The objectives of this study were to determine leaf wa­
ter deficit conditions. Water potential or high membrane integrity will with­
stand drought injury. To maintain normal growth and physiological functions, plants should
maintain a relatively high water content in the proto­
plasm with sufficient mem­
brane activity. Under drought conditions, membrane integrity is greatly altered likewise un­
der high temperature. This is widely used, with other parameters, as indicator for drought resistance.
Plants by maintaining high water potential or high membrane integrity will with­
stand drought injury. This ability is an important factor in determining plant performance under drought stress. The objectives of this study were to determine leaf wa­
ter potential, membrane integrity and phosphatasic ac­
tivity responses to drought of six barley varieties under water deficit conditions.

**ABSTRACT**

The ability of six barley varieties to withstand water deficit was assessed by studying the leaf water potential, cell membrane integrity and the acid phosphatasic enzymes activity. Under drought conditions, Martin variety maintained and adequate leaf water potential, however "Roho" performed poorly under these conditions.

The cell membrane integrity of Martin, Faiz and Tej was less affected by drought or induced osmotic shock by polyethylene glycol (PEG), than Raibane, Aurore-Esperance and Roho varieties. For the enzymatic activity, we observed and increase of phosphatasic activity for both Aurore-Esperance and Roho varieties only.

Changes in water regimes induced different reactions in plant acid phosphatase. Varieties, with greater sensitivity to water stress showed an in­
crease in these enzymes under stressed conditions.

**RESUMÉ**

Nous avons analysé les capacités de résistance au déficit hydrique chez 6 variétés d'orge, à travers l'étude du potentiel hydrique foliaire, de la ré­
sistance protoplasmique (intégrité des membranes cellulaires) et de l'ac­
tivité enzymatique hydrolytique phosphatique.

Les variétés d'orge testées sont: "Martin", "Faiz", "Tej", "Rai"bane", "Au­
rore-Esperance" et "Roho". Elles sont habituellement cultivées en Tunisie.

En conditions de stress hydrique, réalisé par suspension d'arrosage, la variété Martin garde un potentiel hydrique foliaire élevé lui assurant une croissance peu affectée en comparaison avec les autres variétés testées et en particulier en comparaison avec la variété Roho.

De même l'intégrité des structures membranaires est moins affectée chez Martin, Faiz et Tej que chez les autres variétés soumises à la contrainte hydrique par suspension d'arrosage ou par choc physiologique, au polyéthylène glycol (PEG).

Sur le plan enzymatique, nous avons observé une augmentation de l'ac­
tivité des phosphatases acides uniquement chez les variétés Aurore-Es­
péance et Roho.

La réponse à la contrainte hydrique des 6 variétés d'orge testées montre une augmentation de l'activité des phosphatases acides corréllée à la plus grande sensibilité du sujet au stress hydrique.

**MATERIALS AND METHODS**

Plant materials and growth conditions

Six varieties of barley (Hordeum vulgare, L. cv: Martin, Raihane, Roho, Au­
rore-Esperance, Tej and Faiz) supposed to have dif­
ferent level of drought re­
sistance were used. Plants were grown in a growth chamber with 12 hours photoperiod and a 25/22 °C day/night temperature at a constant 70% relative humidity. water stress was applied to plants after 15 days of growth at field ca­
pacity as described by Ben Naceur et al., (1991).The stress treatments consisted of no watering for a period of 25 days. At the end of these treatments the plants reached the 6 leaf-stage.

Leaf water potential

A protable pressure cham­
ber of Scholander was used to measure the leaf water potential. The third
developed leaf from the top was cut and immediately placed into the apparatus for measurement. The equi­
librium pressure required to bring out water to the cut leaf collar cross-section was recorded as the leaf water potential.

Measurement of cell structure integrity

We determined the percentage of cell integrity (PI) in leaves of different barley which were subjected to osmotic shock induced by polyethyleneglycol (PEG-600). Thirty leaf discs were removed using a punch (5 mm in diameter). The leaf discs were washed in distilled water, soaked in 50 ml of PEG solution (20%) and then sub­
jected to osmotic treatment for 2 hours. They were
rinsed in distilled water and then soaked for 4 hours in 50 ml of water. Cell electrolytes were released into the water during the soaking phase (Ben Naceur et al; 1991; Ben Naceur and al., 1994). The design was a randomized complete bloc with each variety replicated five times.

The percentage of cell integrity was measured in two phases at 25 °C:

— measurement of the free conductivity (FC): Which, for the check, correspond to the residual permeability of tonoplast to ions; however, for the treatment we took the residual permeability as well as the additional permeability induced by the treatments.

— measurement of total conductivity (TC): after the first measurement, samples (leaf discs + 50 ml of water) were autoclaved for 20 minutes at 121 °C. The samples were then allowed to rest for a few hours at 25 °C. The new conductivity values were then measured and related to total conductivity.

The percentage of structure integrity was determined using the following formula:

\[ PI = \left(1 - \frac{FC}{TC}\right) \times 100, \]

as reported by Henchi, (1987).

Measurement of phosphatasic activity

The activity of phosphatasic acid (Ec. 3. 1. 3. 2), extracted from third barley leaves, was measured as previously described Tanaka and al., (1989) and Kaneko and al., (1990). We modified the method so that it will be more suitable for our case. We determined the adequate conditions of preparing leaf extract, grounding time, duration, speed of centrifugation and pH optima.

5 ml of tris-maleic buffer 0.1 M, pH 5.8 were added to 100 mg of fresh leaf and were ground with “ultra-tur­rax” for 15 minutes. The homogena obtained was cen­trifuged for 20 minutes at 36000 g (rotor sorvall SS-1) and at 4 °C.

0.4 ml supernatant aliquot was incubated at 30 °C into 2 ml tris-maleic buffer, 0.1 M, pH 5.8, and 2 ml p-nitro­phenyl phosphate (PNPP) as substrate. After 5 minutes the reaction was stopped by adding 2 ml NaOH 1 M. The p-nitrophenol (PNP) formed at the time of PNPP hydrolysis was measured by spectrophotometer at \( \lambda = 400 \) nm.

RESULTS

Leaf water potential

The leaf water potential of different varieties unequally decreased after 25 days of no watering (figure 1). “Martin”, the only variety was able to keep high water potential (~ 13.2 bars) at the end of the experiment and “Roho” had the least potential value (~ 24.8 bars). However, the other varieties had an intermediate values (figure 1). The decrease of leaf water potential was associated with a change in leaf form or rolling inward of the upper leaf surface as it was described by O’Toole and Cruz (1980).

Statistical analysis and Newman-Keuls test allowed us to have significantly four distinct groups at the probability level of \( p \leq 0.05 \):

- group 1: Martin as resistant variety;
- group 2: Tej, Faïz and Raïhane moderately resistant;
- group 3: Aurore-Esperance moderately sensitive;
- group 4: Roho as sensitive variety.

Membrane integrity

The effect of drought stress on the leaf integrity of different varieties is shown in figure 2.
For this parameter, we measured the rate of cell integrity following osmotic shock induced by PEG-600 (figure 2a) or cessation of watering (figure 2b). In the two cases, we found again the same classification as described earlier: “Martin” followed by “Faiz”; “Tej”; “Raihane”; “Aurore-Esperance” and “Roho.” However with the osmotic treatment the varieties were divided in tow distinct groups:
group 1: Martin, Faiz and Tej
group 2: Raihane, Aurore-Esperance and Roho
But with no watering treatment the varieties fallen into three groups significantly different:
group 1: Martin;
group 2: Faiz; Tej and Aurore-Esperance;
group 3: Roho.
The tow procedures gave the same result for Martin as tolerant variety to stress and Roho as sensitive variety. But it’s important to point out that “Martin” variety has later heading date than the rest. Although, it tolerate stress at the first stage, it can’t adequately avoid terminal drought stress (Van Oosterom and Acevedo, 1992 a).

Discussion and conclusion

This study allowed us to have a good variety discrimination according to their degree of tolerance to water stress. At the end of the experiment, Martin variety kept high leaf water potential (- 13.2 bars) in comparison to Roho (- 24.8 bars). This high potential allowed growth to continue by reducing the side effects of drought. Drought conditions caused partial damage to cell membranes, disrupted their structure and released the intracellular solutes into the intercellular environment. In our study, the protoplasmic resistance was associated to drought tolerance since we had the same classification as for the leaf water potential parameters. Under induced osmotic conditions by PEG-600 or no watering, Martin leaves released little electrolytes compared to the other varieties, especially Roho’s leaves. Martin was able to keep better organized cell structure and greater tolerance to drought. The other tested varieties were intermediate and their ranking changed according to the method used. This may be related to the mechanism of drought tolerance efficiency of each variety. Our results agreed with those reported by Krishnamani et al; (1984), Ben Salem and Vieira Da Silva, (1991) and Irigoyen et al; (1992). Despite that acid phosphatase activity measurement exhibited Martin variety had the greatest tolerance and Roho variety the least tolerance,
this supported the hypothesis that the loss of structure integrity may be attributed to the hydrolysis of membrane phospholipids by phosphatases and/or lipase rather than to the mechanistic destruction of membrane after plasmolyze although the synthesis of new enzyme is not discarded (table 1). It seems that these parameters, when analysed separately, had the same effect, however when analyzed together we could discriminate among resistant and less resistant varieties. Also, they allowed us to distinguish among the resistant strategy's used by these varieties. For example (Martin) variety associate two strategies at the same time which are:

— escape: by maintaining a high leaf water potential — mechanic tolerance: no increase of the phosphatasic activity in the other hand, the varieties (Aurore-Esperance) and (Roho) withstand drought by the mechanism of tolerance more than escape.

Whatever technique used, it appeared that drought resistance was accomplished by several mechanisms at the same time. Various tolerance strategies were often used to counter drought effect. However one or two among the mechanisms will dominate the others and their efficiency change according to the genotypes. This could explain changing rank of intermediate genotypes in our screening and confirm previous findings of Cecarelli and al., (1987).

### Table 1 Reactions of barley varieties subjected to water stress.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Martin</th>
<th>Faïz</th>
<th>Tej</th>
<th>Raihane</th>
<th>Auro-Esperance</th>
<th>Roho</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI after PEG shock</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PI after no watering</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Phosphatasic activity response</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Intensity response 0, +, ++, +++.
With 0 has the lowest intensity.

REFERENCES


