Effect of Water Deficit on Physiological Behavior of Some Collected Tunisian Barley Ecolypes

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Abstract: In Tunisia, the post-anthesis water deficit for cereals takes place almost every year. The identification of tolerant barley ecotypes to this stress is of great importance for crop improvement and yield stability. To fulfill this objective, we evaluated the response of 6 barley ecotypes to moderate and severe stress (one week and three weeks of withholding irrigation). After 7 days of water stress, Souhli, Sidi Bouzid and Tozeur 1 ecotypes maintained higher leaf water potential allowing them to keep hydrated tissue cells, a significant accumulation of proline and a high peroxidases activity which allowed them to withstand the effect of oxidative stress and preserve their chlorophyll content. However, the other ecotypes showed lower water potentials. Although the peroxidases activity decreased for Sidi Bouzid and Souhli ecotypes and remained moderate for Tozeur 1 under severe stress compared to what was registered after moderate stress, it remained important compared to their control. This must be due to an acclimation of these ecotypes to water stress. However, for Northern ecotypes, we recorded a high reduction of leaf water potential and chlorophyll content which was associated with lower accumulation of proline content and moderate or significant peroxidases activity related to stress intensity showing their lack of tolerance to water stress.

Key words: Barley, water stress, proline, chlorophyll and peroxidases activity

INTRODUCTION

Cultivated barley (Hordeum vulgare L.) occupies a significant place on a worldwide scale considering its precocity, its potential yield in unfavorable zones and its use in animal and human feeds and in the brewery. In Tunisia, the barley culture remains marginalized compared to that of wheat. In fact, research was more interesting especially in durum and bread wheat with 30 registered cultivars compare with only 15 barley cultivars (El Felah, 1998). Face to insufficiency of meat, milk and its derivatives, the efforts were multiplied to ensure significant forage production, which meets the farmers' needs. For that, selection of productive and adapted barley cultivars to the Mediterranean climatic conditions became necessary.

In the Mediterranean countries, plants are subjected to water deficit which can reach severe levels in summer (Pereira and Chaves, 1993). To survive under these conditions, the plants undertake various mechanisms and morphological, anatomical, physiological and biochemical adaptations to maintain their growth and development (Chaves et al., 2003). Drought tolerance is a complex mechanism (Hoekstra et al., 2001), which allows plants to grow and survive and then maintain a good productivity (Chaves et al., 2002).

Soil water deficit reduces leaf water potential and induces plants to close their stomata. This mechanism is necessary to reduce water loss through transpiration and maintain turgidity and growth (Cornic, 2000).

Under prolonged stress, the plant reacts by slowing down its photosynthesis, which reduces the synthesis and accumulation of organic matter and growth (Ort et al., 1994). Ioaki-Iturbe et al. (1998) showed that severe water deficit inhibited photosynthesis and decreased chlorophyll quantity and β carotene of Pisum sativum L. Severe stress also affects cellular membrane integrity allowing the loss of aqueous solutions to external middle (Hale and Oroott, 1987).

Due to water stress, some plants maintain their tissues hydrated by osmotic adjustment, which is caused by accumulation of small molecules of low molecular
weights (Virgona and Barlow, 1991; Tamura et al., 2003). This phenomenon is regarded as being a significant mechanism for several vegetable species to tolerate water deficit (Chimenti et al., 2002; Wang et al., 2003).

The accumulated dissolved substances act like cytoplasmic osmolytes facilitating the contribution and the retention of water (Hare et al., 1998; McNeil et al., 1999) and stabilizing the macromolecules, the organoids and the structures (proteins, membranes, chloroplasts and liposome) against the damage caused by stress conditions (Bohnert and Jensen, 1996; Hare et al., 1998). Proline accumulation is considered as an important phenomenon and it was thoroughly studied (Ben Naceur et al., 1992). Bergareche et al. (1993) showed a correlation between proline accumulation in wheat and barley and tolerance to water stress. For Al Hakim and Monneveux (1993), the proline plays a significant role in membrane and enzymatic protection for some genotypes of tetraploid and hexaploid wheats.

Water deficit causes the generation of entities and/or free radicals, which are toxic for plants. Feltzer et al. (2002) showed that H$_2$O$_2$ is toxic for chloroplasts even at low concentrations. It inhibits the enzymes of Calvin cycle and thus reduces the CO$_2$ photosynthetic assimilation. The presence of oxygen active species can also induce chlorophyll decomposition and lipid peroxidation (Osmond et al., 1997).

To survive in such conditions and to avoid oxidant effects, plants recourse to enzymatic and nonenzymatic antioxidant systems. Antioxidative enzymatic activities generally become augmented in stressed plants (Meloni et al., 2003). They are thought to contribute towards protection of the cell structures against the reactive oxygen species generated by stress conditions (Hernández et al., 2000). Among the enzymatic antioxidant system, the peroxidases (POX) (EC 1.11.1.7) that are homoproteins of approximately 50 kDa and are present as multiple isozymes in plant tissues. POX are associated with biochemical and physiological processes as growth, cell formation, fruit development and ethylene biosynthesis (Matamoros et al., 2003). POX are also considered among the main antioxidant defenses to various stresses in plants (Mittler, 2002). In fact, Lima et al. (2002) showed an increase in the activities of the superoxide dismutases, the catalases and peroxidases for the tolerant clone of Coffea canephora compared with the sensitive ones. This could be correlated with an extreme protection against the oxidative stress of the tolerant clone, judged by lower rate of the lipide peroxidation and by the lower flux of electrolytes observed in the latter. The increase activity of the antioxidant system, which limits cellular damage, could be an important factor in stress tolerance.

The objective of this study was to study leaf water potential, proline content, chlorophyll content and peroxidases activity of several barley ecotypes under water stress and to determine the potential tolerance of these barley ecotypes to water deficit.

**MATERIALS AND METHODS**

Six local barley ecotypes were collected from various bioclimatic zones of Tunisia: two from the southern arid zone (Tozeur 1 and Kebili 1), Two ecotypes from the center semi-arid climatic stage (Souili and Sidi Bouzid) and two ecotypes originated from the north sub-humid zone (Kelibia 1 and Jendouba 1). The experiment was carried out in National Agronomic Research Institute of Tunisia in 2003.

**Control of the culture:** Sowing was carried out in 112 vegetation pots of 10 L filled with a substrate composed of 1/4 sand and 1/4 peat. Two treatments (Field capacity and withholding irrigation) with four replications per ecotype were imposed. At the full tillering stage, 56 pots were water stressed by withholding irrigation for 21 days. The others were kept at their field capacity. All measured variables were determined from the top third leaf.

The date of stress application was taken as starting date; the other dates are D1 and D2 (after 7 and 21 days of withholding irrigation, respectively).

**Leaf water potential:** Leaf water potential ($\Psi_w$) was two fold determined, at 7 and 21 days of water deficit by estimating xylem-pressure potential in a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) (Scholander et al., 1965). It was measured at the midday on the third uppermost fully expanded leaf from four randomly selected plants per ecotype.

**Proline content:** Proline accumulation was determined as described by Monneveux and Nemmar (1986). Optical density was read at 528 nm using a spectrophotometer (Bio-Rad Smart SpecTM 3000). Proline concentrations were calculated on a fresh weight basis (μg proline (g FW)$^{-1}$) using proline standard (0-0.2 mg mL$^{-1}$) curve.

**Chlorophyll content:** The uppermost fully expanded fresh leaves were taken and washed with distilled water. Ten leaf discs of 38.46 mm$^2$ surface were cut and placed in a tube containing 5 mL of ethanol for two weeks. Chlorophyll contents were calculated according to Arnon (1949) modified method.

**Specific peroxidases activity:** Peroxidases extraction was dealt according to slightly modified Kristensen et al.
(1999) method. One hundred mg of fresh leaves were crushed in the presence of liquid nitrogen in a 50 mM phosphate buffer at pH 7, to which 100 mM KCl, 1M NaCl, 1 mM CaCl₂, 0.1% Triton X-100 and 1% PVP were added. The mixture was centrifuged at 15000 rpm for 30 min at 4°C. The supernatant was kept at -20°C for further analyses.

The kinetic study was carried out according to Vallejos (1983) method in 1 mL mixture containing vegetable extract, 0.1% of guaiacol as an electron donor, 0.1 M phosphate buffer (pH 7) and 30% of H₂O₂. The rate of hydrogen peroxide oxidation was determined with the absorption coefficient (26.6 mM⁻¹ cm⁻¹ at 470 nm). OD evolution was at 470 nm; using spectrophotometer (Bio-Rad Smart Spec™ 3000), was followed during 12 min.

**Protein determination:** The total protein concentration was determined according to Bradford (1976) method. Bovine Serum Albumin (BSA) dilution series used as standard at the range between 0-1 mg mL⁻¹. OD was measured at the wavelength 595 nm.

**Statistic analysis:** The data were analyzed using ANOVAs and subsequent comparison of means was performed using the Newman-Keuls test at 5% probability. Multiple regression values were computed with Statistica software.

**RESULTS**

**Leaf water potential:** Leaf water potential is illustrated in Fig. 1 which shows significant differences among ecotypes in the control treatment (after 1 week and/or 3 weeks).

When the ecotypes were subjected to water deficit, leaf water potential fell independently from the stress duration.

Under moderate and severe stress, Souhli, Sidi Bouzid and Tozzer 1 ecotypes were slightly affected and kept humid tissues. On the other hand, Kelibia 1, Kebili 1 and Jendouba 1 showed a drop in leaf water potential of 3.6, 4.5 and 4.67 folds compared with their respective controls, having therefore dry tissues.

Fig. 1: Effect of water deficit on leaf water potential of six barley ecotypes collected from different Tunisian regions

Fig. 2: Effect of water deficit on proline accumulation (mg g⁻¹ FW) in six local barley ecotypes
Variation of proline concentration: The study of proline content shows variability among the ecotypes in the controls and in the treatments (Fig. 2). In the controls, Kebili 1 and Kelibia 1 ecotypes had the highest proline contents while Tozeur 1 and Sidi Bouzid had the smallest ones. Jendouba 1 and Souhli had an intermediate level.

Under moderate stress (7 days of withholding irrigation), a similar behavior to the control was found. However, under severe stress, the ecotypes expressed various behaviors and were classified in three groups significantly different at *p<0.05. Tozeur 1 and Kebili 1 formed the first group. The second group included Sidi Bouzid and Jendouba 1 ecotypes and the third group gathered Souhli and Kelibia 1. We also remark that Tozeur 1, Kelibia 1 and souhli showed important increases of 3.14 and 2.48 folds, respectively.

Chlorophyll variation under stress conditions: During the first date, Jendouba 1, Kebili 1, Kelibia 1 and Sidi Bouzid ecotypes had no significant difference in the control chlorophyll (a) content (Table 1). On the other hand, after one week from the tillering stage, Tozeur 1 was significantly the most rich whereas Jendouba 1 was the poorest. After a moderate stress, the chlorophyll contents diminished for all ecotypes compared to their controls. The ecotypes Sidi Bouzid and Souhli showed weak reduction rates of 5.16 and 5.2%, respectively compared to the other studied ecotypes. After severe stress, Kebili 1 and Souhli were able to maintain significantly higher chlorophyll content than the others whereas, Jendouba 1's chlorophyll content was the lowest despite the fact that it represented a weak reduction rate of 8.93%.

Peroxidases activity variation under stress conditions: In control conditions, peroxidases showed a variable activity for the two dates of (Table 2). Tozeur 1, Kelibia 1 and Jendouba 1 ecotypes had the highest activity whereas Souhli and Kebili 1 had the lowest one.

Under moderate stress and compared to controls, peroxidases activity increased in all ecotypes; however, this increase was significantly important for Sidi Bouzid, Souhli and Jendouba 1 ecotypes. When the stress was prolonged, there was always an increase of peroxidases activity for all ecotypes compared to control ones. In fact, a significant increase rate occurred in Sidi Bouzid and Kebili 1 with 1.43 and 1.2986%, respectively. On the other hand, the activity dropped in Sidi Bouzid and Souhli compared to what was determined after moderate stress. However, Kebili 1 ecotype kept a relatively steady activity (slight increase compared to what was recorded during the moderate stress).

DISCUSSION

Leaf water potential was a good indicator of species tolerance to water stress. It was used by Gahtoni-Chettri and Lales (1990) to evaluate the tolerance of some bread wheat genotypes to drought. These authors showed that higher leaf water potential was linked to the most tolerant variety to drought. In present case, the ecotypes Souhli, Tozeur 1 and Sidi Bouzid showed a high water potential which kept a relatively adequate tissue hydration under moderate and severe stress. The other ecotypes had lower potential, indicating tissue dehydration under stress conditions.

Low water potential induced cell membrane damage and enzyme inactivation leading to electrolytes lost. In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment (Ketchum et al., 1991). Several studies showed

<p>| Table 1: Mean chlorophyll (Chl) (a) content (µg mm⁻² leaf tissue), percentage decrease rate and chlorophyll a/b ratio |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>Chlorophyll content (µg mm⁻²)</th>
<th>Tozeur 1</th>
<th>Kebili 1</th>
<th>Kelibia 1</th>
<th>Sidi Bouzid</th>
<th>Souhli</th>
<th>Jendouba 1</th>
<th>Kelibia 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control</td>
<td>0.55³</td>
<td>0.49⁵</td>
<td>0.47⁷</td>
<td>0.49²</td>
<td>0.44⁰</td>
<td>0.44⁰</td>
<td>0.44⁰</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0.45⁶</td>
<td>0.40⁸</td>
<td>0.45⁹</td>
<td>0.41²</td>
<td>0.42⁰</td>
<td>0.41⁰</td>
<td>0.41⁰</td>
</tr>
<tr>
<td></td>
<td>Decrease (%)</td>
<td>14.90</td>
<td>17.24</td>
<td>5.16</td>
<td>5.20</td>
<td>16.11</td>
<td>13.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chl a/b ratio</td>
<td>2.841</td>
<td>2.353</td>
<td>3.261</td>
<td>2.810</td>
<td>2.885</td>
<td>2.689</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>0.51³</td>
<td>0.45⁵</td>
<td>0.42⁸</td>
<td>0.45⁴</td>
<td>0.45⁴</td>
<td>0.45⁴</td>
<td>0.48⁸</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0.38⁴</td>
<td>0.42⁹</td>
<td>0.36¹</td>
<td>0.41⁸</td>
<td>0.34²</td>
<td>0.36²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decrease (%)</td>
<td>25.800</td>
<td>5.37</td>
<td>15.510</td>
<td>24.740</td>
<td>8.94</td>
<td>24.054</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chl a/b ratio</td>
<td>2.470</td>
<td>2.696</td>
<td>2.897</td>
<td>3.389</td>
<td>2.802</td>
<td>2.984</td>
<td></td>
</tr>
</tbody>
</table>

The ecotypes were at full tillering stage when withholding irrigation was dealt. Chlorophyll concentration was quantified after one week and three weeks of water deficit. In each row, the values represented by the same lower-case are not significantly different at p<0.05 according to the Newman-Keuls test.

<p>| Table 2: Specific peroxidase (POX) activity (µmol min⁻¹ mg⁻¹) of six local barley ecotypes under water deficit of 7 and 21 days of withholding irrigation |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>POX activity (µmol min⁻¹ mg⁻¹)</th>
<th>Tozeur 1</th>
<th>Kebili 1</th>
<th>Kelibia 1</th>
<th>Sidi Bouzid</th>
<th>Souhli</th>
<th>Jendouba 1</th>
<th>Kelibia 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control</td>
<td>1.164±0.08</td>
<td>0.658±0.05</td>
<td>0.744±0.05</td>
<td>0.669±0.06</td>
<td>0.934±0.18</td>
<td>1.044±0.074</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1.307±0.18</td>
<td>0.838±0.11</td>
<td>2.371±0.123</td>
<td>1.596±0.1</td>
<td>1.167±0.08</td>
<td>1.804±0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase (%)</td>
<td>12.28</td>
<td>27.36</td>
<td>218.68</td>
<td>138.56</td>
<td>124.95</td>
<td>72.7</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>1.164±0.01</td>
<td>0.690±0.02</td>
<td>0.714±0.16</td>
<td>0.795±0.1</td>
<td>1.14±0.014</td>
<td>1.032±0.032</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.513±0.08</td>
<td>1.586±0.09</td>
<td>1.724±0.1</td>
<td>1.373±0.052</td>
<td>2.51±0.09</td>
<td>2.315±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase (%)</td>
<td>116.45</td>
<td>129.86</td>
<td>143.16</td>
<td>72.7</td>
<td>120.17</td>
<td>124.71</td>
<td></td>
</tr>
</tbody>
</table>

Means and confidence intervals were estimated at p = 0.05.
that in addition to its role as osmoprotector intervening in the osmotic adjustment, proline also protects tissues’ structure under stress (Virgona and Barlow, 1991). In deed, Voetberg and Sharp (1991) had shown that in apical region of maize roots growing at a water potential of -1.6 Mpa, proline concentration reached approximately 120 mM and accounted for up to 50% of the osmotic adjustment.

Monneveux and Nemmar (1986) working on durum wheat under water deficit conditions, showed a significant proline accumulation in genotypes tolerant to water deficit. Bergareche et al. (1993) also showed a positive correlation between proline accumulation and barley tolerance to water deficit confirming the results in this study.

In this framework, this study showed that after seven days water deficit, only the ecotype Kelibia 1 had low leaf water potential (-2.07 to -2.4 MPA) and accumulated a high amount of proline. The other ecotypes kept their proline content similar to those recorded in the controls. When the stress was prolonged (21 days), the ecotypes Souilhi, Sidi Bouzid and Tozeur1 accumulated significant proline quantity compared to their controls. These ecotypes also showed an increase in their leaf water potential compared with the others. This proline accumulation would play an osmoprotector effect and would maintain a high water potential and tissue hydration.

Water stress affected photosynthesis and decreased chlorophyll content (Iñaki-Ibarbe et al., 1998; Ladjal et al., 2000). Their finding was similar to the results obtained from this study when water was withheld for barley plants. Water deficit disturbed photosynthesis and generated free radicals, which induced lipid membrane peroxidation of the tylocoydes and thus chlorophyll degradation (Osmond et al., 1997).

According to Lima et al. (2002) the increase of peroxidases activity would protect plants against oxidative stress effect and would play a major role in plants tolerance to water deficit. In this context, the watering suspension caused an increase in peroxidases activity in all ecotypes and especially in Sidi Bouzid and Souilhi.

Under severe stress, this activity decreased in these two ecotypes and continued to increase in the others. Despite this reduction, the activity remained always superior to that recorded for their controls.

Considering all the variables studied, the ecotypes Sidi Bouzid, Souilhi and Tozeur 1 originated from Centre and South of the country; characterized by aridity conditions; showed more tolerance to stress by maintaining a high water potential, a significant proline accumulation and a moderate fall in chlorophyll content.

These three ecotypes showed a peroxidase activity increase indicating a stress situation, followed by a decrease in activity which could be explained by an adaptation to this situation (case of Sidi Bouzid and Souilhi) or a slight increase in activity compared with the others (case of Tozeur 1). These results confirm that the scavenging system forms the primary defence line in protecting the chloroplasts against free radicals. In tolerant plant species, POX activity was found to be higher, enabling plants to protect themselves against the oxidative stress (Scalo et al., 1995), whereas such activity was not observed in sensitive plants (Peters et al., 1989). Our observation are also in agreement with the study of Acar et al. (2001) who recorded an increase in peroxidases activity in barley after 6 days of stress and a steady activity after 12 days of withholding irrigation. The results of this study also confirm those of Loggini et al. (1999).

The ecotype Kebil 1 is moderately tolerant to water deficit since it was able to maintain an intermediate leaf water potential, proline accumulation and peroxidase activity. The other ecotypes originated from north of Tunisia were the most sensitive to water stress within all the physiological parameters studied.

In conclusion, response of plants to water deficit is complex because it disturbs several mechanisms at the same time. Therefore, to select the most tolerant ecotype, it is necessary to study several variables at the same time and analyze their relationships. The results of this study recognized that Sidi Bouzid, Souilhi and Tozeur 1 collected from semi-arid to arid bioclimatic stage were the most tolerant barley ecotypes to water deficit. Also, utility of local germplasm that can be used in future breeding programs.

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REFERENCES


