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 (\textit{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, \textit{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 \textit{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 (\$\approx 250$ mm) (Bannayan \textit{et al.}, 2010), grain yield of small grain cereals which are grown in these areas can be largely influenced by terminal drought stress (González \textit{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 \textit{et al.}, 2007). In literatures, it is well documented that grain number is a more...
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 occurs. 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 m² 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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin</th>
<th>Spike type</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fajr 30</td>
<td>Iran</td>
<td>Six-rowed</td>
<td>Ligne131/Gerbel//Alger-Ceres/3/Jonoob</td>
</tr>
<tr>
<td>Nosrat</td>
<td>Iran</td>
<td>Six-rowed</td>
<td>Karoon/Kavir</td>
</tr>
<tr>
<td>PBYT 46</td>
<td>Iran</td>
<td>Six-rowed</td>
<td>PETUNIA 2/3/GLORIA- BAR-COME//ESPERANZA/4/CABUYA</td>
</tr>
<tr>
<td>PBYT 97</td>
<td>Morocco</td>
<td>Six-rowed</td>
<td>Ligne527/NK1272/JLB70-063/3/IPA99</td>
</tr>
</tbody>
</table>

International Center for Agricultural Research in the Dry Areas (ICARDA)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin</th>
<th>Spike type</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morocco</td>
<td></td>
<td>Two-rowed</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Month</th>
<th>Minimum temperature (°C)</th>
<th>Maximum temperature (°C)</th>
<th>Total monthly precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>0.8</td>
<td>0.2</td>
<td>30.4</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
<td>-2.2</td>
<td>25.6</td>
</tr>
<tr>
<td>December</td>
<td>-4.4</td>
<td>-4</td>
<td>20.4</td>
</tr>
<tr>
<td>January</td>
<td>-2</td>
<td>-6</td>
<td>19.8</td>
</tr>
<tr>
<td>February</td>
<td>-1.4</td>
<td>-6</td>
<td>28.2</td>
</tr>
<tr>
<td>March</td>
<td>6.2</td>
<td>-0.8</td>
<td>32.4</td>
</tr>
<tr>
<td>April</td>
<td>15.6</td>
<td>12.4</td>
<td>39.2</td>
</tr>
<tr>
<td>May</td>
<td>19.8</td>
<td>18.8</td>
<td>43</td>
</tr>
</tbody>
</table>
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 $\chi^2$ 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.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Grain area (cm²)</th>
<th>Grain perimeter (cm)</th>
<th>Major axis length of grain (cm)</th>
<th>Minor axis length of grain (cm)</th>
<th>Grain filling duration (Day)</th>
<th>Number of grain per spike</th>
<th>1000-grain weight (g)</th>
<th>Grain yield (Kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010-2011 Genotypes</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Yousof</td>
<td>0.160 a</td>
<td>0.129 a</td>
<td>0.654 a</td>
<td>0.605 a</td>
<td>0.961 a</td>
<td>0.904 a</td>
<td>0.268 ab</td>
<td>0.330 b</td>
</tr>
<tr>
<td>Fajr 30</td>
<td>0.136 cd</td>
<td>0.128 a</td>
<td>0.600 b</td>
<td>0.594 ab</td>
<td>0.898 ab</td>
<td>0.881 ab</td>
<td>0.243 c</td>
<td>0.235 b</td>
</tr>
<tr>
<td>Noorat</td>
<td>0.151 abc</td>
<td>0.134 a</td>
<td>0.644 a</td>
<td>0.623 a</td>
<td>0.956 a</td>
<td>0.929 a</td>
<td>0.254 abc</td>
<td>0.334 b</td>
</tr>
<tr>
<td>PBYT 46</td>
<td>0.155 ab</td>
<td>0.143 a</td>
<td>0.628 ab</td>
<td>0.599 ab</td>
<td>0.915 ab</td>
<td>0.880 ab</td>
<td>0.273 a</td>
<td>0.255 a</td>
</tr>
<tr>
<td>PBYT 97</td>
<td>0.125 d</td>
<td>0.113 b</td>
<td>0.561 c</td>
<td>0.561 b</td>
<td>0.817 c</td>
<td>0.836 bc</td>
<td>0.249 bc</td>
<td>0.220 b</td>
</tr>
<tr>
<td>Morocco</td>
<td>0.141 bcd</td>
<td>0.135 a</td>
<td>0.598 bc</td>
<td>0.562 b</td>
<td>0.870 bc</td>
<td>0.821 c</td>
<td>0.259 abc</td>
<td>0.261 a</td>
</tr>
<tr>
<td>Mean CV (%)</td>
<td>0.145</td>
<td>0.130</td>
<td>0.616</td>
<td>0.591</td>
<td>0.903</td>
<td>0.875</td>
<td>0.258</td>
<td>0.239</td>
</tr>
<tr>
<td>2011-2012 Genotypes</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Yousof</td>
<td>0.148 a</td>
<td>0.116 ab</td>
<td>0.670 a</td>
<td>0.625 a</td>
<td>0.977 ab</td>
<td>0.952 a</td>
<td>0.295 a</td>
<td>0.338 b</td>
</tr>
<tr>
<td>Fajr 30</td>
<td>0.147 a</td>
<td>0.111 ab</td>
<td>0.662 a</td>
<td>0.660 a</td>
<td>1.006 a</td>
<td>1.014 a</td>
<td>0.283 a</td>
<td>0.227 b</td>
</tr>
<tr>
<td>Noorat</td>
<td>0.138 a</td>
<td>0.111 b</td>
<td>0.645 ab</td>
<td>0.653 a</td>
<td>0.931 abc</td>
<td>1.042 a</td>
<td>0.287 a</td>
<td>0.205 c</td>
</tr>
<tr>
<td>PBYT 46</td>
<td>0.135 a</td>
<td>0.132 a</td>
<td>0.634 ab</td>
<td>0.649 a</td>
<td>0.904 ccd</td>
<td>0.965 a</td>
<td>0.294 a</td>
<td>0.265 a</td>
</tr>
<tr>
<td>PBYT 97</td>
<td>0.125 a</td>
<td>0.108 b</td>
<td>0.621 ab</td>
<td>0.586 a</td>
<td>0.852 cd</td>
<td>0.904 a</td>
<td>0.296 a</td>
<td>0.222 bc</td>
</tr>
<tr>
<td>Morocco</td>
<td>0.137 a</td>
<td>0.108 b</td>
<td>0.605 b</td>
<td>0.587 a</td>
<td>0.854 d</td>
<td>0.882 a</td>
<td>0.312 a</td>
<td>0.236 b</td>
</tr>
<tr>
<td>Mean CV (%)</td>
<td>0.138</td>
<td>0.15</td>
<td>0.643</td>
<td>0.629</td>
<td>0.920</td>
<td>0.960</td>
<td>0.295</td>
<td>0.232</td>
</tr>
</tbody>
</table>

#### Least Significant Differences (LSD)

- Genotypes (G): 0.0085 ***
- Treatment (T): 0.0049 ***
- Year (Y): 0.0049 ***
- GxT: 0.012 ns
- GxY: 0.012 ns
- Txy: 0.007 ns
- GxTxY: 0.017 ns

**Grain area (cm²)**

- Least Significant Differences (LSD)
  - Genotypes (G): 0.0085 ***
  - Treatment (T): 0.0049 ***
  - Year (Y): 0.0049 ***
  - GxT: 0.012 ns
  - GxY: 0.012 ns
  - Txy: 0.007 ns
  - GxTxY: 0.017 ns

**Grain filling duration (Day)**

- Least Significant Differences (LSD)
  - Genotypes (G): 0.0085 ***
  - Treatment (T): 0.0049 ***
  - Year (Y): 0.0049 ***
  - GxT: 0.012 ns
  - GxY: 0.012 ns
  - Txy: 0.007 ns
  - GxTxY: 0.017 ns

**Number of grain per spike**

- Least Significant Differences (LSD)
  - Genotypes (G): 0.0085 ***
  - Treatment (T): 0.0049 ***
  - Year (Y): 0.0049 ***
  - GxT: 0.012 ns
  - GxY: 0.012 ns
  - Txy: 0.007 ns
  - GxTxY: 0.017 ns

**Thousand grain weight (g)**

- Least Significant Differences (LSD)
  - Genotypes (G): 0.0085 ***
  - Treatment (T): 0.0049 ***
  - Year (Y): 0.0049 ***
  - GxT: 0.012 ns
  - GxY: 0.012 ns
  - Txy: 0.007 ns
  - GxTxY: 0.017 ns

**Grain yield (Kg ha⁻¹)**

- Least Significant Differences (LSD)
  - Genotypes (G): 0.0085 ***
  - Treatment (T): 0.0049 ***
  - Year (Y): 0.0049 ***
  - GxT: 0.012 ns
  - GxY: 0.012 ns
  - Txy: 0.007 ns
  - GxTxY: 0.017 ns

### Notes:

- All values are means of three replications.
- Significant differences were determined using the least significant difference (LSD) test at the 0.05 probability level.
- The values followed by different letters in each column and year within the same trial are significantly different at the 0.05 probability level.
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 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       | 2010-11 | 0.73**| 1                 |                   |           |                |                |              |                |                 |                    |                     |         |
| length           | 2011-12 | 0.36* | 1                 |                   |           |                |                |              |                |                 |                    |                     |         |
| Minor axis       | 2010-11 | 0.81**| 0.19**            | 1                 |           |                |                |              |                |                 |                    |                     |         |
| length           | 2011-12 | 0.77**| -0.29**           | 1                 |           |                |                |              |                |                 |                    |                     |         |
| Perimeter        | 2010-11 | 0.82**| 0.98**            | 0.35**            | 1         |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | 0.67**| 0.88**            | 0.14**            | 1         |                |                |              |                |                 |                    |                     |         |
| Starch content   | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | 0.18**| -0.31**           | 0.44**            | -0.09**   | 1               |                |              |                |                 |                    |                     |         |
| Protein content  | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | -0.30**| -0.02**          | -0.25**          | -0.07**   | -0.26**        | 1               |              |                |                 |                    |                     |         |
| Total sugars     | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | 0.06**| -0.18**           | 0.20**            | 0.01**    | 0.23**         | 0.07**         | 1             |                |                 |                    |                     |         |
| Sucrose content  | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | 0.03**| -0.36**           | 0.28**            | -0.22**   | 0.36**         | 0.25**         | 0.16**        | 1               |                |                    |                     |         |
| Glucose content  | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | -0.21**| -0.09**          | -0.21**          | -0.07**   | -0.36**        | 0.33**         | 0.05**        | 1               |                |                    |                     |         |
| Fructose content | 2010-11 | nd    | nd                | nd                |           |                |                |              |                |                 |                    |                     |         |
|                  | 2011-12 | -0.16**| -0.18**          | -0.05**          | -0.17**   | 0.36**         | 0.05**         | 0.27**        | 0.74**         | 0.47**         |                    |                     |         |
| 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**          | 0.85**          | 0.13**    | 0.64**         | -0.35**        | 0.04**        | 0.38**         | -0.29**       | 3.07**            | 1                     |         |
| Yield            | 2010-11 | 0.47**| 0.27**          | 0.43**          | 0.35**    | nd             | nd             | nd           | nd             | nd             | nd                 | 0.64**       | 1       |
|                  | 2011-12 | 0.57**| -0.25**          | 0.80**          | 0.14**    | 0.63**         | -0.24**        | 0.31**        | 0.45**         | -0.22**       | 2.15**            | 0.91**           | 1       |

* and **: Significant at P<0.05 and P<0.01, respectively and nd: Not-significant.
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 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), 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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ويزگی های فیزیکی و کیفی دانه‌های جو تحت شرایط نش خشکی انتهایی

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چکیده

نتش خشکی محدودیت‌اتصلی برای عملکرد گیاه زراعی می‌باشد، که به‌ویژه به زمان و قفور آخر می‌تواند تعداد دانه و وزن دانه‌ها را کاهش دهد. جو است که اجزای دانه آن تشکیل می‌دهند. و در زمان‌های مختلف گیاه مرحله از خشکی (Hordeum vulgare L.) دانه‌ها که توسط نش خشکی تحت تأثیر قرار می‌گیرد هنوز به درستی بررسی نشده است. در این

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مطالعه، زنوتیپ‌های جو (α=0.05) با سطوح مختلف تحمل به خشکی در یک آزمایش مزرعه‌ای دو ساله، تحت شرایط مطلق آبیاری و نش خشکی انجام گردید. به منظور اندوزه‌گیری خواص فیزیکی دانه‌ها، تصویر دیجیتالی گرفته شد و برخی خصوصیات مورفولوژیکی با استفاده از جمه‌ای پردازش تصویر نرم افزار متلب به دست آمد. همچنین، وزن‌گرمی‌های بیوشیمیایی دانه‌ها اندوزه‌گیری گردید. نتایج نشان داده‌اند که وزن هزار دانه بیشتر بوده و صفات عمیق شده و جون کیفیت دانه نیز به طور معنی‌داری تحت تأثیر نش خشکی قرار گرفته (p<0.01). تحت شرایط نش خشکی در مرحله گلدهی، محتوای ببتیستن دانه کاهش و محتوای پروتئین در کله زنوتیپ‌ها افزایش یافته، اما زنوتیپ‌های حساس به خشکی به طور قابل توجهی افزایش ویترین در دو مرحله محتوای پروتئین نشان دادند. علاوه بر این، زنوتیپ‌ها در میزان فند کل، ساکارز، گلکز و فروکتوز نشان دادند. نش خشکی، اندوزه‌دانه و در نهایت وزن هزار دانه زنوتیپ‌های جو را با طریق کاهش مساحت و محور گاوانی دانه‌ها تحت تأثیر قرار داده. همچنین به وزن هزار دانه و محور گاوانی دانه، مساحت دانه، محتوای نشانه و ساکارز معنی دارنده (p<0.01). در هر دو شرایط، نتایج ثابت می‌کند که برخی خصوصیات مربوط به اندوزه‌دانه به ویژه محور گاوانی و مساحت ممکن است به عنوان صفات مفید برای تخمین وزن هزار دانه و وزن‌گرمی‌های بیوشیمیایی جو به کار رود.