Evaluation the water stress tolerance of ten durum wheat genotypes by some physiological parameters

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Abstract

Wheat is important food for more than 35% of the world's population and its cultivation is mainly limited to such areas with water scarcity. The current study involves studying changes in the accumulation of glycine betaine, membrane stability, chlorophyll, protein and Malon (MDA) Di Aldelyde of ten varieties of durum wheat under stresses. The results of this study showed a significant decrease in the accumulation of glycine betaine, relative water content, membrane stability, chlorophyll content, MDA. The study showed that varieties responded to water stress with different mechanisms in different proportions between varieties to maintain the vital functions of the durum varieties studied.

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Introduction

Drought is a worldwide problem constraining global crop production serious (Dhande et al., 2004; Glombitza et al., 2004). Drought is a complex physical-chemical process, in which many biological macro molecules and small molecules are involved, such as nucleic acids (DNA, RNA, micro RNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements (Apel et al., 2004; Casati et al., 2004). Water availability mostly affects accumulation of some organic compatible solutes such as sugars, betaines and proline, which adjust the intercellular osmotic potential, is early reaction of plants to water stress. Sairam and Saxena (2000) reported that oxidative stress which caused metabolic damage in water stress, increases lipid peroxidation, resulting in greater membrane injury and pigment bleaching. Zlatev and Stoyanov (2005) suggested that the proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. Vendruscolo et al. (2007) found that Malon dialdehyde (MDA) is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. The physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency and develop rapidly for the purpose of crop production, yield improvement and yield stability under water stress conditions, developing of drought tolerant varieties is the best option to select a new varieties (Siddique et al., 2000).

Materials and methods

Plant materials

Ten durum wheat genotypes (Vitron, GTA, Waha, Cirta, Bidi, Wahbi, OTB4, Ter1-3, F4, Bousselem) were provided by experimental fields, as cultivars weren’t in a similar pedigree and were suitable in field of station. These experiments were carried during 2014-2015 in the experiment was conducted under green house (Bio pol, Chaabat Erssas), University of the Brothers Mentouri Constantine, Algeria, by using of split plot design that main factors contains 100% Fc and 50% Fc and sub factors contains ten Varieties of wheat in four replications.

Determination of glycine betaine content

The glycine betaine content was measured by the method of (Grieve et Grattan, 1983). The plant material (0.5g) was grinded with 20mL Distilled water for 48 h at 25 °C Leave in the refrigerator until the day of the heat treatment 0.5 ml of H.sub.2 SO.sub.4 are added and left to the ice laying 1 hour Measured by the spectrophotometer at 365 nm.

Determination of content lipid peroxides

The level of lipid per oxidation was measured in terms of Malonyl Dialdehyde (MDA) content, a product of lipid peroxidation following method of Heath and Packer (1968).

The plant material (0.3g) was grinded with 3 mL of 0.1% Trichloroacetic Acid (TCA). The homogenate was centrifuged at 1000g for 20 min. A 0.5 mL aliquot of the supernatant was mixed with 1.5 mL solution of 20% TCA containing 0.5% Thiobarbituric Acid (TBA).

The mixture was incubated in a boiling water bath for 30 min then quickly cooled in an ice bath and then warming to room temperature. The extinction was measured at 532 nm and 600 nm.

Determination of chlorophyll

Drought stress treated and control leaf samples were extracted with 80% Acetone and absorbance of supernatants were measured spectrophotometrically. Chlorophyll A was determined at wavelength 663 nm and A at 645 nm, and total chlorophyll at 652 nm.

Determination of protein

Determination of soluble protein contents were determined according to Bradford (1979) methods, used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue.

Statistical analysis

Statistical analysis was carried out with the SAS statistical computer package (SAS, Verison, 9). Experimental data were analyzed with the protected Duncan’s test p<0.05 level.
**Results and discussion**

*Glycine betaine*

The results of the present study showed positive effect of drought stress on glycine betaine accumulation, membrane stability.

Our results show that the variety Cirta has a maximum concentration (3%). The minimum value is marked by the Vitron and Bousselem varieties with 0.3%, -0.2% respectively, with an 0.85 (Fig. 1).

<table>
<thead>
<tr>
<th>Génotypes</th>
<th>Polyphénols</th>
<th>Protéines</th>
<th>Glycine betaine</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The control plants</td>
<td>Stressed plants</td>
<td>The control plants</td>
<td>Stressed plants</td>
</tr>
<tr>
<td>Vitron</td>
<td>0.040</td>
<td>0.045</td>
<td>0.370</td>
<td>0.255</td>
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<tr>
<td>Gta dur</td>
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<td>0.050</td>
<td>0.343</td>
<td>0.313</td>
</tr>
<tr>
<td>Waha</td>
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<td>0.045</td>
<td>0.310</td>
<td>0.187</td>
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<tr>
<td>Cirta</td>
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<td>0.050</td>
<td>0.320</td>
<td>0.257</td>
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<tr>
<td>B17</td>
<td>0.070</td>
<td>0.080</td>
<td>0.297</td>
<td>0.328</td>
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<tr>
<td>Wahbi</td>
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<td>0.050</td>
<td>0.341</td>
<td>0.252</td>
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<tr>
<td>Oth4(3)</td>
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<td>0.060</td>
<td>0.250</td>
<td>0.243</td>
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<tr>
<td>Ter(2-1)</td>
<td>0.070</td>
<td>0.080</td>
<td>0.258</td>
<td>0.249</td>
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<td>F4/3</td>
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<td>0.050</td>
<td>0.332</td>
<td>0.286</td>
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<tr>
<td>Bousselem</td>
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<td>0.060</td>
<td>0.286</td>
<td>0.160</td>
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<td>Minimum</td>
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<td>0.040</td>
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<td>0.080</td>
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<td>0.056</td>
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<tr>
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<td>0.0134</td>
<td>0.043</td>
<td>0.050</td>
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</tbody>
</table>

These results are confirmed in the ANOVA test, which reveals no significant differences for this parameter. Our study shows that the local variety Cirta recorded a significant accumulation of glycine betaine, this accumulation of betaine could contribute to osmoregulation in natural storage batteries, and however osmoprotection seems to be responsible for the tolerance to abiotic stresses in transgenic plants Zlatev and Stoyanov (2005). Important work on glycine betaine suggested its varied roles in plants. New evidence suggests that the contribution of differential expression of endogenous genes in glycine betaine mediated stress tolerance in plants. Additional work to determine whether transcriptome modifications are direct targets of glycine betaine or are produced from metabolic adjustment in transgenic plants. Analysis of variance (ANOVA) showed a significance in the stressed varieties P <0.05 (Table 1).

The figure 2 shows that the controls exhibit a fairly high stability percentage which varies from (86.60%) in the Cirta variety to a low percentage (46.57%) in the Wahbi variety with an average (62.77%).

On the other hand, in the stresses, the variety B17 registered a large percentage (41.43%) and the variety Bousselem showed the low percentage (51.49%) with an average (106.76%). The results show that the leaves retain an important structural integrity despite the presence of salt which causes a physiological dryness. This ability to maintain the integrity of its membranes seems to be associated with mechanisms of avoidance of saline stress.
Indeed, in many plants, there has been a disorganization of the ultra-structure of the walls caused by stress (BLUM, 1981). These alterations may result from mechanical destruction by plasmolysis. Analysis of variance (ANOVA) showed a very high significance in the ten control and stressed varieties $P < 0.001$ (Table 1).

Chlorophyll variations are shown in the histograms of Fig. 3. These results show that the Ter (2-1) variety registered the large value (15.14%), but the Vitron variety marks the low value (9.2%). For the stresses, the F4/3 variety has a higher value (17.72%), whereas the inferior value (10.19%) is marked by the variety Gat Hard for an average (12.76%). Chlorophyll is a plant pigment responsible for the green coloring of plants. This pigment, which is found in plant cells, is used with other pigments by plants to perform photosynthesis. This process allows the plant to use the energy of the sun to convert carbon dioxide ($\text{CO}_2$) and water into oxygen and organic matter.

The chlorophyll content is considered as a suitable parameter of abiotic stress tolerance (salinity, drought) in several species (Srivastava et al., 1988). Analysis of variance (Anova) showed a high significant difference in ten genotypes and under controlled and stressed conditions $P < 0.01$ (Table 1).
Stress increased the concentration of Malondialdehyde (MDA) in the genotypes studied (Fig. 4). The maximum value is noted in the stressed variety B17 of 32,756 μmol g-1MF by contribution to the controls. While the stressed Vitron variety has a minimum value of 11.54 μmol g-1MF as a contribution to the controls. The mean is 23,080 μmol g-1MF between the stratified varieties and 7,249 μmolg1MF between the control varieties. MDA plays an abiotic stress indicator and therefore can be used as a bio marker for oxidative stress (Lakhdhar-Chaabouni et al., 2007, Funes et al., 2005, Giguère et al., 2003). Analysis of variance (ANOVA) showed a very high significant difference in the ten genotypes studied and under controlled and stressed conditions $P < 0.001$. 

In this study stress decreased the concentration of proteins in the genotypes studied (Fig. 5). This decrease is marked in the Boussemel variety under stressed condition with a minimum value of 0.160 mg/ml per serving of the controls. Whereas the variety B17 in stressed condition marked, a maximum value of 0.328 mg/ml per serving of the controls. The mean was 0.253 mg/ml between the ten stressed varieties and 0.311 mg/ml between the ten control varieties. Analysis of variance (ANOVA) showed a very high significant difference in the ten genotypes studied and under controlled and stressed conditions $P < 0.001$. 

Fig. 3. The effect of drought on leaf chlorophyll.

Fig. 4. The effect of drought on leaf MDA.
Conclusion
Our study showed a difference response of the ten genotypes studied under controlled and stressed conditions for all parameters. The stressed condition reduced significantly relative water content for all genotypes. In addition, chlorophyll content and glycine betaine content are significantly decreasing under stressed conditions. The results of this study suggest that some osmoregulators is preferable in wheat if water supply is limiting.

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