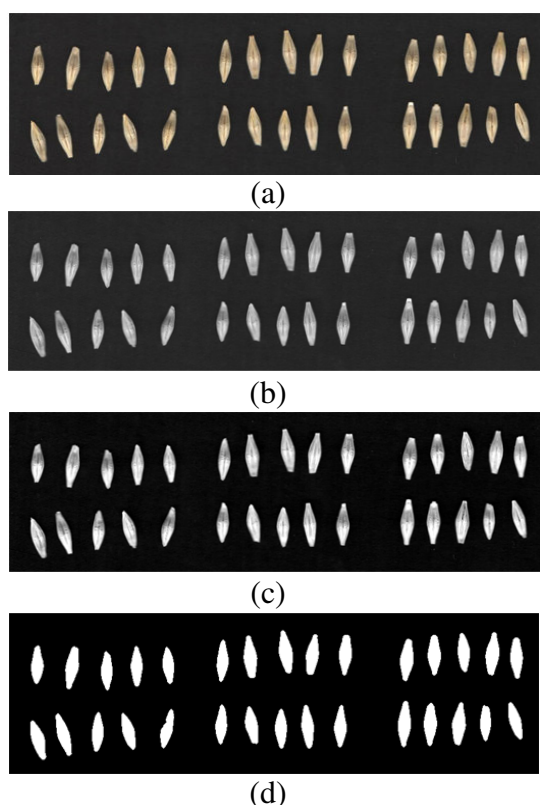| Month	Minimum temperature (°C)		Maximum temperature (°C)		Total monthly precipitation (mm)	
	2010- 2011	2011- 2012	2010- 2011	2011- 2012	2010- 2011	2011- 2012
October	0.8	0.2	30.4	29.6	0.2	2.9
November	0	-2.2	25.6	19	0.2	12.8
December	-4.4	-4	20.4	23.4	9.3	10.9
January	-2	-6	19.8	21	13.8	1.1
February	-1.4	-6	28.2	29.4	5.2	0.2
March	6.2	-0.8	32.4	30.8	4.7	6
April	15.6	12.4	39.2	35.3	0.3	0.2
May	19.8	18.8	43	41	0	0

Figure 1. Image analysis steps of barley grains. (a) Input image; (b) Grayscale image; (c) Adjusted grayscale image, and (d) Binary image.

Figure 2. The histogram of gray-scale image of barley grains.

protein concentration was determined using a standard curve with samples of known protein concentrations (Bovine serum albumin). Then the absorbance at 595 nm was recorded using a spectrophotometer (Cary 300 Scan, Santa Clara, California, USA).

Grain Water-soluble Carbohydrates

Total soluble sugar content was measured using the phenol-sulfuric acid method with some modification (AOAC, 1995). First 1.5 ml of 80% ethanol (v/v) 65°C was added to 0.03 g barley grain flour, and centrifuged for 10 minutes (Eppendorf 5417 R) in 3,000 rpm; the supernatant was transferred into a test tube. This stage was double repeated with hot distilled water (65°C), and in each time, the supernatant was transferred in to the same test tube. Furthermore the test tube was placed in
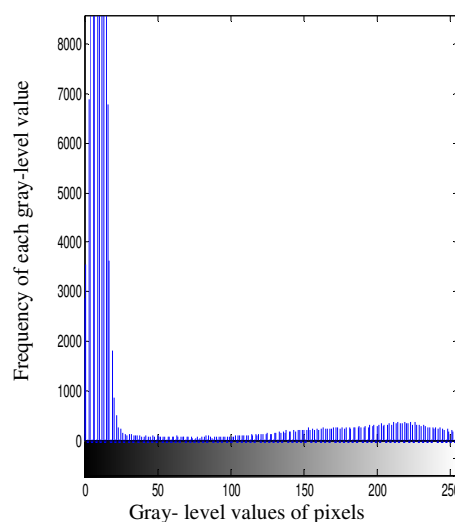
the oven at 45°C to be completely evaporated to reach dryness. Then, 1 ml of distilled water was added to the dried extract and mixed for 20 minutes. This was continued by mixing 500 µl of the solution, 1,250 µl sulfuric acid 98% and 250 µl phenol 5% (w/v) together in a new test tube, and leaving in room temperature for 45 minutes. Finally, its absorbance was recorded at 485 nm using a micro plate reader (Infinite M 200 pro). A calibration curve, in which *D*-glucose was used as standard, was prepared.

The rest of 500 µl of the solution was filtered through a 0.45 µm PTFE filter (Waters, Milford, MA, U.S.A), and 20 µl of each sample was injected to the HPLC system (Knauer, Wissenschaftliche Geräte GmbH, Germany), RI detector with a flow rate of 1 ml min⁻¹. The three sugars (sucrose, glucose and fructose) were separated on an analytical column (Eurokat –H column, column temperature 40°C) using a mobile phase of sulfuric acid solution (0.02N). Sugars were quantified from standard curves.

Grain Starch

Starch concentration was measured based on acidic hydrolysis method with some modification (AOAC, 1995). After

separating supernatants in sugar measurement, the residual pellets were placed in an oven at 50°C to dry. Then 4.5 ml distilled water and 6 ml perchloric acid 52% (v/v) were added to pellets in test tubes. The test tubes were placed over the night in a cold room (4°C). On the next day, samples were filtered with Whatman paper (Cat No. 1440 125) in a new test tube and finally the solution was brought up to 30 ml by adding distilled water. It was developed by mixing 500 µl of the solution, 1,250 µl sulfuric acid 98% and 250 µl phenol 5% (w/v) together in a new test tube, and leaving it in room temperature for 45 minutes, Finally, its absorbance was recorded at 485 nm using a micro plate reader (infinite M200 pro, Tecan Trading AG, Switzerland).

Statistical Analysis

Analysis Of Variance (ANOVA) was carried out with SAS (Statistical Analysis System ver. 9.1) and means were compared using by the *LSD* test (P< 0.05). The homogeneity of variance in grain quality traits was tested with Bartlett's χ^2 test and it was not significant, therefore combined analysis was performed.

RESULTS

Grain Yield

The differences between treatments, genotypes and their interactions (ANOVA) were highly significant in grain yield (P< 0.01) (data not shown; means are summarized in Table 3). Drought stress caused a significant reduction in grain yield of all genotypes in two growing seasons (Table 3), but the highest reduction in grain yield was obtained from Fajr 30 (95.79 and 87.83% in the first and second year, respectively) and Nosrat (94.48 and 88.59% in the first and second year, respectively) in both years (Table 3). Interestingly, these two genotypes had higher grain yield than the

others in well-watered conditions during two growing seasons (Table 3). It means that low yield potential genotypes had lower grain yield reduction in response to drought stress. These results are in accordance with the findings of Rizza *et al*. (2012) who reported "higher yield potential associated to lower stability".

Also, there was a significant difference among 1,000-grain weight of genotypes and drought stress significantly affected this trait (P< 0.01). When the barley plants were exposed to terminal drought stress, the 1,000-grain weight of all of genotypes decreased (Table 3). The data presented in Table 3 showed that the highest reduction (59.12%) in 1,000-grain weight occurred in Fajr 30 in 2011-2012, while the lowest reduction (9.32%) was in Morocco in the drought stress compared to the well-watered treatment in 2010-2011 (Table 3). The comparison of 1,000-grain weight in two growing seasons showed that higher reduction in this trait in drought stress conditions occurred in 2011-2012 (Table 3). It seems that it is due to more reduction in grain filling duration in drought stress conditions in the second year (Table 3).

The number of grain per spike was significantly affected by treatments, genotypes and their interactions in two growing seasons (P< 0.01). Drought stress significantly reduced the number of grain per spike in all genotypes (Table 3). In both conditions and two growing seasons, Morocco as a two-rowed barley cultivar had the lowest grain number per spike, but the highest reduction in the number of grains per spike was obtained from Nosrat (35.09% in the first year) and Fajr 30 (44.84% in the second year) (Table 3).

Image Analysis

Results of image analysis in both growing seasons showed that area and minor axis length of grains were significantly affected by drought stress (P< 0.01), but the effect of

Table 3. Physical properties of grain, grain filling duration, number of grain per spike, 1000-grain weight and grain yield of different barley genotypes in 2 years of field experimentation with Well-Watered (WW) and terminal Drought Stress (DS) trials. Different letters in each column within the same year indicate significant differences (P< 0.05) according to *LSD* Test.

	Grain area (cm²)		Grain perimeter (cm)		Major axis length of grain (cm)		Minor axis length of grain (cm)		Grain filling duration (Day)		Number of grain per spike		1000- grain weight (g)		Grain yield (Kg ha⁻¹)	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
2010- 2011 Genotypes																
Yousof	0.160 a	0.129 a	0.654 a	0.605 a	0.961 a	0.904 a	0.268 ab	0.230 b	50.0 a	36.3 a	45.69 a	38.28 a	38.77 a	28.73 b	7384.0 bc	2038.06 a
Fajr 30	0.136 cd	0.128 a	0.606 b	0.594 ab	0.898 ab	0.881 ab	0.243 c	0.235 b	44.3 b	29.7 a	43.48 ab	28.60 bc	31.90 c	28.43 b	8390.3 ab	352.91 d
Nosrat	0.151 abc	0.134 a	0.648 a	0.623 a	0.956 a	0.929 a	0.254 abc	0.234 b	46.3 b	30.3 a	41.66 abc	27.04 c	34.83 b	30.37 ab	8684.4 a	478.96 d
PBYT 46	0.155 ab	0.140 a	0.628 ab	0.599 ab	0915 ab	0.880 ab	0.273 a	0.255 a	51.0 a	36.3 a	37.63 bc	31.70 b	37.57 a	29.87 ab	5829.7 d	1478.89 b
PBYT 97	0.125 d	0.113 b	0.561 c	0.561 b	0.812 c	0.836 bc	0.249 bc	0.220 b	52.7 a	37.0 a	35.07 c	31.86 b	32.52 c	21.40 c	7392.5 bc	1512.50 b
Morocco	0.141 bcd	0.133 a	0.598 bc	0.562 b	0.876 bc	0.821 c	0.259 abc	0.261 a	44.0 b	36.0 a	22.92 d	17.87 d	35.40 b	32.10 a	6923.4 cd	1151.94 c
Mean	0.145	0.130	0.616	0.591	0.903	0.875	0.258	0.239	48.1	34.3	37.74	29.23	35.16	28.48	7434.06	1168.88
CV (%)	6.86	5.66	3.74	3.48	4.27	3.58	4.31	3.99	3.87	19.84	10.17	6.69	2.74	5.40	9.24	6.14
2011- 2012 Genotypes																
Yousof	0.148 a	0.116 ab	0.670 a	0.625 a	0.977 ab	0.952 a	0.295 a	0.238 b	49.7 ab	23.0 a	35.07 b	29.95 a	36.13 b	20.53 a	5284.9 ab	891.9 b
Fajr 30	0.147 a	0.118 ab	0.682 a	0.660 a	1.004 a	1.014 a	0.283 a	0.227 b	42.0 bc	15.7 b	42.42 a	23.40 b	31.80 c	13.0 d	5768.2 ab	701.9 b
Nosrat	0.138 a	0.111 b	0.645 ab	0.653 a	0.931 abc	1.042 a	0.287 a	0.205 c	44.0 bc	15.3 b	39.03 ab	30.10 a	37.27 ab	18.27 b	6727.0 a	767.6 b
PBYT 46	0.135 a	0.132 a	0.634 ab	0.649 a	0.904 bcd	0.965 a	0.294 a	0.265 a	48.0 abc	22.7 a	43.07 a	28.90 a	37.47 ab	20.40 a	5113.7 b	776.0 b
PBYT 97	0.125 a	0.104 b	0.621 ab	0.598 a	0.852 cd	0.904 a	0.298 a	0.222 bc	53.3 a	21.3 a	38.30 ab	29.09 a	30.13 d	17.50 b	6196.4 ab	1519.8 a
Morocco	0.137 a	0.108 b	0.605 b	0.587 a	0.850 d	0.882 a	0.312 a	0.236 b	39.7 c	12.0 c	18.77 c	17.37 c	37.80 a	16.33 c	5145.7 b	857.4 b
Mean	0.138	0.115	0.643	0.629	0.920	0.960	0.295	0.232	46.1	18.3	36.11	26.47	35.10	17.57	5705.99	919.10
CV (%)	9.39	8.99	5.21	7.64	4.78	9.89	5.54	4.63	10.25	8.68	8.88	7.53	2.23	2.48	13.91	13.51

Least Significant Differences (LSD)

	Grain area (cm²)	Grain perimeter (cm)	Major axis length of grain (cm)	Minor axis length of grain (cm)	Grain filling duration (Day)	Number of grain (Per spike)	Thousand grain weight (g)	Grain yield (Kg ha⁻¹)
Genotypes (G)	0.0085 ***	0.0273 ***	0.0478 ***	0.0101 ***	3.5625 ***	2.3634 ***	0.8356 ***	437.05 ***
Treatment (T)	0.0049 ***	0.0158 *	0.0276 ns	0.0058 ***	2.0568 ***	1.3645 ***	0.4824 ***	252.33 ***
Year (Y)	0.0049 ***	0.0158 ***	0.0276 ***	0.0058 ***	2.0568 ***	1.3645 **	0.4824 ***	252.33 ***
G×T	0.012 ns	0.0386 ns	0.0676 ns	0.0142 *	5.0381 ns	3.3424 ***	1.1818 ***	618.09 ***
G×Y	0.012 ns	0.0386 ns	0.0676 ns	0.0142 ns	5.0381 ns	3.3424 ***	1.1818 ***	618.09 ns
T×Y	0.007 ns	0.0223 ns	0.039 *	0.0082 ***	2.9087 ***	1.9297 ns	0.6823 ***	356.85 ***
G×T×Y	0.017 ns	0.0546 ns	0.0956 ns	0.0201 *	7.1249 ns	4.7268 *	1.6713 *	874.11 *

drought stress was not significant on major axis length and perimeter. Genotypes varied significantly in area, major axis length, minor axis length and perimeter (P< 0.01) In general, physical properties of grains were lower in drought stress than well-watered conditions except in 2011-2012 in major axis length where the difference was not significant (Table 3). The differences between well-watered and drought stress conditions in area and minor axis length traits were higher in the second year than in the first year (Table 3). In both growing seasons, the highest grain areas in well-watered and drought stress conditions were obtained from Yousof and PBYT 46, respectively (Table 3).

Grain Protein

Grain protein content was affected by both drought stress and genotypes, significantly (P< 0.01). In both well-watered and drought stress conditions, the highest and lowest values of protein in grain were obtained from PBYT 97 and Morocco, respectively (Figure 3-a). Grain protein content of PBYT 97 line measured in 2011–2012 was significantly higher than the other genotypes in both well-watered and drought stress conditions (Figure 3-a).

In this study, although genotypes remarkably varied in grain protein content, but it should be noted that one of the genotypes was different in terms of morphological characteristics. Morocco, as a two-rowed barley cultivar, had less protein content compared to the other genotypes (six-rowed genotypes). In agreement with other studies (Bleidere, 2008), grain protein content was higher in six-rowed barley genotypes than two-rowed barley genotypes. Grain protein content increased in drought stress condition compared to well-watered condition (Table 3). The highest (46.88%) and the lowest (1.51%) increase in protein content were obtained from Fajr 30 and PBYT 97, respectively (Figure 3-a).

Grain Starch

Starch content was significantly affected by genotypes, treatments and their interaction (P< 0.01). Indeed, drought stress decreased grain starch content in all genotypes (Figure 3-b), and the highest and the lowest grain starch was obtained from PBYT 46 line in normal and drought conditions, respectively (Figure 3-b). So, PBYT 46 line had the most reduction (38.19%) in grain starch content in the terminal drought stress condition compared to the well-watered condition (Figure 3-b).

Grain Water-Soluble Carbohydrates

The differences in total water-soluble carbohydrates, sucrose, glucose and fructose among genotypes and between treatments, as well as their interaction, were highly significant (P< 0.01). The reaction of barley genotypes to drought stress differed in grain sugar content (Figure 4), and the value of total water soluble carbohydrates increased significantly in Morocco, but reduced in the other ones (Yousof, Fajr 30, Nosrat, PBYT 46 and PBYT 97) (Figure 4). These findings indicated that the levels of sucrose, glucose and fructose increased in Morocco genotype under drought stress condition (Figure 4).

Correlation

In both growing seasons, there was a high positive significant correlation between 1,000-grain weight and grain area as well as minor axis length (P< 0.01) (Table 4). Also, according to the significant correlation between 1,000-grain weight and grain yield (P< 0.01), high significant correlations were observed between grain yield and grain area as well as minor axis length (P< 0.01) (Table 4).

Grain yield and 1,000-grain weight were positively correlated with starch and sucrose content of grains (P< 0.05 and P< 0.01), but there was a negative correlation between

Table 4. Pearson correlation coefficients among measured traits on 6 barley genotypes in control and terminal drought stress conditions in field experimentation of Yazd-Iran (2010-2011, 2011-2012).

Grain Parameters	Years	Area	Major axis length	Minor axis length	Perimeter	Starch content	Protein content	Total sugars	Sucrose content	Glucose content	Fructose content	Thousand grain weight	Yield
Area	2010-11	1											
	2011-12	1											
Major axis length	2010-11	0.73^{**}	1										
	2011-12	0.36^{*}	1										
Minor axis length	2010-11	0.81^{**}	0.19^{ns}	1									
	2011-12	0.77^{**}	-0.29^{ns}	1									
Perimeter	2010-11	0.82^{**}	0.98^{**}	0.35^{*}	1								
	2011-12	0.67^{**}	0.88^{**}	0.14^{ns}	1								
Starch content	2010-11	nd	nd	nd	nd	1							
	2011-12	0.18^{ns}	-0.31^{ns}	0.44^{**}	-0.09^{ns}	1							
Protein content	2010-11	nd	nd	nd	nd	nd	1						
	2011-12	-0.30^{ns}	-0.02^{ns}	-0.25^{ns}	-0.07^{ns}	-0.26^{ns}	nd						
Total sugars	2010-11	nd	nd	nd	nd	nd	nd	1					
	2011-12	0.06^{ns}	-0.18^{ns}	0.20^{ns}	0.01^{ns}	0.23^{ns}	0.07^{ns}	nd					
Sucrose content	2010-11	nd	nd	nd	nd	nd	nd	nd	1				
	2011-12	0.03^{ns}	-0.36^{*}	0.28^{ns}	-0.22^{ns}	0.36^{*}	0.25^{ns}	0.16^{ns}	nd				
Glucose content	2010-11	nd	nd	nd	nd	nd	nd	nd	nd	1			
	2011-12	-0.21^{ns}	-0.09^{ns}	-0.21^{ns}	-0.21^{ns}	-0.07^{ns}	-0.36^{*}	0.33^{*}	0.05^{ns}	nd			
Fructose content	2010-11	nd	nd	nd	nd	nd	nd	nd	nd	nd	1		
	2011-12	-0.16^{ns}	-0.18^{ns}	-0.05^{ns}	-0.17^{ns}	0.36^{*}	0.05^{ns}	0.27^{ns}	0.74^{**}	0.47^{**}	nd		
Thousand grain weight	2010-11	0.78^{**}	0.39^{*}	0.80^{**}	0.51^{**}	nd	nd	nd	nd	nd	nd	1	
	2011-12	0.66^{*}	-0.24^{ns}	0.85^{**}	0.13^{ns}	0.64^{**}	-0.35^{*}	0.04^{ns}	0.38^{*}	-0.29^{ns}	0.07^{ns}	1	
Yield	2010-11	0.47^{**}	0.27^{ns}	0.43^{**}	0.35^{*}	nd	nd	nd	nd	nd	nd	0.64^{**}	1
	2011-12	0.57^{**}	-0.25^{ns}	0.80^{**}	0.14^{ns}	0.63^{**}	-0.24^{ns}	0.31^{ns}	0.45^{**}	-0.22^{ns}	0.15^{ns}	0.91^{**}	1

* and **: Significant at $P < 0.05$ and $P < 0.01$, respectively and ns: Not-significant.
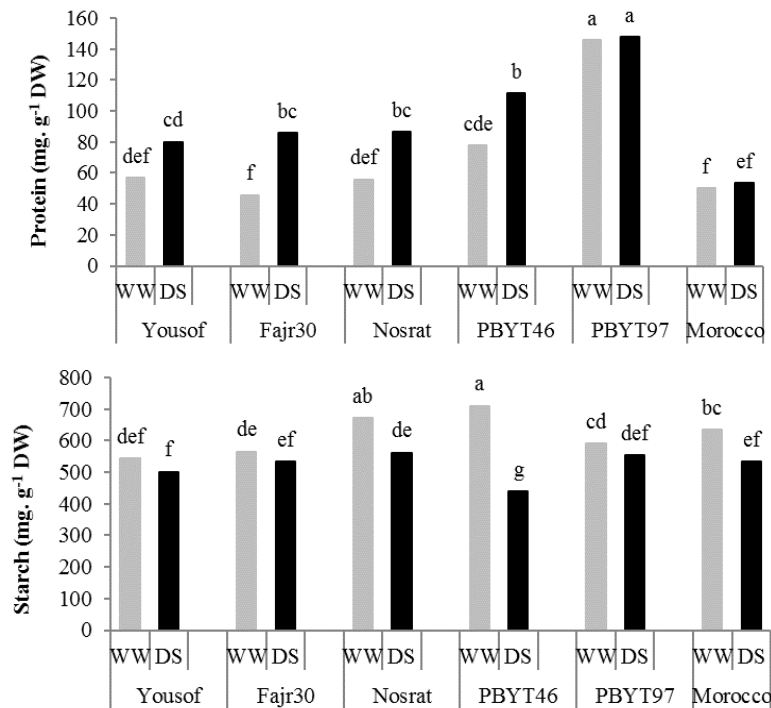
Figure 3. Grain protein content (a) and grain starch content (b) of different barley genotypes in Well-Watered (WW) and terminal Drought Stress (DS) conditions in field experimentation of Yazd- Iran (2011-2012). Means for each trait, followed by a similar letter are not significantly different at *P*<0.05 according to *LSD* Test.
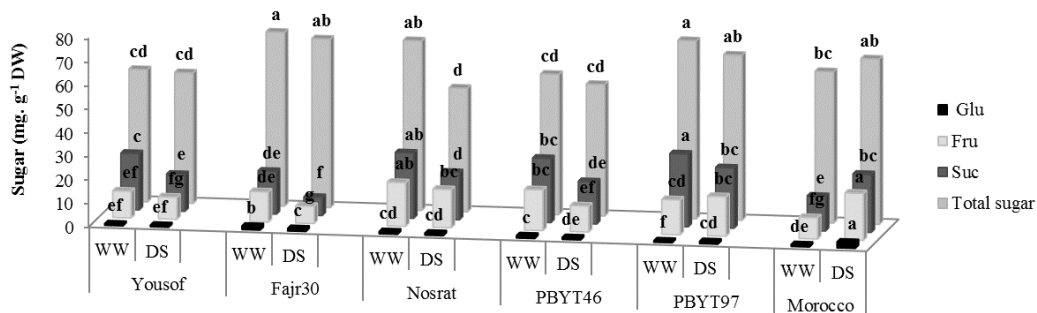

Figure 4. Grain total sugar, sucrose, glucose and fructose content of different barley genotypes in Well-Watered (WW) and terminal Drought Stress (DS) conditions in field experimentation of Yazd- Iran (2011-2012). Means for each trait, followed by a similar letter are not significantly different at *P*< 0.05 according to *LSD* Test

1,000-grain weight and grain protein content (P< 0.05) (Table 4), so it seems that larger grains have less protein contents. Even under well-watered condition, correlation between grain protein content and 1,000-grain weight was significantly negative (P<

0.01) (the correlation results of well-watered condition are not presented separately).

Correlation coefficients among physical property traits showed that perimeter was positively correlated with major axis length in both years (P< 0.01) (Table 4), and it

seems that drought stress did not reduce major axis length or the perimeter of grains. In contrast, drought stress decreased minor axis length of grains (Table 3). Also, there was a significant positive correlation between area and minor axis length as well as major axis length (Table 4), so any decrease in minor and major axis length of grains led to decrease in grain area. Also, major axis length was negatively correlated with sucrose content ($P< 0.05$) (Table 4).

DISCUSSION

Drought stress is one of the most important abiotic stresses limiting crop yield and production (Alqudah *et al.,* 2011). Although grain number is primarily a more noticeable factor than grain size in determining yield (Dolferus *et al.,* 2011), but grain weight may play a more decisive role than grain number per spike in terminal drought stress condition (Cossani *et al.,* 2009). Since grain weight is associated with its physical properties in well-watered and drought stress conditions, therefore the estimation of grain size by image processing, as a relatively new technique in agricultural studies, can considerably be an efficient way to predict grain weight in both conditions. However, the exact changes in barley grain physical properties under terminal drought stress are still not clear. In this study, it is well documented that barley genotypes varied significantly in area, major axis length, minor axis length and perimeter of grains. It has been reported that size-dependent features varied among Indian wheat varieties (Shouche *et al.,* 2001). The area and minor axis length of the grain were reduced by drought stress. On the other hand, drought stress at anthesis stage had no significant effect on major axis length and perimeter of grains. It seems that the grain filling process affected grain width more than grain length, thus drought stress caused reduction in the accumulation of photo-assimilates to grains by reducing grain filling duration and because of the positive significant correlation between minor axis length and area, finally the drought stress at anthesis stage

reduced grain area through reducing grain width. Therefore, with respect to our objective, we conclude that area and minor axis length of grains are more sensitive traits to terminal drought stress than perimeter and major axis length of grains. Also, according to the high significant correlations between these two traits with grain yield and 1,000-grain weight, it seems that measurements of area and minor axis length of grains may be an applicable method to predict the grain weight than the other traits.

Furthermore, terminal drought stress at anthesis stage of barley could affect grain biochemical properties. These properties including starch, sugar and protein content of grains are closely linked with human and animal nutrition (You and Izydorczyk, 2007; Baik and Ullrich, 2008; Clarke *et al.,* 2008; Thitisaksakul *et al.,* 2012). The present study underlined that terminal drought stress at anthesis stage primarily increased grain protein content in barley genotypes. Similar results about increasing protein content in winter wheat were reported by Zhao *et al*. (2005). In addition, increased grain nitrogen concentration associated with drought stress has been reported in barley (Maleki Farahani *et al*., 2011). Data also showed that the drought-sensitive genotypes namely Fajr 30 and Nosrat had interestingly higher enhancement in protein content (46.88% and 35.33%, respectively) than the other ones. Apparently, more increase in grain protein content in the drought stress compared to the well-watered treatment can be a reliable indicator of sensitivity to drought stress. On the one hand, according to the negative significant correlation between grain protein content and 1,000-grain weight, and on the other hand, according to the positive significant correlation between 1,000-grain weight and grain physical properties, it is possible that measuring physical properties of grains could be a novel method for distinguishing drought-tolerant and drought-sensitive genotypes from each other.

In contrast to increased protein in drought stress condition, starch content did not elevate in drought stress and terminal drought stress

significantly decreased starch content in all genotypes. Drought stress not only decreased grain filling duration (Samarah *et al.,* 2009), but also reduced the number of endosperm cell (Fábián *et al.,* 2011), therefore starch content in the grain will be reduced because of these two reductions. Also, drought stress could reduce the amount of starch by affecting biosynthetic enzyme activity like Soluble Starch Synthase (SSS) (Ahmadi and Baker, 2001b), which is positively correlated with the rate of starch synthesis in wheat grains (Keeling *et al.,* 1993). Moreover, according to the positive correlation between starch and minor axis length of the grain (r= +0.44) and 1,000-grain weight (r= +0.64) (P< 0.01), it is concluded that (maybe) the larger the grain is, the greater the starch will be. The results were in agreement with Fox *et al.* (2006) who previously reported that the smaller grains usually produce lower levels of starch. Also, the high significant correlation between 1,000-grain weight and minor axis length (P< 0.01) suggests that the minor axis length of grains may be serving as an appropriate estimator of starch content and 1,000-grain weight.

In the current work, the reaction of genotypes to drought stress differed in sugar content. Under drought stress condition, the level of sucrose, glucose and fructose decreased in all genotypes except in Morocco. This is probably because of the morphological and physiological differences between two-rowed and six-rowed barley genotypes. Also, the uniform behavior of the other genotypes was ascribed to their same origin. The origin of all genotypes used in this study is Iran, but Morocco is originally from ICARDA (International Center for Agricultural Research in the Dry Area). In addition, to our knowledge, the current study is the first report demonstrating different reactions of two-rowed and six-rowed barley genotypes to terminal drought stress in terms of total water-soluble carbohydrates, sucrose, glucose and fructose, thus, this evidence needs to be tested with further research studies on larger groups of genotypes.

Drought stress caused a significant reduction in 1,000-grain weight and grain yield in all the barley genotypes. Grain weight, as the major component in estimating the total yield, can be influenced by grain filling rate and duration (Ahmadi and Baker, 2001a; Samarah *et al.,* 2009). According to the positive and significant correlation between 1,000-grain weight and starch content in grains, it is assumed that drought stress reduced the starch content through which affected the 1,000-grain weight. Because more than half of the grain dry weight consisted of starch (Jung *et al.,* 2008), any reduction in starch accumulation following drought stress resulted in the decrease in cereal grain weight (Duffus, 1992). Also, drought stress at grain filling stage induces early senescence which shortens the period of grain filling (Plaut *et al.,* 2004). Thus, any reduction in both rate and duration of grain filling in which starch accumulated resulted in grain weight reduction (Nicolas *et al.,* 1985). Also, this reduction in 1,000-grain weight could be explained by changing sucrose content in grain. Sucrose content of barley grain was affected by terminal drought stress in this study, and since sucrose is one of the principal factors affecting cell division (Ahmadi and Baker, 2001a), so it is obviously clear that any reduction in grain weight may be related not only to filling progression but can also be attributed to cell division process. In the drought stress condition, consistent with the lowest amount of sucrose, fructose in Fajr 30 had the most reduction in 1,000-grain weight (59.12%) in 2011- 2012.

Finally, our results clearly demonstrated that drought stress had an influential role on grain physical properties such as area and minor axis length of grains by changing the biochemical properties of the grain. Considering that the biochemical and physical characteristics of barley grains play a determining role in marketing and food quality produced with barley (Baik and Ullrich 2008), thus more studies in the field of discrimination of grains with higher quality based on their digital images in different stress conditions can be recommended. Image processing can be used as an accurate, sensitive and non-destructive technique for predicting biochemical contents of grains to identify compatible varieties for

different environmental conditions. According to the results of this paper and also considering the climate condition in Iran which is affected by terminal drought stress, genotypes with low grain yield stability (Fajr 30 and Nosrat) should be replaced with other genotypes.

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REFERENCES

1. Ahmadi, A. and Baker, D. A. 2001a. The Effect of Water Stress on Grain Filling Processes in Wheat. *J. Agric. Sci.,* **136:** 257-269.

2. Ahmadi, A. and Baker, D. A. 2001b. The Effect of Water Stress on the Activities of Key Regulatory Enzymes of the Sucrose to Starch Pathway in Wheat. *Plant Growth Regul.,* **35:** 81-91.

3. Alqudah, A. M., Samarah, N. H. and Mullen, R. E. 2011. Drought Stress Effect on Crop Pollination, Seed Set, Yield and Quality. In: *"Alternative Farming Systems, Biotechnology, Drought Stress and Ecological Fertilization, Sustainable Agriculture Reviews 6"*, (Ed.): Lichtfouse, E.. Springer, PP. 193- 215.

4. AOAC. 1995. *Official Methods of Analysis of AOSC International.* 16th Edition,. AOAC, Arlington, USA, 452 PP.

5. Baik, B. K. and Ullrich, S. E. 2008. Barley for Food: Characteristics, Improvement, and Renewed Interest. *J. Cereal Sci.,* **48:** 233-242.

6. Bannayan, M., Sanjani, S., Alizadeh, A., Lotfabadi, S. S. and Mohamadian, A. 2010. Association between Climate Indices, Aridity Index, and Rainfed Crop Yield in Northeast of Iran. *Field Crop. Res.,* **118:** 105-114.

7. Bleidere, M. 2008. Genetic and Environmental Effect on the Grain Quality of Spring Barley. *Latvian J. Agron. (Agronomijas vēstis).,* **11**: 33-38.

8. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.,* **72**: 248-254.

9. Burger, W. C. and LaBerge, D. E. 1985. Malting and Brewing Quality. In: *"Barley"*, (Ed.): Rasmussen, D. C.. American Society of Agronomy, Madison, PP. 367–401.

10. CGC. 2006. *Official Grain Grading Guide.* Canadian Grain Commission, Winnipeg Canada. Available in http://www.grainscanada.gc.ca/oggg-gocg/2013/06-barley-2013-eng.pdf.

11. Clarke, B., Liang, R., Morell, M., Bird, A., Jenkins, C. and Li, Z. 2008. Gene Expression in a Starch Synthase IIa Mutant of Barley: Changes in the Level of Gene Transcription and Grain Composition. Funct. *Integr. Genom.,* **8:** 211-221.

12. Cossani, C. M., Slafer, G. A. and Savin, R. 2009. Yield and Biomass in Wheat and Barley under a Range of Conditions in a Mediterranean Site. *Field Crop. Res.,* **112:** 205-213.

13. Dolferus, R., Ji, X. and Richards, R.A. 2011. Abiotic Stress and Control of Grain Number in Cereals. *Plant Sci.,* **181**: 331-341.

14. Duffus, C. 1992. Control of Starch Biosynthesis in Developing Cereal Grains. *Biochem. Soc. Trans.,* **20**: 13-18.

15. Fábián, A., Jäger, K., Rakszegi, M. and Barnabás, B. 2011. Embryo and Endosperm Development in Wheat (*Triticum aestivum* L.) Kernels Subjected to Drought Stress. *Plant Cell Rep.,* **30**: 551-563.

16. Fox, G. P., Kelly, A., Poulsen, D., Inkerman, A. and Henry, R. 2006. Selecting for Increased Barley Grain Size. *J. Cereal Sci.,* **43:** 198-208.

17. González, A., Martin, I. and Ayerbe, L. 2008. Yield and Osmotic Adjustment Capacity of Barley under Terminal Water-stress Conditions. *J. Agron. Crop Sci.,* **194**: 81-91.

18. Jung, K. H., An, G. and Ronald, P. C. 2008. Towards a Better Bowl of Rice: Assigning Function to Tens of Thousands of Rice Genes. *Nat. Rev. Genet.,* **9**: 91-101.

19. Keeling, P., Bacon, P. and Holt, D. 1993. Elevated Temperature Reduces Starch Deposition in Wheat Endosperm by Reducing the Activity of Soluble Starch Synthase. *Planta,* **191:** 342-348.

20. Majumdar, S. and Jayas, D. 2000. Classification of Cereal Grains Using Machine

Vision. IV. Combined Morphology, Color, and Texture Models. *Trans. ASAE*, **43**: 1689-1694.

21. Maleki Farahani, S., Chaichi, M. R., Mazaheri, D., Tavakkol Afshari, R. and Savaghebi, Gh. 2011. Barley Grain Mineral Analysis as Affected by Different Fertilizing Systems and by Drought Stress. *J. Agr. Sci. Tech.,* **13:** 315-326.

22. Nicolas, M. E., Lambers, H., Simpson, R. J. and Dalling, M. J. 1985. Effect of Drought on Metabolism and Partitioning of Carbon in Two Wheat Varieties Differing in Drought-Tolerance. *Ann. Bot.,* **55:** 727-742.

23. Plaut, Z., Butow, B., Blumenthal, C. and Wrigley, C. 2004. Transport of Dry Matter into Developing Wheat Kernels and Its Contribution to Grain Yield under Post-anthesis Water Deficit and Elevated Temperature. *Field Crop. Res*., **86:** 185-198.

24. Rizza, F., Ghashghaie, J., Meyer, S., Matteu, L., Mastrangelo, A. M. and Badeck, F. W. 2012. Constitutive Differences in Water Use Efficiency between Two Durum Wheat Cultivars. *Field Crop. Res*., **125**: 49–60

25. Samarah, N., Alqudah, A., Amayreh, J. and McAndrews, G. 2009. The Effect of Late☐terminal Drought Stress on Yield Components of Four Barley Cultivars. *J. Agron. Crop Sci*., **195:** 427-441.

26. Schulman, A. H., Runeberg-Roos, P. and Jääskeläinen, M. 2000. Grain Filling and Starch Synthesis in Barley. *Dev. Crop Sci.,* **26**: 147-167.

27. Shouche, S., Rastogi, R., Bhagwat, S. and Sainis, J. K. 2001. Shape Analysis of Grains of Indian Wheat Varieties. *Comput. Electron. Agric*., **33**: 55-76.

28. Thitisaksakul, M., Jiménez, R. C., Arias, M. C. and Beckles, D. M. 2012. Effects of Environmental Factors on Cereal Starch Biosynthesis and Composition. *J. Cereal Sci.,* **56**: 67-80.

29. Ugarte, C., Calderini, D. F. and Slafer, G. A. 2007. Grain Weight and Grain Number Responsiveness to Pre-anthesis Temperature in Wheat, Barley and Triticale. *Field Crop. Res.,* **100:** 240-248.

30. Walker, C. K. and Panozzo, J. F. 2012. Measuring Volume and Density of a Barley Grain Using Ellipsoid Approximation from a 2-D Digital Image. J. Cereal Sci., 55: 61-68.

31. You, S. and Izydorczyk, M. 2007. Comparison of the Physicochemical Properties of Barley Starches after Partial α-Amylolysis and Acid/Alcohol Hydrolysis. *Carbohydr. Polym.,* **69:** 489-502.

32. Zhao, C., Liu, L., Wang, J., Huang, W., Song, X. and Li, C. 2005. Predicting Grain Protein Content of Winter Wheat Using Remote Sensing Data Based on Nitrogen Status and Water Stress. Int. J. *Appl. Earth Obs. Geoinf.,* **7:** 1-9.

33. Zadoks, J. C., Chang, T. T. and Konzak, C. F. 1974. A Decimal Code for the Growth Stages of Cereals. *Weed Res.,* **14:** 415-421.

ویژگی‌های فیزیکی و کیفی دانه‌های جو تحت شرایط تنش خشکی انتهایی

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چکیده

تنش خشکی محدودیت اصلی برای عملکرد گیاه زراعی می‌باشد، که بسته به زمان وقوع آن، می‌تواند تعداد دانه و نیز وزن دانه‌ها را کاهش دهد. جو (.Hordeum vulgare L) یکی از غلات متحمل است که اجزای دانه آن نقش مهمی در تغذیه انسان و دام دارد؛ اما، ویژگی‌های فیزیکی و بیوشیمیایی دانه‌ها که توسط تنش خشکی تحت تأثیر قرار می‌گیرد هنوز به درستی بررسی نشده است. در این

مطالعه، ژنوتیپ‌های جو (n=۶) با سطوح مختلف تحمل به خشکی در یک آزمایش مزرعه‌ای دو ساله، تحت شرایط مطلوب آبیاری و تنش خشکی انتهایی مورد مطالعه قرار گرفتند. به منظور اندازه‌گیری خواص فیزیکی دانه‌ها، تصاویر دیجیتالی گرفته شد و برخی خصوصیات مورفولوژیکی با استفاده از جعبه ابزار پردازش تصویر نرم افزار متلب به دست آمد. همچنین، ویژگی‌های بیوشیمیایی دانه‌ها اندازه‌گیری گردید. نتایج نشان داد نه تنها اندازه و وزن هزار دانه بلکه کیفیت دانه نیز به طور معنی‌داری تحت تأثیر تنش خشکی قرار گرفت ($p<0.01$). تحت شرایط تنش خشکی در مرحله گلدهی، محتوای نشاسته دانه کاهش و محتوای پروتئین در کلیه ژنوتیپ‌ها افزایش یافت، اما ژنوتیپ‌های حساس به خشکی به طور قابل توجهی افزایش بیشتری در درصد محتوای پروتئین نشان دادند. علاوه بر این، ژنوتیپ‌ها در میزان قند کل، ساکارز، گلوکز و فروکتوز تفاوت داشتند. تنش خشکی، اندازه دانه و در نهایت وزن هزار دانه ژنوتیپ‌های جو را از طریق کاهش مساحت و محور کوچک دانه‌ها تحت تأثیر قرار داد. همبستگی بین وزن هزار دانه و محور کوچک دانه، مساحت دانه، محتوای نشاسته و ساکارز معنی‌دار بود ($p<0.01$). در هر دو شرایط، نتایج تأکید می‌کند که خصوصیات مربوط به اندازه دانه به ویژه محور کوچک و مساحت ممکن است به عنوان صفات مفیدی برای تخمین وزن هزار دانه و ویژگی‌های بیوشیمیایی جو به کار رود.