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REGULAR ARTICLE

BEHAVIOR OF TUNISIAN LOCAL BARLEY ACCESSIONS UNDER PROGRESSIVE WATER DEFICIT: PHYSIOLOGICAL AND BIOCHEMICAL APPROACHES

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SUMMARY

Leaf water potential, percentage of membrane integrity and pigments chlorophyll content provide information on plant water status, on cell membranes integrity and on its photosynthetic capacity particularly under water stress conditions. These parameters were used to differentiate the behavior of 14 local barley accessions subjected to various intensities of stress (one week, two weeks and three weeks). The Principal Component Analysis (PCA) of the collected data at the end of each week revealed that the accessions behavior varies with the water deficit period. In fact, some are tolerant during the first and/or second week of stress and subsequently they are affected with a very substantial reduction in their chlorophyll pigments and their percentage of membrane integrity after three weeks. Others appear to be sensitive during the first week of stress and became tolerant under severe stress. This tolerance is manifested by the maintenance of membrane integrity, high content of chlorophyll pigments, significant proline accumulation and important specific activity of peroxidases. The study also showed that the 14 accessions exhibit two behavior types: i) significant decrease in leaf water potential with proline accumulation (constitutive osmotic adjustment) to keep cells turgid and ii) trivial drop of leaf water potential (osmotic adjustment of adaptive type). Moreover, variability in the different accessions behavior to water deficit seems to be linked to their geographical origin especially that supposed tolerant accessions are mostly from South and Central Tunisia characterized by severe aridity.

Key words: Barley; water stress; behavior; accessions; Tunisia

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1. Introduction

Leaf water potential, representing the hydrous status of plant tissues, is an indicator of species sensitivity or tolerance to water deficit. It was used by Gharti-Chhetri and Lales (1990) to evaluate the tolerance of several wheat genotypes to drought. These authors have shown that the highest leaf water potential is related to the variety tolerance to water deficit. Likely, Chaves et al., (2002) reported that low leaf water potential is due to the low soil water extraction, the low water flow rates in the plant and the intense evapotranspiration. These water potential variations were considered as indicators of plant adaptive strategies to water stress (Chaves et al., 2003). In dry conditions, lower plant water potential induces a significant loss of leaves turgidity (Levitt 1980; Turner 1986). It induces a disorganization of cell membranes especially those of thylakoids and increases, consequently, cell permeability which can be estimated by electrolytes leakage which is generally proportional to the stress damage (Hsissou 1994; Ladjal et al., 2000). Hence, the damage index is considered as sensitivity/tolerance indicator to stress. When this index is lower, membranes' integrity is reserved and the plant is qualified tolerant to stress (Kocheva and Georgiev, 2003). The water deficit causes also loss of chlorophyll pigments (Ladjal et al., 2000). The decrease in chlorophyll and protein concentrations could be considered as typical symptoms of oxidative stress caused by water deficit (Moran et al., 1994).

The turgor maintenance is performed by depolymerization of carbohydrate reserved macromolecules or by neo-synthesis of small molecules decreasing the water osmotic potential (Virgona and Barlow, 1991). This osmotic adjustment is considered as an important mechanism of water deficit tolerance used by several plant species (Chimentiet al. 2002; Wang et al., 2003). The accumulated solutes not only proceed as cytoplasmic osmolytes facilitating water transport and retention but also as protectors stabilizing macromolecules, organelles and structures (proteins, membranes, chloroplasts and liposomes) against stress damage (Al Hakimi and Monneveux, 1993; Bohnert and Jensen, 1996; Hare et al., 1998). Among these osmolytes, proline accumulation in wheat and barley organs (except glumes) is positively correlated with their tolerance to water stress (Bergareche et al., 1993).

Several unfavorable environmental conditions (drought, salt stress...) limit CO_2 fixation and reduce the $NADP^+$ regeneration in the Calvin cycle. Therefore, photosynthetic photon transport is, in one hand, reduced and, on the other hand, accompanied by superoxide radicals (O_2^-), singlet oxygen ($O_{\cdot 2}$) and hydroxyl radicals (OH^-) formations (López-Huertas et al., 1997). To overcome the oxidative effect, caused by water deficit, plants use anti-oxidants such as peroxidases which eliminate H_2O_2 and $O_{2\cdot}$ accumulated in respiratory chain even under normal water conditions. These oxidants are more abundant in stressed plants where they engender considerable damage.

Table 1 Accessions origin, bioclimatic stage and rainfall (Monthly Bulletin of the National Meteorological Institute from 1975 to 2004)

Accessions	Origin	Bioclimatic stage	Rainfall (mm)
Tozeur 1 (A1)	Tozeur	Saharian	150
Tozeur 2 (A2)	Tozeur	Saharian	150
Kébilli 1 (A3)	Kébilli	Saharian	150
Kébilli 2 (A4)	Kébilli	Saharian	150
Kébilli 3 (A5)	Kébilli	Saharian	150
Kasserine (A6)	Kasserine	Arid superior	300
Sidi Bouzid (A7)	Sidi Bouzid	Arid superior	300
Jendouba 1(A8)	Jendouba	Humid inferior	800
Jendouba 2 (A9)	Jendouba	Humid inferior	800
Souihli (A10)	Sahel	Semi-arid inferior	400
Martin (A11)	Introduced from Algeria (1931)		
Kalaâ (A12)	Kalaât El Andalous	Sub-humid	600
Kélibia 1(A13)	Kélibia	Sub-humid	600
Kélibia 2 (A14)	Kélibia	Sub-humid	600

This study aims to show (i) the influence of water deficit on leaf water potential, chlorophyll pigments (a and b) and membrane integrity variations in some Tunisian local barley accessions, (ii) the effect of water deficit on proline accumulation and peroxidases specific activity and (iii) genetic variability of barley accessions based on their tolerance to drought.

2. Materials and methods

Plant material

Thirteen barley accessions belonging to different bioclimatic zones of Tunisia were studied. 'Martin' variety, characterized by its productive and adaptive performance, was considered as model (Table 1).

Methods

Experimental design

Sowing was carried out in the 'Institut Natioanal de la Recherche Agronomique de Tunisie' INRAT experimental station (36°51', 10°11') under semi-arid climate. 112 pots, filled with seven kg of substrate composed by 3/4 soil (Table 2) and 1/4 peat each, were used and randomly installed under field conditions with eight replications per accession. Each pot contained four barley seedlings which were regularly irrigated in order to ensure their vegetative growth until full tillering stage. At this moment, water treatment was applied using two regimes (100 % field capacity 'control' and water deficit 'irrigation withheld') for each accession. The control plants were regularly irrigated every 3 days, while stressed plants were not irrigated during three weeks. At the end of each week, measurements of leaf water potential (POT), percentage of membrane integrity (PIM), chlorophyll (a) and (b) (Chl a and b) content, accumulated proline (PRO) and specific peroxidase activity (ASP) were carried out on leaves situated in the 3rd level counted from the terminal leaf for control and stressed plants.

Table 2 Physical proprieties, chemical composition and hydrous characteristic of INRAT soil

Physical properties (% of dry matter)	Chemical composition (%)	Hydrous characteristics (%)
Clay = 16 Fine silt = 19,5 Big silt = 14 Fine sand = 27,5 Big sand = 20	Active lime = 13 Total nitrogen = 0,08 Total P_2O_5 = 4 Assimilable P_2O_5 a = 0,01 Total K_2O = 3,87 Assimilable K_2O = 0,94 pH = 8,2	F.C = 22 P.W.P = 18 R.W.C = 4

F.C: Field Capacity; P.W.P: Permanent Wilting Point; R.W.C: Reserve Water Capacity

Measurements

Leaf water potential

The leaf water potential (POT) was measured using the pressure chamber of Scholander et al. (1965).

Membrane integrity

The percentage of membrane integrity was determined using Kocheva and Georgiev (2003)'s method. 30 leaf discs (38.46 mm^2) were placed in test tubes containing 5 ml of distilled water which was continuously and gently shaken then, after 4 hours of incubation, the electric conductivity (EC) was determined. The total electrical conductivity (TEC) was measured after autoclaving the tubes for 20 minutes at 121° C to ensure total leakage of tissue's electrolytes. The percentage of membrane integrity was calculated for control and treated plants as PIM = [1 - (EC/TEC)] x 100.

Chlorophyll

Chlorophyll content was determined using Arnon (1949)'s method and formula.

Proline

Proline quantification in leaves was done according to Monneveux and Nemmar (1986)'s method using 100 mg of fresh leaves per sample.

Specific activity of peroxidases

Extraction was performed according to Brian et al., (1999)'s method. 100 mg of frozen leaves were ground with mortar in 5 ml of 50 mM phosphate buffer (containing 100 mM KCl, 1 M NaCl, 1 mM $CaCl_2$, 0.1% Triton X-100 and 1% Poly Venyl Pyrrolidine; pH 7.0). The homogenate was centrifuged at 15000 rpm and 4 ° C for 30 min. The supernatant was used for peroxidases activity determination according to Vallejos (1983). One unit of peroxidases activity is defined as the amount of the enzyme that caused an increase of 0.1 in the absorbance at 470 nm by the gaïacol.

Statistical analysis

A principal component analysis (PCA) was applied on two data sets for control and stressed plants at the end of each week. Each matrix contains 14 accessions in rows and the measured parameters in columns. All these PCA were performed using SPSS 11.5 software (SPSS Inc., 2002).

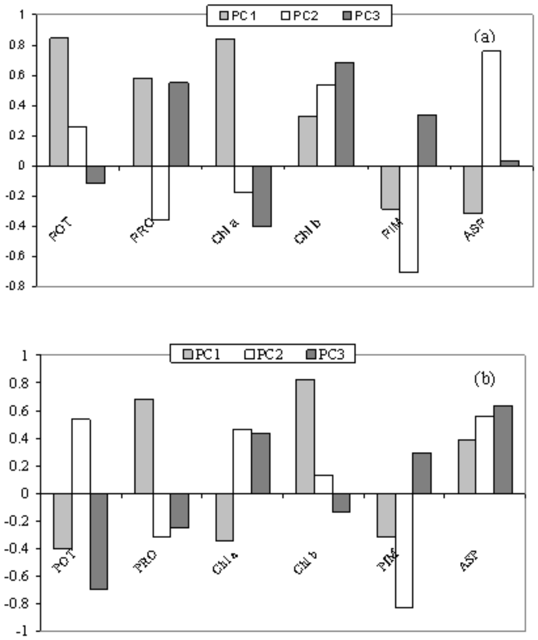
3. Results

First week

PCA applied to the parameters of control plants (Fig. 1a) shows three axes with eigenvalue superior to 1. These axes summarized 78.3% of the percentage of cumulative variance. The first axis (34.01%) shows a high positive correlation of POT, PRO and Chl (a). The negative side of this axis is marked by ASP and PIM. The second axis (26.57%) is mainly marked by ASP and Chl (b) on its positive side and PIM and PRO on its negative one. The last axis (17.71%) showed the presence of Chl (a) on the negative side and Chl (b) and PRO on its positive side.

A second PCA applied on treated plants' parameters (Fig. 1b) shows three axes with eigenvalue superior to 1 and 75.74 % of cumulative variance. The first axis (28.02%) is represented by Chl (b) and PRO on its positive side and POT and Chl (a) on the negative one. The second axis (26.96%) is marked by POT, ASP and Chl (a) on the positive side and PIM on its negative one. On the third axis (20.76%), ASP and Chl (a) are on the positive side while POT mainly dominates the negative one.

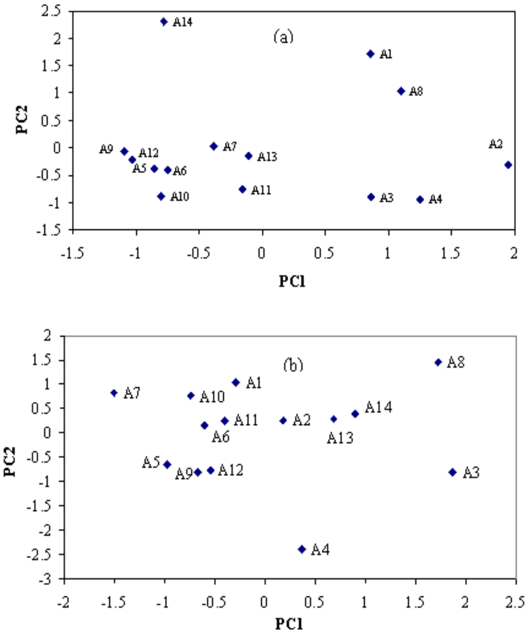
Fig. 1 Loading plot of the studied variables during the first week of water stress application on the three principal components (PC1, PC2 and PC3). (a): Control; (b): Stressed



The distribution of the 14 control accessions on the first two PCA axes is shown in Figure 2a. On the positive side of axis 1 are A2, A4, A8, A3 and A1. These accessions are characterized by a highest POT, Chl (a) and PRO. On the negative side of this axis are A9, A12, A5, A14, A10 and A6 characterized by a highest PIM and ASP and a lowest POT, Chl (a) and PRO. The accessions A14, A1 and A8 have the highest ASP, Chl (b) and POT in the positive side of axis 2. On the negative side, A4, A3 and A10 are present and showing the highest PIM, PRO and Chl (a).

The distribution of the 14 stressed accessions on the two PCA axes (Fig. 2b) shows that A3, A8 and A14 are characterized by the highest Chl (b), PRO and ASP on the positive side of PC1. On its negative side, characterized by an increasing POT, Chl (a) and PIM, A7, A5, A9 and A10 are dominating. POT, ASP and Chl (a) mainly represent the positive side of the second axis and characterize A1, A7 and A10. On its negative side, A4 is marked by the highest PIM and PRO.

Fig. 2 Score plot of barley accessions in a plane defined by the first two principal components (PC1 and PC2) during the first week of water stress application. (a): Control, (b): Stressed

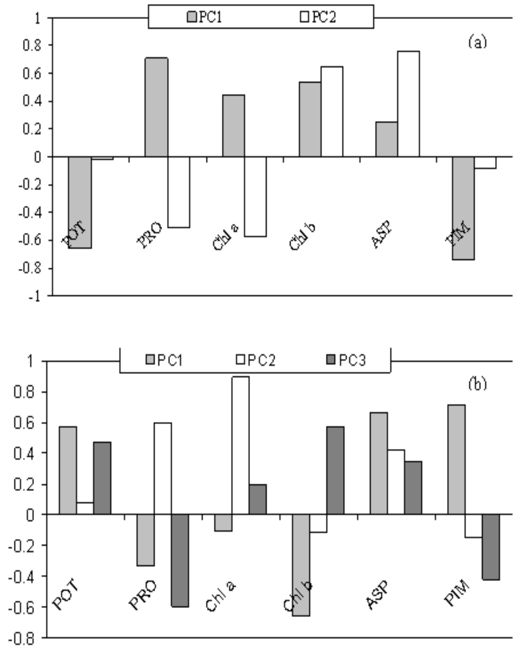


Second week

After two weeks of stress application, PCA on control plants (Fig. 3a) shows two axes with eigenvalue superior to 1 and 60.46% of cumulative variance. The first axis (33.86%) shows a high positive correlation of PRO and Chl (a and b). The negative side is marked by PIM and POT. The second axis (26.6%) is mainly marked by ASP and Chl (b) on its positive side and Chl (a) and PRO on its negative one.

Figure 3b shows that PCA, applied to the parameters of treated plants, revealed three axes with eigenvalue superior to 1 and 74.22% of cumulative variance. The first axis (30.52%) is defined by PIM, ASP and POT on its positive side. On the negative one, Chl (b) and PRO are more correlated. The second axis (23.03%) is determined by Chl (a), PRO and ASP on its positive side and PIM and Chl (b) are very low correlated on the negative one. The third axis (20.67%) is marked by Chl (b), POT and ASP on the positive side and PRO and PIM on the other side.

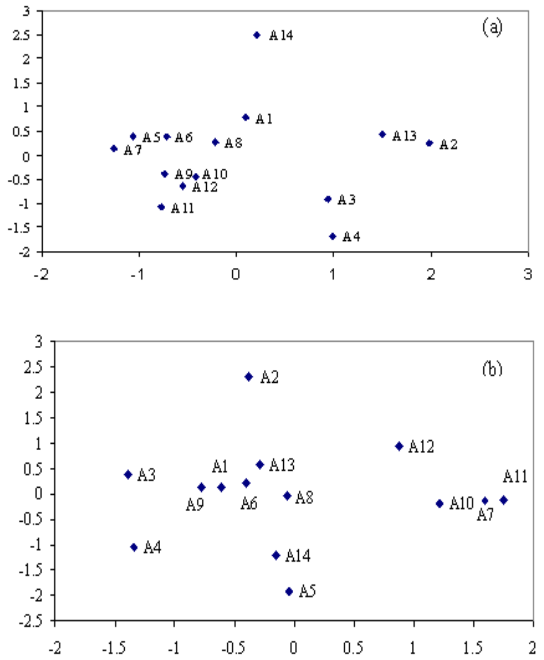
Fig. 3 Loading plot of the studied variables during the second week of water stress application on the three principal components (PC1, PC2 and PC3). (a): Control; (b): Stressed



The control accessions distribution on the PC1-PC2 plan, represented by Figure 4a, shows that A2, A13, A4 and A3 are located on the positive side of PC1 characterized by the dominance of PRO, Chl (a and b). On its negative side, A7, A5, A11, A9 and A6 showed the highest PIM and POT. On the positive side of PC2, A14 and A1 have the highest ASP and Chl (b) while A4, A11, A3 and A12 have the highest Chl (a) and PRO since they are located on PC2 negative side.

Figure 4b shows the distribution of stressed accessions on the PC1-PC2 plan. A11, A7, A10 and A12 have the highest PIM, ASP and POT since they are located on the positive side of PC1. The negative is distinguished by A3, A4, A9 and A1 with the highest Chl (b) and PRO. The PC2 positive part is mainly represented by A2 having the highest Chl (a), PRO and ASP however, its negative side is marked by A5, A14 and A4 with the highest PIM and Chl (b).

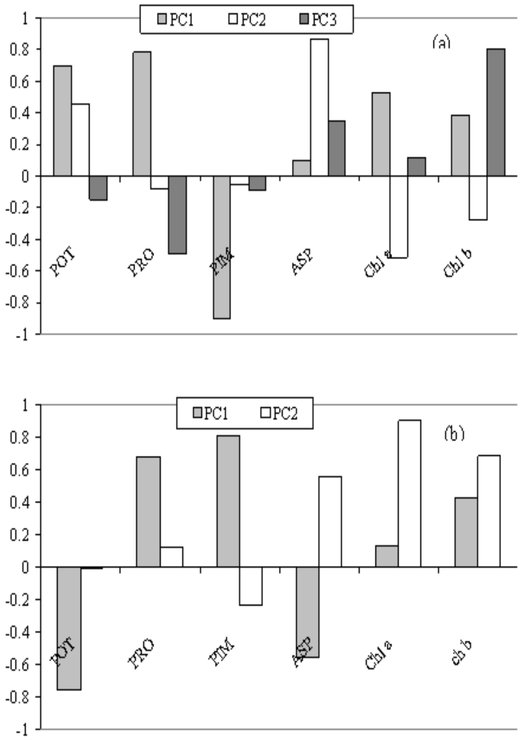
Fig. 4 Score plot of barley accessions in a plane defined by the first two principal components (PC1 and PC2) during the second week of water stress application. (a): Control, (b): Stressed



Third week

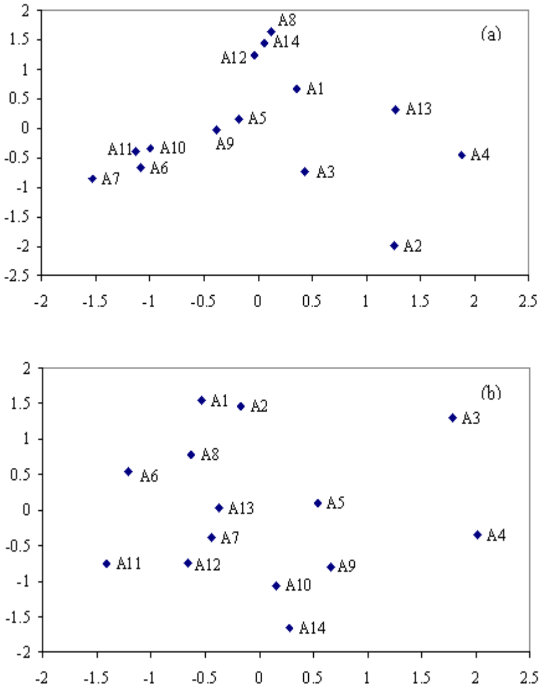
The analysis of control plants' parameters after three weeks of treatment using PCA revealed three axes with eigenvalue superior to 1 and explaining 78.44% of the total variance (Fig. 5a). PC1 (39.2%) is dominated by PRO, POT and Chl (a) on its positive side and PIM on the negative one. PC2 (21.75%) has ASP and POT on its positive side and Chl (a and b) on the other side. PC3 (17.5%) is dominated by Chl (b) and ASP on its positive side and by PRO on the negative one. The PCA of stressed plants (Fig. 5b) carries with two axes (64.29%). PC1 (36.64%) is defined by PIM and PRO on the positive side and POT and ASP on its negative side. PC2 (27.66%) is determined by a positive correlation of Chl (a and b) and ASP.

Fig. 5 Loading plot of the studied variables during the third week of water stress application on the three principal components (PC1, PC2 and PC3). (a): Control; (b): Stressed



On the positive side of axis 1 (Fig. 6a), A4, A2, A13 and A3 are characterized by the highest PRO, POT and Chl (a). In contrast, accessions showing the highest PIM are A7, A11, A6 and A10. The control plants, marked by the highest ASP and POT, distributed on the positive side of PC2 are A8, A14 and A12. In opposition, A2, A3 and A7 are marked by Chl (a and b). Distribution of stressed plants (Fig. 6b) showed that A4 and A3 are on the positive side of PC1 with the highest PIM, PRO and Chl (b). A11 and A6, distinguished by the highest POT and ASP, are located on the negative side of this axis. For PC2, A1, A2 and A3 with the highest Chl (a and b) and ASP are distributed on its positive side while A14 and A10 are on the negative one.

Fig. 6 Score plot of barley accessions in a plane defined by the first two principal components (PC1 and PC2) during the third week of water stress application. (a): Control, (b): Stressed



As a result of all PCA on the stressed plant data, two groups are identified: i) the first group, consists of A5, A6, A7, A9 and A11, is characterized by a high leaf water potential and ii) the second group, formed by A1, A2, A3, A4, A8, A10, A12 and A14, is characterized by a very low leaf water potential with a significant proline accumulation.

4. Discussion and Conclusion

The plant response to water deficit is complex because it depends on severity and duration of stress as well as the development stage during which the stress occurred (Aidaoui, 1994). Difficulties encountered include the highly dynamic character of the plant water status and the complex interactions between water stress and environmental variables. Water stress disturbs all plant functions (metabolism and physiological processes) that control growth and plant development and affects yield and its components as well as product quality (Passioura, 1997). Water deficit affects various plant biochemical processes, leaf water potential, chlorophyll content and net photosynthesis (Thomas, 2003).

Main results show that after one week, the studied accessions exhibited different responses vis-à-vis water deficit. In fact, A5, A9 and A10 were able to maintain their POT, Chl (a) and PIM. This indicates that these accessions were able to overcome drought and are considered, therefore, tolerant to water stress. During the second week, these accessions showed a decrease in their chlorophyll pigments' content and preserved their POT and PIM. Also, A7 and A11 kept their tissues well hydrated and well integrated membranes. However, A2, A3 and A4 were able to maintain high chlorophyll pigments' content and a significant proline accumulation. After three weeks, mainly A3 kept PIM, chlorophyll pigment concentrations, high proline accumulation and high ASP. It seems that the fourteen studied accessions present two standard behaviors under water deficit. The first is characterized by high leaf water potential representing accessions developing mechanisms to avoid water deficit. The second is characterized by low leaf water potential representing accessions that can tolerate water deficit. This result is in contradiction with those of Ben Salem (1993) showing that barley varieties are characterized by avoidance and esquive of water deficit.

Liu et al., (2004) showed that the tolerant wheat variety kept high relative water content compared to the sensitive one and it was able to maintain high leaf water potential. This is in agreement with our results which indicate that the tolerant accessions (A5 and A9) were able to maintain high leaf water potential during the first two weeks of stress application. A decrease in chlorophyll pigment content (chl (a), chl (b) and total chl) in plants subjected to severe stress compared to their control was reported by Harinasut et al., (2000). However, under moderate stress, chl (a) remains stable, while chl (b) decreases significantly and proportionally with stress intensity. This is confirmed by our study showing that A5 and A9, supposed tolerant to water deficit on the basis of their high content of chl (a) during the first week, decreased their content of chlorophyll pigments in contrast to A2, A3 and A4 during the second week of stress. On the other hand, proline accumulation is positively correlated with the osmotic potential indicating tolerance to water stress (Iannucci et al. 2000; Neelam and Ajay, 2005). Likely, Slama (2002) showed that the wheat variety accumulating more proline is more productive under water deficit conditions. These results corroborate with our finding showing that A2, A3 and A4 accumulated high amounts of proline after two weeks of water deficit. Only A3 and A4 kept their high proline content after three weeks of stress.

Water deficit decreases cell membranes integrity especially that of thylakoids and increases cell permeability which can be estimated throughout electrolyte leakage generally proportional to the damage caused by stress (Hsissou 1994; Ladjal et al., 2000). According to Ben Naceur (1994), species tolerant to water deficit possess more integrated membranes and less electrolytes leakage than sensitive ones. Only A3 and A4 maintained important percentages of membrane integrity. According to Aouad (1997) and Khales and Baaziz (2005), increased peroxidases activity depends on the duration and severity of stress. They also noted that this increase is triggered in tolerant plants. Likely, Niedzwiedz-et al., (2004) showed a correlation between free radicals production and the efficiency of antioxidant system in wheat plants tolerant to drought stress. In our study, only A3 showed a significant increase in specific peroxidases activity. Drought resistance depends on the plant constitutive characteristics especially maintenance of leaf water potential, membrane structures' integrity and chlorophyll content. Various mechanisms are responsible for maintaining water status and membrane integrity including proline accumulation and antioxidant system activation such as peroxidases. Consequently, A3 is the most tolerant barley accession to water deficit. It can be used in future programs of barley genetic improvement. Therefore, important consideration should be given to collection and conservation of local material for breeding, in order to maintain and preserve local barley germplasm from genetic erosion.

Plant species and varieties have many adaptation forms to harsh climatic conditions. These natural abilities are used in crop improvement. To achieve this goal, we must rely on improved varieties and rustic bases. Studying the effect of abiotic stress on cereal cultivars shows that adaptive genes are associated with plant response. In barley and other crops, there is considerable variation in response to abiotic stress to explore in local germplasm and wild species. This interdependence of genetics and physiology is important in determining the share of participation of genotype (G), of environment (E) and their interaction (GxE) on the species phenotypic behavior.

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