SCREENING OF SIX BARLEY VARIETIES FOR DROUGHT RESISTANCE BY USING LEAF WATER POTENTIAL, MEMBRANE STABILITY, AND PHOSPHATASIC ACIVITY PARAMETERS

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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, Faïz and Tej was less affected by drought or induced osmotic shock by polyethylene glycol (PEG), than Raïhane, 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 increase in these enzymes under stressed conditions.

RÉSUMÉ

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 résistance protoplasmique (intégrité des membranes cellulaires) et de l'activité enzymatique hydrolitique phosphatasique.
Les variétés d'orge testées sont: "Martin", "Faïz", "Tej", "Raïhane", "Aurore-Espérance" 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, Faïz 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'activité des phosphatases acides uniquement chez les variétés Aurore-Espérance et Roho.
La réponse à la contrainte hydrique des 6 variétés d'orge testées montre une augmentation de l'activité des phosphatases acides corrélée à la plus grande sensibilité du sujet au stress hydrique.

Breeding higher-yielding crops for dry environments has been, in the past, accomplished by selecting directly for yield (Good and Maglagan, 1993).
Another approach to improve drought resistance in plants was done by identifying the physiological mechanisms involved to insure this resistance and then by screening these genotypes at early stage at the laboratory level (Van Oosterom and Acevedo, 1992 b).
To maintain normal growth and physiological functions, plants should maintain a relatively high water content in the protoplasm with sufficient membrane activity.
Under drought conditions, membrane integrity is greatly altered likewise under high temperature. This is widely used, with other parameters, as indicator for drought resistance.
Plants by maintainig high water potential or high membrane integrity will withstand drought injury.
This ability is an important factor in determining plant performance under drought stress.
The objectives of this study were to determine leaf water potential, membrane integrity and phosphatasic activity responses to drought of six barley varieties under water deficit conditions.

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MATERIALS AND METHODS

Plant materials and growth conditions

Six varieties of barley (*Hordeum vulgare*, L. cv: Martin, Raïhane, Roho, Aurore-Esperance, Tej and Faïz) supposed to have different level of drought resistance 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 capacity 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 chamber 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 equilibrium 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 subjected to osmotic treatment for 2 hours. They were

rinsed in distilled water and than 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 desing was a randomized complete bloc with each variety replicated five times.
The percentage of cell integrity was measured in two phases at 25 °C:
— easurement 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 = (1-FC/TC) · 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).

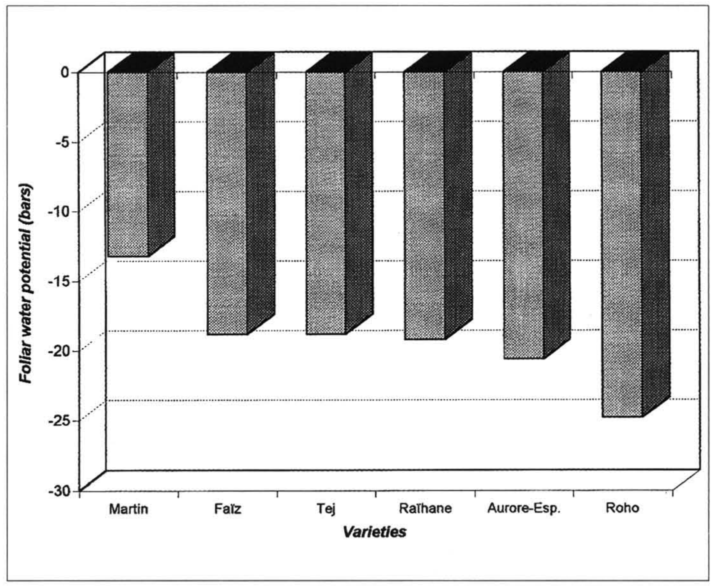

Figure 1 - Change in foliar water potential of six barley varietis subjected to water deficit.

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**.

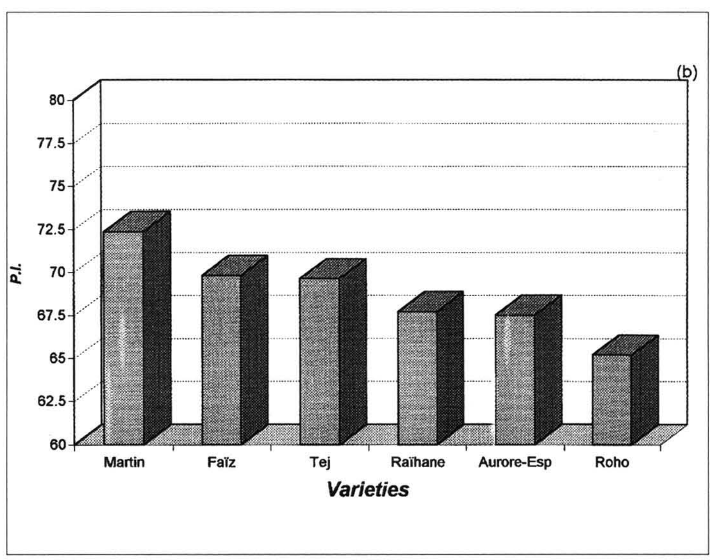

Figure 2a

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 classfication as described earlier: "Martin" followed by "Faïz"; "Tej"; "Raïhane"; "Aurore-Esperance" and "Roho".
However with the osmotic treatment the varieties were divided in tow distinct groups:
group 1: Martin; Faïz and Tej
group 2: Raïhane; Aurore-Esperance and Roho
But with no watering treatment the varieties fallen into three groups significantly different:
group 1: Martin;
group 2: Faïz;Tej and Aurore-Esperance;
group 3: Roho.
The tow procedures gave the same result for Martin as totalerant 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).

Acid phosphatase activity

The acid phosphatase activity was determined just before and after osmotic stress application. The results are shown in **figure 3**, expressed in percent of activity increases. The PEG stress did not increase significantly the phosphatase activity of Martin, Faïz and Tej varieties. However the increase of activity of Roho and Aurore-Esperance varieties was significantly different from the initial stage.
group 1: martin, Faïz and Tej
group 2: Raïhane
group 3: Aurore-Esperance
group 4: Roho
Whatever criteria used, Martin variety showed more tolerance to drought stress, and Roho to be more sensitive. Faïz, Tej and Raïhane were moderately tolerant since they don't have the same sustainaible classification. Aurore-Esperance was moderately sensitive since it was in most of the cases among the lowest groups.

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 Rohôs 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,

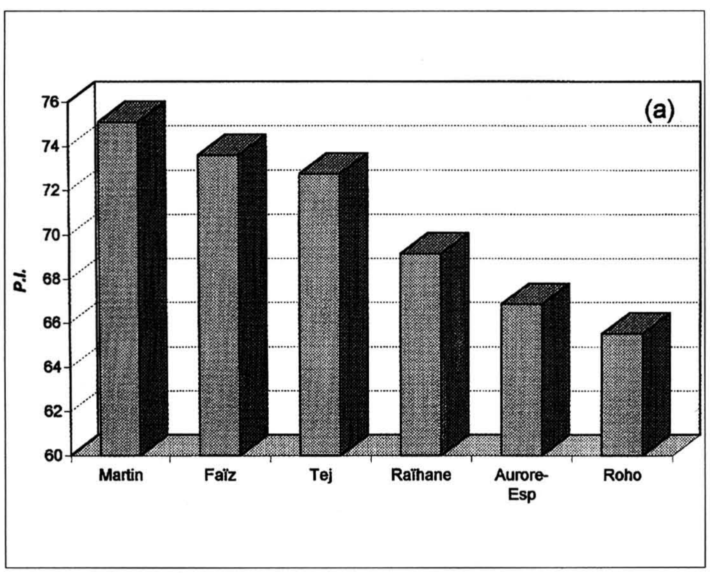

Figure 2b - Change in membrane integrity of six barley varieties subjected to water stress induced by osmotic shock with PEG-600 (a) or by no watering (b).

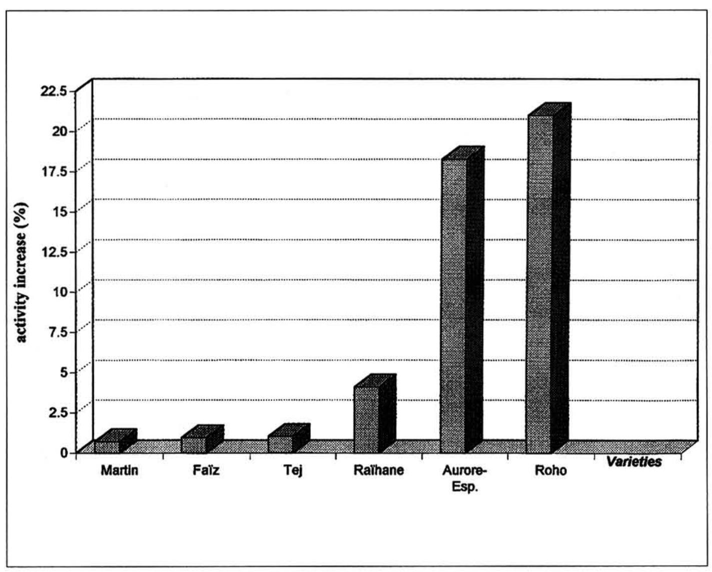

Figure 3 - Acid phosphatase activity expressed in % of control of six bartley varieties subjected to osmotic shock.

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

	Martin	Faïz	Tej	Raïhane	Auro -Esperance	Roho
Ψf	+	++	++	++	+++	++++
PI after PEG shock	+	+	+	++	++	++
PI after no watering	+	++	++	++/+++	++/+++	+++
Phosphatasic activity response	0	0	0	+	++	+++

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

this supported the hypothesis that the loss of structure integgrity may be attributed to the hydrolysis of membrane phospholipidis by phosphatases and/or lipase rather than to the mechanic 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 exemple (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-Esperrance) and (Roho) withstand drought by the mechanism of tolerance more than escape.

Whatever technique used, it appearred 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 Ceccarelli and al., (1987). Our study was carried at the stage of barley seedlings (40 days after seeding). The result showed that the variety (Martin) was particularly resistant to water deficit which is in concordance with the work reported by Maamouri et al. (1988), who dealt with the whole cycle of the plant. However under our experimental conditions the (Roho) variety was less tolerant to water deficit and more vulnerable to drought damage. But because its early heading date, it ensures a good avoidance of terminal drought stress.

This pattern is preferable in Mediterranean environments where the risk of terminal drought is frequently. "Roho" variety requires a minimum of water at the seedling stage to ensure good early growth vigour (Icarda, 1989), in order to fulfil it's grown cycle under drough conditions. ●

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