The Effects of Water Deficit on Crop Yield and the Physiological Characteristics of Barley (*Hordeum vulgare L.*) Varieties

E. Mamnouie¹, R. Fotouhi Ghazvini ¹, M. Esfahany¹* and B. Nakhoda²

ABSTRACT

The effects of water deficit on grain yield, yield components and the physiological characteristics of barley (*Hordeum vulgare L.*) varieties were studied in a split plot design during the 1999-2000 growing season in Karaj, Iran. Five irrigation levels [0, 25, 50, 75, and 100% crop water requirements (CWR)] and six barley varieties Karoon × Kavir, Reihani (drought tolerant), Torkman, C-74-9 (intermediate), Kavir × Badia and Gorgan-4 (2 rowed type, drought sensitive) were arranged in the main plots and sub plots respectively. Results showed that water deficit induced stress and this decreased the grain yield and yield components. Water deficit increased the canopy temperature and those varieties showed a higher canopy temperature under non-stress conditions, performed better under drought conditions. Severe stress reduced the chlorophyll content (SPAD values) considerably, but the differences were not significant between the 50, 75 and 100% CWR treatments. In addition, no significant differences were observed in the chlorophyll content of barley varieties under drought conditions. The effect of irrigation on the photochemical efficiency of photosystemII (Fv/Fm) and the other fluorescence parameters for all varieties were significant. Although the Fv/Fm values were not significant in barley varieties at any level of irrigation, in general Karoon × Kavir and Reihani varieties showed a better performance under water deficit conditions. Proline content was significantly different in various irrigation treatments, but its accumulation at any level of irrigation did not differ significantly in barley varieties. It was concluded that the higher canopy temperature (less negative ΔT) under well irrigated conditions and higher grain yield, 1000-grain weight, Fv/Fm values under water stress conditions could possibly be the proper criteria for screening the drought tolerant barley genotypes under field or laboratory conditions.

Keywords: Barley (*Hordeum vulgare L.*), Canopy temperature, Drought tolerance, Photosystem II, SPAD, Water deficit.

INTRODUCTION

Water deficit (drought) at different stages of barley’s life cycle, (from seed germination to maturity) may adversely affect the final yield of the crop. The effect of drought on the yield of cereals depends on the duration and the severity of the stress [9]. Long-term drought under high temperature conditions, coinciding with the reproductive stage of the plant, may cause a decrease in the size and number of the grains, and eventually its yield.

Drought may cause abortion of the embryonic sac, dehydration of the style and pollen and, hence, interference in pollination [8, 9]. Singh, et al. [10] found that drought reduces the number of the grains/spike and thereby yield [11]. Water deficit at the tillering stage had no significant effect on the number of grains on spikes but, at the flowering stage, it reduced grain weight significantly [9].

¹. Faculty of Agriculture, University of Guilan, Rasht, Islamic Republic of Iran.
². Seed and Plant Improvement Institute, Karaj, Islamic Republic of Iran.
* Corresponding author.
Since drought may affect osmotic adjustment and canopy temperature, these two can be used as an index for screening varieties of barley to drought stress. Varieties that showed warmer canopies under irrigated conditions had a lower stomatal conductance and higher yield under drought conditions [2]. Liu and Zhang [6] reported that, under water deficit, the difference between air and canopy temperatures (ΔT) in wheat varieties was significantly higher at spike exertion stage but it had no effect on the yield. Under irrigated conditions, the reduction in the yield was significantly related to ΔT. Chlorophyll content is positively correlated with the photosynthesis rate and soluble proteins [3, 13]. The chlorophyll content which was measured by SPAD. 502 correlated to the photosynthesis of leaves (R²=0.77**) [7]. A significant correlation between the SPAD reading and spectrophotometric method of chlorophyll determination (R² = 0.95**) was also reported [13]. In addition, chlorophyll fluorescence has been accepted as a reliable index for drought tolerance screening of barley varieties. This phenomenon is a criterion for thylacoide membrane integrity and electron transfer efficiency from photosystem II to photosystem I [7]. The photochemical efficiency of photosystem II is determined by the Fv/Fm ratio, which is reduced during periods of drought stress.

Free proline accumulation can also be used as another index for screening the tolerance of varieties [5]. Water stress may cause a disorder in the mitochondrial membrane and proline oxidation to glutamate might therefore be decreased. Proline accumulation by way of the glutamic pathway may help osmotic adjustment, energy production and further absorption of the released ammonia from protein breakdown [11].

The objectives of this experiment were to evaluate the effects of water deficit on the physiological characteristics of barley varieties in order to exploit them for drought tolerance screening.

MATERIALS AND METHODS

Field experiments were conducted at the Seed and Plant Improvement Institute (SPII), Karaj (Iran) (35°N Latitude) during the growing season of 1999-2000 in a split plot design with three replications. Five levels of irrigation: 0, 25, 50, 75, and 100% of crop water requirements and six barley varieties, namely: Karoon × Kavir, Reihani (drought tolerant), Torkman, C-74-9 (intermediate), Kavir × Badia and Gorgan-4 (2-rowed type) (sensitive) - all obtained from Cereals Department of Seed and Plant Improvement Institute (SPII)- were laid out in the main plots and sub plots respectively. Seeds were sown in 16 rows in 10 × 2.5 m plots (400 plants. m⁻²) in Autumn. Plants were irrigated twice after sowing equally (1100 m³/ha) and irrigation treatments were applied after the plants established and after their further growth in Spring (since April, 4). Their evapotranspiration potential (ETP) was determined according to:

ETP=Ep × Kp    (1

Kp = Evaporation from Class A Evaporation pan

Ep = Evaporation from Class A Evaporation pan

Kp = Evaporation pan coefficient (0.85 in karaj) (obtained from Meteorology Station of Karaj).

Evapotranspiration of the crop (ETC = Crop water requirement) was calculated according to the following equation:

ETC = ETP × Kc    (2

Kc = Crop coefficient (0.96, 1.12, 1.15, 0.95 and 0.65 for growth stages of all barley varieties; stem elongation, booting, heading, anthesis and grain filling, in karaj). (Data obtained from Cereals Dept. - Seed & Plant Development Inst. (SPII))

The amount of irrigation water for each treatment (T in mm) was calculated as follows:

T= ETC × Percent of crop water requirements for each treatment (mm)    (3

Irrigation water was applied according to 80% irrigation efficiency and effective rainfall (twice during growing season; 3.5 and 2.5 mm in April and May, respectively) at
10-day intervals and its volume was measured using a WSC\(^1\) Phloem type III fixed in the irrigation ditches. The total amount of irrigation water was 0, 2107.2, 3739, 5786 and 6450 m\(^3/\)ha applied 0, 4, 5, 5 and 5 times for 0, 25, 75 and 100% of crop water requirements, respectively.

Grain yield was determined by harvesting the eight middle rows of each plot at the maturity stage and the yield components (number of grains/spike and number of spikes m\(^{-2}\)) were determined from the samples taken randomly (10 spikes from the remaining parts of each plot) at the dough stage. Canopy temperature was measured between 12:00-2 pm. using an infrared thermometer [Kane - May Co. UK] at anthesis for determination of the \(\Delta T\) (temperature difference between canopy and ambient air). The apparatus was targeted at the canopy with a 10-20\(^{\circ}\) angle from four directions and at least 1 m distance after initial calibration. Ambient air temperature was measured using a mercury thermometer and \(\Delta T\) calculated as following:

\[
\Delta T = T_{\text{canopy}} - T_{\text{air}}
\]

The effect of water deficit on the chlorophyll content of flag leaves was determined using a portable chlorophyll meter (SPAD 502-Minolta Co. Japan) at the heading stage. The measurements were performed twice with an interval of one week between each and 30 flag leaves were chosen randomly and the SPAD value recorded. Chlorophyll fluorescence and the parameters of \(F_0\), \(F_v\), \(F_m\) and \(F_v / F_m\) (Fluorescence Initial, Fluorescence Maximum and Fluorescence Variable, respectively) were measured on the flag leaves of barley varieties by means of a plant stress meter (Biomonitor 1989, UK) at the heading stage. Measurements were carried out on 30 flag leaves from each plot five days after every irrigation, subsequent to the canopies approximate clousure. The broadest part of the flag leaves was clipped in the measuring chamber of the apparatus and exposed to 650 nm wavelength radiation and the \(F_0, F_m\) and \(F_v\) values were measured after initial calibration. All measurements were carried out at 10 am in order to avoid the effects of dew and air humidity. The free proline content was determined according to Bates \textit{et al.} [1] at different irrigation levels in 15 flag leaves from each plot at the heading stage. 0.5 gr of leaf samples were crushed in a precooled mortar and pestle with a small amount of sand and 10 ml 3\%\,(W/V) solfocalicylic acid. Leaf extracts, Ninhydrin and glacial acetic acid (equal amounts) were incubated in a water bath for one hour and the proline isolated by adding 4 ml Toluene to each and measured in 520 nm in a spectrophotometer (M 330- 230 Jenway – UK). The proline content was determined against its standard curve and converted to \(\mu\)g proline in 1gr fresh weight. All

\begin{table}
\centering
\begin{tabular}{lccccccccc}
\hline
\textbf{S.O.V.} & \textbf{Mean Squares (MS)} & \textbf{Irrigation} & \textbf{Variety} & \textbf{Irrigation} & \textbf{SPAD} & \textbf{F0/Fm} & \textbf{Proline} \\
\hline
\textbf{Irrigation} & 348.75** & 121803.74** & 246.19** & 0.43** & 256.71** & 229.6** & 0.349** & 36412.7** \\
\textbf{regime} & \textbf{Ea} & 4.599 & 1868.86 & 10.72 & 0.013 & 1.47 & 7.517 & 0.001 & 19.36 \\
\textbf{Ea} & 35.823** & 93934.95** & 577.72** & 0.06** & 16.29** & 30.5** & 0.31** & 33.11* \\
\textbf{Variety} & 9.206** & 8748.98** & 11.83** & 0.008* & 4.19** & 3.247** & 0.003** & 7.79** \\
\textbf{Irrigation} & 7.543 & 1648.37 & 8.15 & 0.007 & 0.96 & 7.67 & 0.001 & 14.38 \\
\textbf{× variety} & \textbf{Eb} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} \\
\textbf{Irrigation} & \textbf{× variety} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} & \textbf{n.s} \\
\hline
\textbf{*}, ** Significant at 0.05 and 0.01, respectively
\textbf{n.s} Non significant
\end{tabular}
\caption{Summery analysis of variance for barley varieties parameters recorded at different irrigation levels.}
\end{table}
data were analysed by MSTATc, SAS and SPSS softwares and the Excel software was used for graphical descriptions.

RESULTS AND DISCUSSION

Grain Yield and Yield Components

An analysis of variance showed that water deficit significantly reduced the 1000-grain weight, number of spikes, m⁻², number of grains/spike and grain yield (Tables 1 and 2). The 1000-grain weight and its size reduction is possibly due to a decrease in the assimilation rate and lower photoasimilate translocation to physiological sinks. Water deficit is known to reduce the 1000-grain weight by shortening the grain-filling period [3, 9]. These findings are in agreement with Mohammad et al. [9] who found that water stress at the flowering stage reduced 1000-grain weight significantly. There were significant differences in the number of grains/spike between barley varieties (Table 2). However, differences were not significant between 50, 75 and 100% crop water requirements, suggesting that moderate water deficit had no effect on the abortion of young florets and did not reduce the number of grains/spike. The number of grains/spike of the Gorgan-4 variety was the lowest due to its 2-rowed type (distichum) spike feature. Water deficit reduced the number of spikes m⁻² in barley varieties (Table 2). The average number of spikes m⁻² was 494 in Gorgan-4 which was related to its higher tillering capacity as 2-rowed barley and the lowest spikes m⁻², an average of 324, was observed in the Reihani variety. The grain yield of barley varieties at different irrigation levels is shown in Tables 2 and 3. Grain yields in the Karoon × Kavir and Kavir × Badia varieties were the highest under well-irrigated condition, while the Karoon × Kavir and Reihani varieties had the highest grain yield under stress conditions (Table 3). Moffat et al [8] also showed that water stress at the reproductive growth stage reduced the grain yield in wheat significantly.

Canopy Temperature

Water deficit had a significant effect (P ≤ 0.01) on the canopy temperature difference between canopy and ambient air (ΔT)
Negative and positive $\Delta T$s under well irrigated and water stress conditions are shown in Figure 1. Negative $\Delta T$s indicated a cooler canopy and positive $\Delta T$s indicated a warmer canopy. With regard to the heat absorbing nature of transpiration, the cooler canopy (negative $\Delta T$) is equivalent to a higher transpiration rate and higher stomatal conductance, while the warmer canopy (positive $\Delta T$) indicated a lower transpiration rate and higher stomatal resistance. A higher water pressure deficit induces higher stomatal resistance and, so, a higher leaf surface temperature. The $\Delta T$ increment due to water stress has been reported in wheat [4]. $\Delta T$ values for Karoon × Kavir and Reihani varieties were both -1°C under well irrigated conditions (Table 3) which had the highest grain yield under drought conditions (0.3 and 0.32 Kg.m$^{-2}$ respectively). It seems that a considerable relationship could be observed between higher canopy temperature (less negative $\Delta T$) under well irrigated conditions and higher grain yield under stress conditions.

<table>
<thead>
<tr>
<th>Irrigation Regime</th>
<th>Variety</th>
<th>No. Spikes/m$^2$</th>
<th>Grain yield (Kg/m$^2$)</th>
<th>$\Delta T$(°C)</th>
<th>$F_v/F_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Torkman</td>
<td>340 ghi jklm nop</td>
<td>0.479 cdefghijklmn op</td>
<td>-3.32 mn</td>
<td>0.72 abc</td>
</tr>
<tr>
<td></td>
<td>Karoon × Kavir</td>
<td>472 cdef ghijklmn op</td>
<td>0.685 a</td>
<td>-1.32 mn</td>
<td>0.72 abc</td>
</tr>
<tr>
<td></td>
<td>Reihani</td>
<td>374 fghi jklm nop</td>
<td>0.578 abcd e fghi jklmn</td>
<td>-1.32 mn</td>
<td>0.69 abc</td>
</tr>
<tr>
<td></td>
<td>C-74-9</td>
<td>328 ghi jklm nop</td>
<td>0.597 abcd e fghi jklmn</td>
<td>-3.32 mn</td>
<td>0.72 abc</td>
</tr>
<tr>
<td></td>
<td>Gorgan-4</td>
<td>680 a</td>
<td>0.507 bcd e fghi jklmn</td>
<td>-5 n</td>
<td>0.77 a</td>
</tr>
<tr>
<td></td>
<td>Kavir × Badia</td>
<td>577 b</td>
<td>0.665 ab</td>
<td>-4.3 n</td>
<td>0.77 a</td>
</tr>
</tbody>
</table>

| 75%                | Torkman       | 397 efgh jklm nop| 0.422 efghijklmnop      | 3 efg          | 0.64 cde  |
|                   | Karoon × Kavir| 378 fgh jklm nop| 0.537 abcd efghijklmnop| 0 hijk         | 0.68 bed  |
|                   | Reihani       | 369 ghi jklm nop| 0.603 abcd e fghi jklmn| -1.32 mn       | 0.62 def  |
|                   | C-74-9        | 339 jghi jklm nop| 0.570 abcd efghijklmnop| -2.7 lmn       | 0.63 de   |
|                   | Gorgan-4      | 580 b            | 0.441 defghijklmnop      | -0.33ijkl      | 0.71 abc  |
|                   | Kavir × Badia | 554.7 bc         | 0.607 abc                | -0.66 jklm     | 0.73 ab   |

| 50%                | Torkman       | 357 ghi jklm nop| 0.370 ghijklmnop        | 2.3 fgh         | 0.57 efg  |
|                   | Karoon × Kavir| 359 ghi jklm nop| 0.478 cdefghijklmnop    | 1.6 ghij        | 0.62 de   |
|                   | Reihani       | 343 ghi jklm nop| 0.512 bcd efghijklmnop  | -0.33 ijkl      | 0.54 gh   |
|                   | C-74-9        | 307 hijkl jklm nop| 0.470 abcd efghijklmnop| 0 hijk         | 0.54 gh   |
|                   | Gorgan-4      | 493 bcde jklm nop| 0.367 ghijklmnop        | 2 ghij          | 0.63 de   |
|                   | Kavir × Badia | 520 bcd e fghi jklm nop| 0.549 abcd efghijklmnop| -0.3 ijkl      | 0.62 def  |

| 25%                | Torkman       | 342 ghi jklm nop| 0.331 hijklmnp q r st uv w xyz | 3 efg          | 0.49 hi   |
|                   | Karoon × Kavir| 341 ghi jklm nop| 0.408 ghijklmnop         | 2.7 efghijklmnop| 0.57 efghi|
|                   | Reihani       | 318 ijk jklm nop| 0.362 ghijklmnop         | 3 efghijklmnop  | 0.54 fgik |
|                   | C-74-9        | 267 ijkl jklm nop| 0.321 ijklmnopqr st uv w xyz | 3 efghijklmnop  | 0.44 ij   |
|                   | Gorgan-4      | 430 defg jklm nop| 0.277 klmnopqrst uv w xyz | 2 cdeijn       | 0.53 gh   |
|                   | Kavir × Badia | 402 egf jklm nop| 0.293 jklmnopqrst uv w xyz | 4.5 defghijklmnop| 0.41 j    |

| 0%                 | Torkman       | 250 jkl jklm nop| 0.188 mno pqrst uv w xyz | 8 a            | 0.41 j    |
|                   | Karoon × Kavir| 300 hijkl jklm nop| 0.301 jklmnopqrst uv w xyz | 7 abc          | 0.47 ij   |
|                   | Reihani       | 219 kl jklm nop| 0.323 jklmnopqrst uv w xyz | 5 cdeijn       | 0.44 j    |
|                   | C-74-9        | 223 kl jklm nop| 0.170 nopqrst uv w xyz    | 5.7 abcd efghijklmnop| 0.33 k    |
|                   | Gorgan-4      | 307 hijkl jklm nop| 0.104 pqrst uv w xyz     | 7.7 ab         | 0.29 k    |
|                   | Kavir × Badia | 213 l jklm nop| 0.132 opqrst uv w xyz     | 7 abc          | 0.31 k    |

*, ** Significant at 0.05 and 0.01, respectively
conditions. Golestani and Assad [4] also reported similar results. They proposed that the mean comparison of ΔTs could not be considered in drought tolerance screening of wheat varieties by itself, while the higher canopy temperature under well irrigated conditions could be exploited as a screening criteria for drought tolerance selection of different genotypes under non-stress conditions.

### Chlorophyll Content

The irrigation regime and varieties of barley both had significant effects on chlorophyll content (SPAD value) (Table 1). Water deficit decreased the chlorophyll content (SPAD value) in barley varieties and its reduction was significant at lower irrigation rates (50.14 and 45.96 at 25% and lower CWR respectively). (Table 2) There were not any significant differences between varieties. Similar results has been reported by Yavad [13].

A positive and significant correlation between the photosynthesis rate and SPAD values has been reported [3]. A lower photosynthesis rate under moderate water stress has been attributed to lower stomatal conductance although, under severe stress, stomatal resistance may accompanied by non stomatal (mesophyll) resistance [7].

It seems that the SPAD value measurement at the heading stage may not be a reliable indicator for screening the drought tolerant varieties, because the pre-anthesis photosynthesis and carbohydrate remobilization ability might have compensated for the current photosynthesis reduction at the grain filling stage. Plant height is an important morphological trait related to carbohydrate remobilization under stress conditions [2, 3]. The higher grain yield of the Karoon × Kavir and Reihani varieties under stress conditions may be related to their taller architecture (58 and 59 cm respectively) and better remobilization performance rather than to current photosynthesis. Sink activity and the grain filling period are two major important factors in carbohydrate remobilization capacity too. Sink activity is the sink’s ability to absorb and utilize carbohydrates on the basis of the sink’s tissue weight. The grain filling period, along with the grain filling rate, determine final grain weight and the former is greatly shortened due to environmental stresses. However, the grain filling period was relatively longer in the Karoon × Kavir...
and Kavir × Badia varieties (45 and 44.5 days respectively) under drought stress which may have enhanced their grain yield.

### Photochemical Efficiency of Photosystem II

Irrigation regimes, the varieties of barley and their interactions all had significant effects on photochemical efficiency ($F_v/F_m$). (Table 1) Irrigation levels had significant effects on all chlorophyll fluorescence parameters especially on the $F_v$ and $F_m$ values which were reduced and increased, respectively, with an increase in stress severity (data was not shown). Such an effect of water stress had been expressed in a significant reduction in the $F_v/F_m$ values (Table 2). The main effects of variety on the $F_v/F_m$ values were different at all irrigation levels and distinctly higher in Karoon × Kavir (0.62) (Table 2). The $F_v/F_m$ values for Karoon × Kavir and Reihani varieties were highest under severe stress condition (0% CWR) (0.47 and 0.44, respectively) which had better yield performance under drought conditions (Table 3). It can be concluded that the adverse effect of water stress on $F_v/F_m$ could be adjusted by the varietal difference between plant types.

It seems that higher photochemical efficiency of Karoon × Kavir played a major role in its drought tolerance. Reports had shown that wheat plants could acclimatise to high temperatures by using higher $F_v/F_m$ retention during reproductive growth. Such an acclimatisation could be related to the protection capability of the QA complex or its protein regeneration capacity in chlorophyll–protein complexes [8] which had led to higher grain yield under stress condition [8].

### Proline Content

An analysis of variance showed that irrigation levels had significant effects on the free proline content of barley varieties and a lower significant difference ($P \leq 0.05$) was observed between the barley varieties (Table 1). Water deficit significantly increased the proline content of all barley varieties to a maximum 152 μg.gr$^{-1}$ Fw, but was at its minimum (43.5 μg.gr$^{-1}$ Fw) under well-irrigated treatment (Table 2).

Irrigation regimes and varieties of barley both had significant effects on proline content and the irrigation effect was more distinct than that of the variety (Table 1). As regards to proline accumulation under environmental stresses and it's role in osmotic adjustment (1, 4, 8 and 12), it seems that it's accumulation procedure were more or less similar in all varieties. In comparison with the results of Lingose and Bergman [5], it may be suggested that the Karoon × Kavir and Reihani varieties were much more tolerant to drought stress with a higher grain yield at 0% CWR (0.31 and 0.323 Kg.m$^{-2}$ respectively).

### CONCLUSION

The present study showed that the higher canopy temperature (less negative $\Delta T$) under well irrigated conditions and higher grain yield, 1000–grain weight and $F_v/F_m$ under water stress conditions were the proper criteria (morphological or physiological markers) for screening the drought tolerant barley genotypes under field or laboratory conditions. Further experiments should be focused on screening for drought tolerance at the early stages of plant growth using such criteria in order to determine the relationship with field experiments and shortening the screening duration.

### REFERENCES


کاهش در تیمارهای ۵۰، ۷۵ و ۱۰۰ درصد آب‌درمانی بیش از حد در شرایط تنش آب تغییرات میزان کلروفیل در ارتفاع مختلف آب‌درمانی آب‌داری شده بود. اثرات آب‌داری بر رشد و وزن گیاهی فتوسنتزی Fv/Fm و سایر اجزای فتوسنتزی (کلیه ارتفاع آب‌درمانی) ارتفاع جو در هر سطح آب‌داری معنی‌دار بود. لیکن در مجموع میزان فتوسنتزی در ارتفاع کارون، کور، و در شرایط تنش آب کمتر از سایر ارتفاع‌ها بود. شرایط آب‌داری باعث تغییرات معنی‌داری در میزان اسید آمینه بروملین اتبایش شده در ارتفاع جو گردو بود. لیکن میزان بروملین اتبایش شده در ارتفاع مختلف جو در سطوح مختلف آب‌داری معنی‌داری نداشت. در مجموع به نظر میرسد که در شرایط آب‌داری کاملاً بالاتر بودن دمای پوشش گیاهی (T = نفاوت دمای بین پوشش گیاهی و انفجار، کمتر، منفی) و در شرایط تنش آب بالاتر بودن عملکرد دانه، وزن هزار دانه و وزن هزار دانه و در سنی خاصی از مؤثرترین شاخص‌های Fv/Fm و تغییرات آب‌داری و وزن هزار دانه در مزارع ایران محسوب می‌شوند.