Botanical Studies (2006) 47: 51-60.

Comparative sensitivity of two Moroccan wheat varieties to water stress: the relationship between fatty acids and proline accumulation

Mimoun EL KAOUA[1,*], Rachid SERRAJ[2], Mohamed BENICHOU[3], and Driss HSISSOU[1]

[1]*Laboratoire de Biotechnologie et Phytopathologie Moléculaire, Département de Biologie, Faculté des Sciences et Techniques Guéliz, Université Cadi Ayyad B.P. 549, Av. Abd el karim El Khattabi, Marrakech, Maroc*
[2]*Crop Physiology Laboratory, International Crops Research Institute for the Semi-Arid Tropics, Po Patancheru 502 324, India, CGIAR-ICRISAT*
[3]*Department of Biology, Laboratory of Biochemistry Cadi Ayyad University, BP2390 Marrakech, Morocco*

(Received October 12, 2004; Accepted August 2, 2005)

ABSTRACT. Membrane lipids, peroxidation, β-carotene content, proline accumulation, and photosynthetic activity were analyzed in two Moroccan wheat (*Triticum aestivum* L.) varieties, Nasma (adapted to irrigated zone) and Tigre (adapted to semi-arid zone). Plants were grown in plastic pots under laboratory irrigation and water stress conditions. One set of plants was subjected to regular water irrigation and another to water stress conditions created by water deprivation. After 30 days of water shortage (corresponding to 8% of field capacity), the leaves' fatty acid contents, especially that of Octadecatrienoic acid (18:3 $\Delta^{9,12,15}$), i.e. the major unsaturated fatty acid, significantly decreased. The decrease was more pronounced in Nasma varieties than in Tigre. The amount of galactolipids, phospholipids, and β-carotene decreased in droughted plants of both local wheat varieties while the content of neutral lipids increased. Lipid peroxidation, assessed as the content of malondialdehyde (MDA), was found to be augmented under stress. (The rate of increase was 41% and 19% in Nasma and Tigre respectively). The amount of proline accumulated in leaf segments of both varieties subjected in vitro to osmotic stress was suppressed by addition of octadecanoic or octadecadienoic acids. The inhibition of photosynthetic capacity under osmotic stress was reduced when fatty acids were added in medium. A positive relationship was observed between proline accumulation and membrane stability. The mechanisms of these physiological responses to water stress are discussed.

Keywords: Lipids; Photosynthetic capacity; Proline; *Triticum aestivum*; Water stress.

Abbreviations: FAME, fatty acid methyl esters; **MGDG**, monogalactosyldiacy-glycerol; **DGDG**, digalactosyldiacy-glycerol; **PH**, phospholipids; **NL**, neutral lipids; **TBARS**, 2-thiobarbituric acid-reactive substances; **TBA**, 2-thiobarbituric acid; **MDA**, malondialdehyde; **RM**, reference medium; **PEG**, Polyethylene glycol; **16:0**, palmitic acid; **16:1**, palmitoleic acid; **18:0**, stearic acid; **18:1**, oleic acid; **18:2**, linolenic acid; **18:3**, linolenic acid.

INTRODUCTION

Photosynthetic CO_2 assimilation is known to be severely inhibited by water stress (Boyer, 1970; Kriedemann, 1986; Vu et al., 1987). This is partly due to the closing of the stomata that also reduces leaf transpiration and prevents the development of excessive water stress in plant tissues. Furthermore, under water stress conditions the chlorophyll contents as well as the carotenes and other foliar pigments can be decreased (Iturbe Ormaetxe et al., 1998). Inhibition of photosynthetic capacity under water stress conditions is related to a decrease in PSII activity with changes in specific intrinsic and extrinsic proteins involved in electron transport. This inhibition is a consequence of reduced oxygen-evolving capacity and additional damage within reaction centers (Eastman et al., 1997). In cotyledons of clusterbean (*Cyamopsis tetragonoloba* L.), water stress induced a quantitative loss in the D1 protein, an increase in thylakoid lipid peroxidation and a decline in the level of β-carotene (Deo and Biswal, 2001). On the other hand, the role of β-carotene in protecting isolated PS II reaction centers from photoinhibition damage has been demonstrated (Yang et al., 2002).

*Corresponding author: E-mail: mkaoua@fstg-marrakech.ac.ma; Tel: 044-43-31-63; Fax: 044-43-31-70.

At the cellular level, cell membranes are one of the first targets of plant stresses (Levitt, 1972). The ability of plants to maintain, under drought, membrane integrity is what determines tolerance towards drought stress (Vieira da Silva et al., 1974). Membrane stability is a widely used criterion to assess crop drought tolerance (Premachandra and Shimada, 1988; Zuily-Fodil et al., 1990). During severe water stress, a decrease in the membrane lipid contents has been observed (Monteiro de Paula et al., 1990). This decrease is mainly due to an activation of degradation processes (Monteiro de Paula et al., 1993). The phospholipase and galactolipase activities have been shown to be stimulated in stressed and senescing plants (Kanituga and Gemel, 1984; O'Sullivan et al., 1987; EL-Hafid et al., 1989). It was also reported that in cotyledons of clusterbean (*Cyamopsis tetragonoloba* L.) the peroxidation of the thylakoid lipids increases under water stress conditions (Deo and Biswal, 2001).

Water stress increases the production of reduced reactive oxygen species (ROS) in chloroplasts (Smirnoff, 1993). In plants, the chloroplasts are a major source of activated O_2 (Asada and Takahashi, 1987; Foyer et al., 1994). The superoxyde radical of O_2 is produced by photoreduction of O_2 at PSI and PSII, and singlet O_2 is formed by energy transfer to O_2 from triplet excited state chlorophyll (Asada and Takahashi, 1987). The negative effects of stress may be partly due to the oxidative damage resulting from the imbalance between production of activated O_2 and antioxidant defence mechanisms (Foyer and Harbinson, 1994). Other subcellular compartments of leaves, such as mitochondria and peroxisomes, are potential generators of O_2 and H_2O_2, mainly as a consequence of electron transport and enzymatic reactions (Del Rio et al., 1992). Under water stress, the formation of ROS is usually exacerbated. Water stress caused a marked increase in oxidative damage to proteins, particularly in mitochondria and peroxisomes. Bartoli et al. (2004) have suggested that mitochondria are the main target for oxidative damage to proteins under drought conditions.

Since drought is a major constraint to cereal production and yield stability in most regions in Morocco, the purpose of this work was to evaluate differences in the response to water stress of two Moroccan wheat varieties, i.e., Nasma (adapted to irrigated zones) and Tigre (adapted to rainfed zones). For that, we report on changes in fatty acids, lipid peroxidation (commonly taken as indicator for oxidative stress), and on the relationship between photosynthetic activity and proline accumulation under osmotic stress. Proline accumulation under osmotic stress conditions was previously supposed to be involved in osmotic adjustment (Delauney and Verma, 1993). In this work, we have evaluated the effect of saturated or unsaturated fatty acids on proline accumulation and photosynthetic activity altered under osmotic stress conditions. Previous studies have shown that proline osmo-induced in canola leaf was suppressed by the addition of PUFAs to stress medium containing PEG (Huguet-Robert et al., 2003).

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of two bread wheat (*Triticum aestivum* L.) varieties that are adapted to irrigated conditions (Nasma) or rainfed semi-arid conditions (Tigre) were provided by SONACOS (National Company for Seeds Marketing in Morocco, Souihla Road, Marrakech City).

Seeds were germinated on moist filter paper placed in glass Petri dishes (9 cm in diameter) in the dark at 25±1°C. Seedlings were transferred at stage 1-2 on Zadoks scale, i.e. two leaves unfolded (Zadoks et al., 1974), into plastic pots (24 cm in height and 20 cm in diameter) containing a mixture of peat, garden soil, and sand (2:1:1 v/v/v) with a water saturation capacity of 75%. Four seedlings per pot and 15 pots for each variety and condition were used. The pots were placed under natural day and night temperatures (20 ± 4°C and 7 ± 3°C, respectively) and under sunlight (10 ± 2 h). All plants were irrigated every two days with 250 ml of tap water.

After appearance of the 5th leaf, the pots were divided into three groups according to water stress treatments: control plant (continuous irrigation), plants subjected to water deprivation corresponding to 15% of field capacity (evaluated by gravimetric measurement) for 15 days, and plants subjected to water deprivation corresponding to 8% of field capacity for 30 days. For all subsequent experiments, leaf material was collected from newly developed leaves during the stress period.

Measurement of leaf pigments

Leaf pigments were extracted from 0.5 g of leaves with acetone/distilled water (90:10 v/v); the chlorophyll contents were determined spectrophotometrically (GBC UV/VIS 916) using a kinetic method of controlled pheophytinization (Laval-Martin, 1985). Addition of 5 µl of HCl (3 N) to 3 ml of the pigment extracts was sufficient to produce total pheophytinization of both chlorophylls.

For the determination of β-carotene contents, 20 ml of pigment extract were saponified by 10 ml of 15% ($w.v^{-1}$) KOH under darkness for 60 min. Carotene pigments were extracted by addition of petroleum ether, and the absorbance of β-carotene in the suspension was measured by a spectrophotometer (GBC UV/VIS 916) at 443 nm. The β-carotene amounts were calculated using the coefficient $\varepsilon = 0.2630\ ml \cdot \mu g^{-1} \cdot cm^{-1}$ (Laval-Martin and Mazliak, 1979).

Photosynthetic oxygen evolution

The photosynthetic oxygen evolution by the leaf tissues, exposed to saturating light (420 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by lamps HMB-160 W) was measured using a modified Clark type electrode (YSI Model 5300) sensitive to a variation of about 3 $\mu M\ O_2 \cdot min^{-1}$. The consistency of photosynthetic response was checked by measuring two successive dark to light (12 min/condition) transitions

for each sample. The experiments were carried out in a temperature-controlled cuvette of 3 ml at 25°C in a buffer (50 mM Tris, pH 7.5; 1 mM $MgCl_2$) containing 40 mM $NaHCO_3$ (El Kaoua and Laval-Martin, 1995) on 50 mg of fresh leaf segments (leaf 5) maintained previously in the same buffer for 15 min. The photosynthetic activity was expressed in µml of $O_2 \cdot$µmol chlorophyll$^{-1} \cdot h^{-1}$.

In comparison with control, the effect of osmotic stress on leaf photosynthetic activity in the suspension medium was evaluated by adding 25% of PEG-10 000 alone or in combination with 25 µM of fatty acids (18:0 or 18:2). This concentration of PEG 10 000 provided - 0.95 MPa osmotic potential. Tap water provided only - 0.05 MPa as measured with an A0300 KNAUER Automatic Semi-Micro Osmometer and calculated using the equation of Michel et al. (1983):

$\Psi = 8.3141 \times 10^{-5} \times (273.15 + °C) \times mosmol/Kg \times 10^{-1} = MPa$

Proline assay

Wheat seedlings were grown for 6 weeks in hydroponic Hoagland's medium in controlled environment chambers at 22°C and under constant illumination provided by TSE standard lamps (Type Preheated F40W/54 daylight) giving about 80 µmol$\cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. During the culture period, the medium was changed eve ry four days. Developed leaves were cut into segments (1.0 / 0.5 mm), and 100 mg was transferred to a reference medium (5 mmol$\cdot l^{-1}$ HEPES, 10 mmol$\cdot l^{-1}$ KCl, 1.5 mmol$\cdot l^{-1}$ $CaCl_2$, pH 6.0) (Huguet-Robert et al., 2003).

Osmotic stress was created by adding 25% (≡ -0.95 MPa) of PEG-10000 to the reference medium. The Petri dishes containing segments of leaves were divided into six groups: (i) stress medium with PEG solution only, (ii) stress medium supplemented with 25 µM of octadecanoic acid, (iii) stress medium supplemented with 50 µM of 18:0, (iv) stress medium supplemented with 25 µM of 18:2, (v) stress medium supplemented with 50 µM of octadecadienoic acid; and (vi) the control group was represented by leaf segments in the reference medium (without PEG). The pH was adjusted to 6.0. The Petri dishes were closed, placed under continuous light, and shaken continuously at 22°C.

Proline extraction and determination

Proline content was determined 5 min after the leaf segments were cut, and the segments were maintained for 6 h or 20 h in the different mediums. Proline was extracted from 100 mg of fresh matter with 3 ml of methanol: chloroform: water (12:5:1, v/v/v). The homogenate was centrifuged at 2,500 *g* for 5 min, and the supernatant was recuperated and used for proline estimation.

Free proline was quantified by spectrophotometer by means of a colorimetric reaction with ninhydrin according to the method of Singh et al. (1973) modified by Paquin and Lechasseur (1979). 1ml of supernatant was transferred to test tube and heated in a water bath until methanol evaporation. 0.33 ml of ninhydrin solution (0.01 g of ninhydrin, 0.166 ml of sulphuric acid 6 M and 0.25 ml of glacial acetic acid), 0.33 ml of glacial acetic acid and 0.33 of water were added to 0.33 ml of sample. Then the tube was cooled to room temperature, and 2 ml of toluene was added. After 30 s of shaking, two phases were separated, and the absorbance of the upper phase was read at 520 nm. Proline concentrations were determined by comparison to a 0- to 300 µg proline standard curve and then expressed on a (µg g^{-1}) DW basis.

Lipids extraction and separation

Leaf tissues (0.5 g FW) were ground in an ice-cold mortar and fixed in 15 ml of boiling 1.5% NaCl solution. Lipids were then extracted with 100 ml of a mixture of chloroform/methanol/water (8:4:1 v/v/v) (El Kaoua et al., 1991). After a strong manual mixing, the emulsion was centrifuged at 5,000 rpm (HEMLE - Type Z383 - Max.Speed 17,000 rpm - Max. RCF 26.810xg - Rotor 220.87 VO1) for 5 min, and the recuperated chloroform hypophase was evaporated at 35°C and stored under nitrogen gas at -20°C until separation and analysis of the different fatty acid classes.

A spot of the chloroform lipid solution was deposited on silica gel TLC plates (Kieselgel 60). One-dimensional separation was performed in a saturated atmosphere with the solvent mixture of acetone, acetic acid, and water (100:2:1 v/v/v) (Christie, 1982). The spots corresponding to the different lipid classes were visualized under UV light after the plate was sprayed with an aqueous solution of rhodamine 6 G (0.02%, w/v). The bands corresponding to galactolipids (MGDG and DGDG), phospholipids, and neutral lipids were identified by their different Rought front of migration (Rf).

Esterification and methylation of fatty acids

Before esterification, an internal standard consisting of 40 µg of 19:0 (nonadecanoic acid, Sigma) was added, followed by sulphuric acid/methanol (2.5/97.5 v/v). After two hours at 65°C, 2 ml of petroleum ether and 1 ml of distilled water were added, and the fatty acid methyl esters (FAME) present in the upper phase were collected and analysed with a gas chromatograph (Varian Star Chromatography Workstation, CP 3380 CG) equipped with a capillary column (Carbowax 20M, 0.35 mm × 25 m) and using nitrogen as carrier gas at a pressure of 5.6 bar. The separation was carried out at 160°C for 50 min, then 210°C for 1 h 30 min; the detector was a flame ionisation type (FID).

The FAMEs were identified by comparison of the retention times with those of known standards, and determination of fatty acid composition was based on percentage of each peak area. The quantitative analysis of the FAME was performed by using an integrator (CP-Scanview; Chromatography Application Database, Version 6.0) based on the known quantity (40 µg) of 19:0.

Determination of malondialdehyde

The peroxidation of leaf lipids was measured by the TBA method, as described by Iturbe-Ormaetxe et al. (1998), in which the reactive substance was quantified as MDA, an end product of lipid peroxidation. Lipid peroxides were extracted from 0.5 g (fw) of leaves with 5 ml of 5% (w/v) metaphosphoric acid and 100 µL of 2% (w/v) butyl hydroxytoluene (in ethanol). After centrifugation (15,000 *g* for 20 min), the chromogen was formed by mixing 0.5 ml of supernatant, 50 µl of 2% (w/v) butyl hydroxytoluene, 0.25 ml of 1% (w/v) TBA (in 50 mM NaOH), and 0.25 ml of 25% (v/v) HCl. The reaction mixtures were incubated at 100°C for 30 min and cooled to room temperature. The chromogen formed was extracted by adding 1 ml of *n*-butanol to the mixture followed by vigorous shaking, the butanol and aqueous phases were separated by centrifugation, and the absorbance of the TBARS was determined as TBA-MDA complex at 532 nm using a spectrophotometer UV (GBC UV/ Vis 916). The amount of MDA was calculated: $\varepsilon = 155.0\ mM^{-1} \cdot cm^{-1}$ (Shalata and Tal, 1998).

Statistical analysis

The calculated mean values ± SD are reported in the tables and figures below. The significance of statistical differences between samples and each treatment were evaluated by the t-test.

RESULTS

Lipids analysis

Under control conditions, the fatty acids consistently identified in the total leaf lipids of the two wheat varieties were 16:0, 16:1, 18:0, 18:1, 18:2 and 18:3. However, 16:0 and 18:3 represent together about 80% of total fatty acids (Table 1).

Water stress caused a decrease of fatty acid content in both Nasma and Tigre varieties. This decrease was more dramatic for the unsaturated 18:3 octadecatrienoic acid in Nasma and was paralleled by an increase in the percentages of saturated fatty acids 16:0 and 18:0 (Table 1). The lipid composition was also affected by water stress in both varieties. Indeed, there was an important decrease in the percent of galactolipids and phospholipids, especially in Nasma, and an increase of neutral lipids under water stress (Figure 1). Lipid peroxidation increased significantly more in stressed Nasma varieties compared to Tigre (Figure 2).

Proline accumulation

When the leaves were cut in segments, the proline content increased about 16 fold in Nasma and threefold in Tigre (Figure 3) due to water loss by tissues after 6 h in reference medium. This new proline content remained unchanged after 20 h in RM, and we have considered this new content as the control. When leaf segments were subjected to osmotic stress imposed by PEG-10000 during 20 h, they accumulated more proline. This accumulation was remarkably higher in Nasma compared to Tigre (Figure 3). Indeed, the proline level increased about 7 and 9 fold in Nasma and Tigre, respectively. This accumulation was significantly lower in either variety when the stress medium was supplemented with 25 µg of 18:0 or 18:2. When the same fatty acid concentrations were increased to 50 µg, the proline content fell significantly in Nasma varieties.

Pigments content

β-carotene content was about twofold higher in Tigre than in Nasma under irrigated conditions (Figure 4). This content was significantly reduced (more than 50%) by water stress in both varieties, but remained two times higher in Tigre than in Nasma (Figure 4).

Table 1. Total fatty acid content ($mg \cdot g\ DW^{-1}$) and fatty acid composition (%) of leaves of two wheat (*Triticum aestivum*) varieties, Nasma and Tigre. The varieties were subjected (Stressed) or not (Control) to water stress, induced by withholding irrigation after the fifth leaf appearance for 30 days. Results are means ± SD (n = 3).

Fatty acids		Nasma		Tigre	
		Control	Stressed	Control	Stressed
16:0	Content	2.73 ± 0. 43	2.43 ± 0.41	2.87 ± 0.27	1.90 ± 0.31
	%	17.3 ± 0.3	28.2 ± 3.1	25.9 ± 1.5	24.2 ± 2.5
16:1	Content	0.57 ± 0.12	0.31 ± 0.10	0.50 ± 0.02	0.30 ± 0.05
	%	3.9 ± 0.3	3.5 ± 1.2	4.5 ± 0.2	4.2 ± 0.3
18:0	Content	0.35 ± 0.05	0.29 ± 0.01	0.24 ± 0.07	0.18 ± 0.03
	%	2.4 ± 0.1	3.5 ± 0.7	2.2 ± 0.5	2.9 ± 0.9
18:1	Content	0.74 ± 0.13	0.47 ± 0.24	0.38 ± 0.02	0.28 ± 0.05
	%	4.3 ± 0.5	5.6 ± 1.7	3.6 ± 0.2	4.6 ± 1.5
18:2	Content	0.65 ± 0.05	0.26 ± 0.15	0.21 ± 0.07	0.20 ± 0.05
	%	4.2 ± 0.2	2.9 ± 0.9	2.3 ± 0.6	2.5 ± 0.4
18:3	Content	9.43 ± 0.18	4.80 ± 1.37	5.59 ± 0.28	4.39 ± 0.73
	%	66.6 ± 2.7	54.8 ± 4.7	60.3 ± 1.9	58.9 ± 2.9

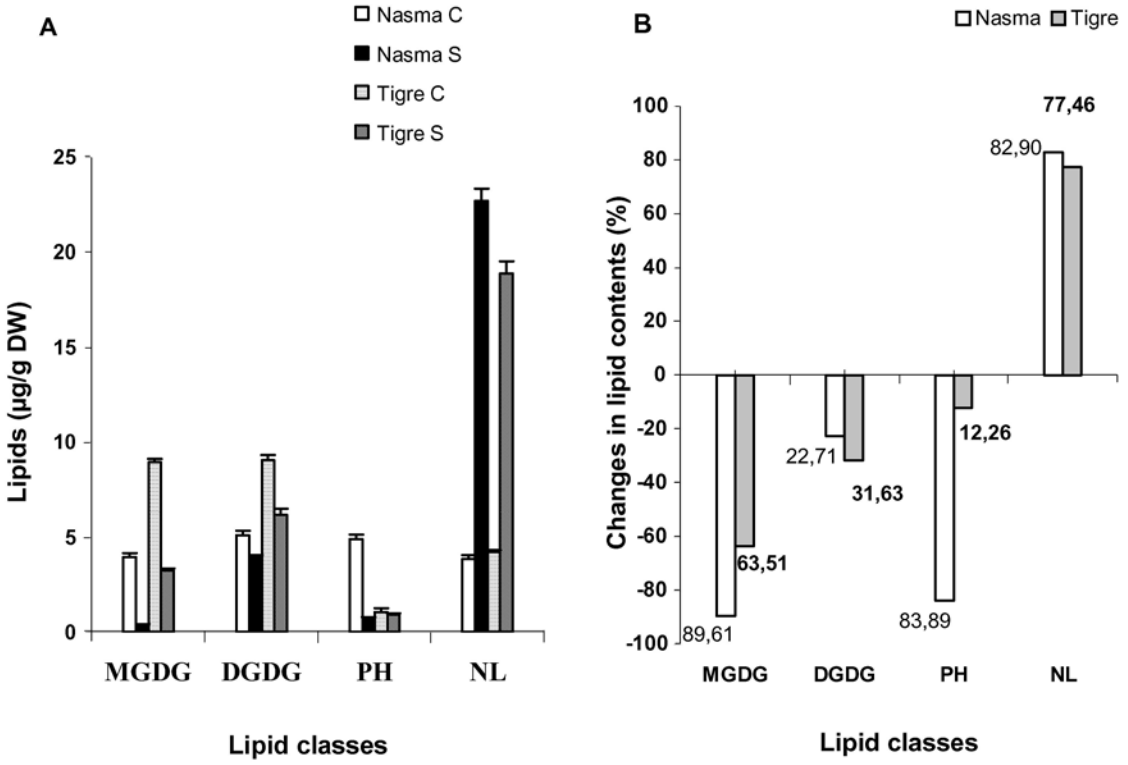

Figure 1. Change in the relative amount of lipid classes (expressed as a percent of control) as related to water stress, induced by withholding irrigation during 30 days, in leaves of two wheat (*Triticum aestivum* L.) varieties: Nasma and Tigre: (A) Result represents the variation of lipid content (in µg·g DW^{-1}) under water stress. (B) Result expressed as a percent of control. The result are means ± S.D. (n=3). C: control; S: stressed.

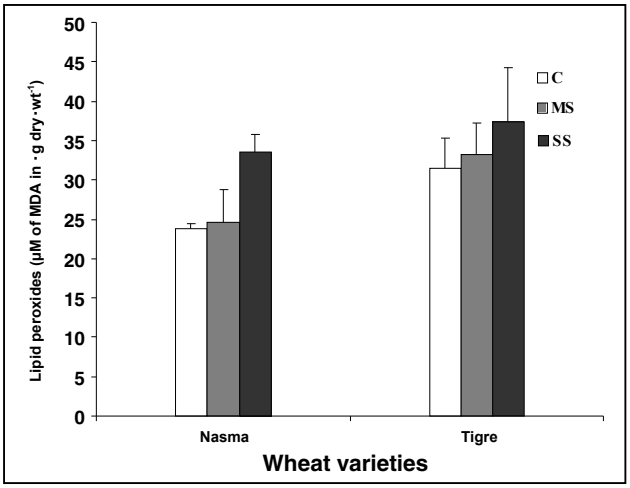

Figure 2. Malondialdehyde (the end product of lipid peroxidation) quantified in leaves from two cultivars of *Triticum aestivum* (Nasma was adapted to an irrigated zone, and Tigre to a semi-arid zone) subjected to moderate (MS) or to severe (SS) stress induced by withholding irrigation after the fifth leaf appearance for 15 and 30 days, respectively. Results represent means ± S.D. (n = 5 separate measurements). (C) = control.

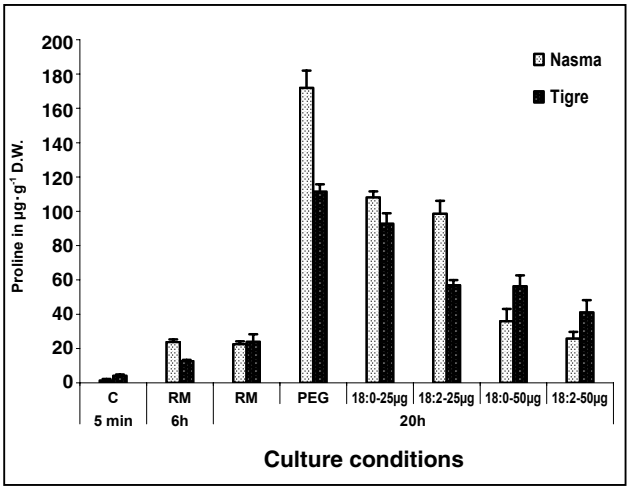

Figure 3. Proline content in leaf segments of two wheat varieties maintained 5 min under ambient conditions (C) or 6 h and 20 h in reference medium (RM). The osmotic stress was created by the addition of 25% PEG10 000 in RM during 20 h supplemented or not with two different concentrations (25 µg or 50 µg) of stearic acid (18:0) or linolenic acid (18:2). Results are means ± S.D. (n=3).

Photosynthetic activity

Photosynthetic activity of leaf fragments, measured by oxygen evolution was more important in Tigre than in Nasma varieties (Figure 5). The PEG-induced osmotic stress resulted in a significant decrease of oxygen evolution in both varieties. However, photosynthesis inhibition was almost complete in Nasma (94%) while in Tigre 65% of activity remained after 15 min of treatment (Figure 5). When the PEG was added with fatty acids, the photosynthesis activity was restored in Nasma. The C18:2 is more effective than C18:0 at establishing this photosynthetic activity.

DISCUSSION

Our results showed that water stress leads to a significant decrease in polar lipids (MGDG and phospholipids) and an increase in neutral lipids in both wheat varieties studied, Nasma and Tigre. Neutral lipid (diacylglycerol and triacylglycerol) accumulation, previously observed in other plants (Martin et al., 1986; Hubac et al., 1989; Dakhma et al., 1995), was considered a defence mechanism of the plant against drought stress (Hubac et al., 1989). It has also been suggested that the free fatty acids, released during water stress by the action of lipases on polar lipids, could be stored in triacylglycerols to avoid oxidation by free radicals and activated oxygen forms (Hubac et al., 1989; Dakhma et al., 1995).

The results showed that the wheat variety Tigre, adapted to rainfed semi-arid conditions, showed a more stable phospholipid content after water stress than did the variety Nasma, which is mostly adapted to irrigated conditions. These results are in agreement with previous studies on *Vigna unguiculata* leaves (Sahsah et al., 1998).

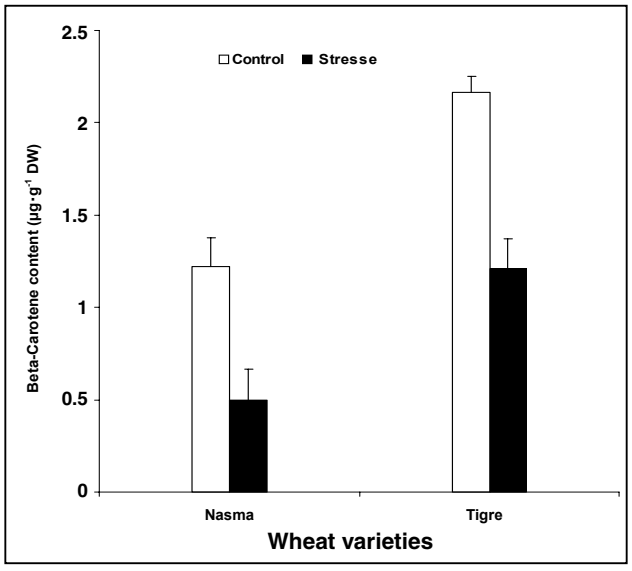

Figure 4. β-carotene (µg·g^{-1} DW) in leaves of two wheat (*Triticum aestivum*) cultivars (Nasma and Tigre) subjected to water stress induced by withholding irrigation for 30 days (8% of field capacity). Results are means ± S.D. (n=3).

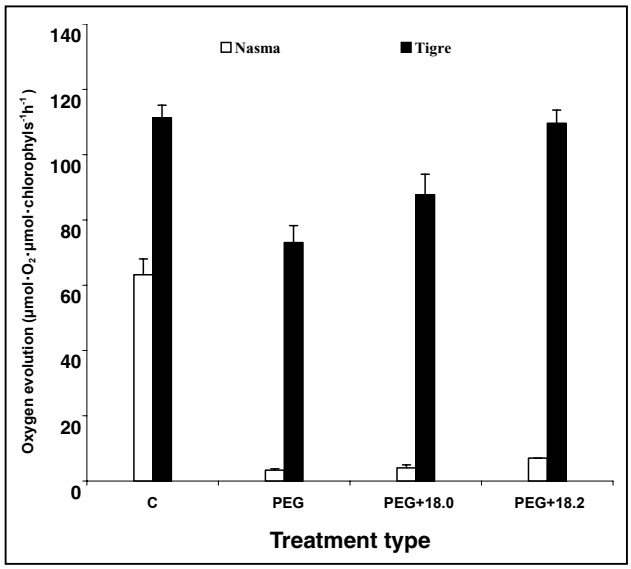

Figure 5. Photosynthetic capacity (µmole O_2·µmole chlorophyll^{-1}·h^{-1}) in leaf discs of two wheat (*Triticum aestivum*) cultivars, Nasma and Tigre. Effect of osmotic stress created by 25% PEG (≡ -0.95 MPa) added or not with fatty acid (octadecanoic, 18:0, or octadecadienoic acid, 18:2) for 15 min in leaf disc suspension medium. Values are the means ± S.D. (n = 5 separate measurements).

The observed decrease in galactolipids localised on the chloroplast envelope, stroma lamellae, and grana system (Bahl et al., 1976) indicates that water stress affects the chloroplast membranes. This could contribute to the inhibition of photosynthesis, which is more important in Nasma than in Tigre. In fact, Nasma showed a larger decrease in MGDG and a higher inhibition of photosynthesis. A decline in MGDG content under water stress conditions has been considered by several authors to be a feature of drought-sensitive cultivars (Hubac et al., 1989; Pham Thi et al., 1990; Quartacci et al., 1995). It was also previously shown in cotton that water stress increased the activity of MGDG-hydrolases (El-Hafid et al., 1989). Similarly, a more pronounced decrease in lipid contents in drought-sensitive than in drought-tolerant cultivars has been observed in cowpea and was explained mainly by an activation of lipid degradation processes (Monteiro de Paula et al., 1993). However, tolerance to water stress may involve different mechanisms, including the capacity to maintain high levels of antioxidants (Sairam et al., 1998). Relative to Tigre, the irrigation-dependant Nasma variety presented a higher degree of peroxidation and contained less β-carotene pigments. It was reported that β-carotenes play a role as antioxidants (Sairam et al., 1998). Carotenoids can directly deactivate singlet oxygen ($^1O_2$) and can also quench the excited triplet state of chlorophyll, thus indirectly reducing the formation of $^1O_2$

species (Sieferman-Harms, 1987; Foyer and Harbinson, 1994).

A higher proline accumulation was observed in the irrigation-dependent variety Nasma, in which higher peroxidation levels and a higher decrease in unsaturated fatty acids were also observed. Proline accumulation in stressed plants is a well documented phenomenon. It has been suggested that this amino acid acts as an organic solute directly involved in osmotic adjustment to osmotic stress and/or in protecting macromolecules under various environmental perturbations (for a review, see Delauney and Verma, 1993). In this work, we noticed that the accumulation of the proline is more important in the most sensitive stress varieties. This accumulation can be correlated with the cell damage; therefore, it is probably one of the consequences of stress.

In addition, our results clearly showed that addition of octadecanoic or octadecadienoic acid decreased osmo-induced proline accumulation significantly and restored photosynthetic activity (Figure 5). This effect of 18:0 or 18:2 addition can be explained by their biosynthesis of octadecatrienoic acid (18:3), an abundant polyunsaturated fatty acid found in the galactolipids of thylakoid membranes. Such a pathway might be involved in the synthesis of polar lipids and membrane stabilization. In addition after its formation, 18:3 might be converted into methyl-jasmonate (Me-JA) via 12-oxophytodienoic acid (12-OPDA) in chloroplast (Vick and Zimmerman, 1984). Me-JA is considered to be a regulator of plant growth and development (Sembdner and Parthier, 1993). Me-JA and other cyclopentanone compounds, mainly jasmonic acid (JA) and its amino acid conjugates, influence a variety of physiological properties, especially those related to stress tolerance (Parthier, 1991). Our results suggested a positive relationship between fatty acids and photosynthetic capacity in the two wheat varieties studied. It is known that a decrease in fatty acid unsaturation results in a decrease of membrane fluidity (Shinitzky, 1984). The water stress induced a decrease in the degree of unsaturation in the phospholipids (Svenningsson and Liljenberg, 1986) and in MGDG (Zuily-Fodil, et al., 1992). On the other hand, drought-induced peroxidative forms of wheat have been shown to attack polyunsaturated fatty acids in common bean (Ferrari-Iliou et al., 1993).

The accumulation of proline would be merely a consequence of membrane disorganization brought on by water stress. This is further supported by the addition of fatty acids, which probably regenerates the oxidized lipids of the membrane. Very recent findings (Huguet-Robert et al., 2003) have shown that osmo-induced proline accumulation in canola leaf discs is inhibited by polyunsaturated fatty acids. The authors suggest that PUFAs were converted to methyl-jasmonate, which could actually behave as a more potent suppressor of the proline response.

In conclusion, our results showed that water stress induced a decrease in polar lipid content and an increase in neutral lipids in both local varieties studied. A strong decrease in phospholipids, galactolipids, unsaturated fatty acids and a higher degree of peroxidation were observed in the irrigation-dependant variety, suggesting more disorganized and unstable membranes. This could be associated with higher metabolic damage as they can be ascertained through proline accumulation. However, further studies are required for the physiological analysis of the interactions between membrane lipids and proline metabolism, and their respective roles in response to drought. Also, we need in the future work to analyse phosphatidylglycerol fatty acids from chloroplasts, especially hexadecenoic acid (16:1 cis and trans) which play important roles in environmental stress.

Acknowledgements. Part of this work was supported by the Biology Department of the Faculty of Science and Technology, Marrakech and by the Project No. p5t1/03 (Centre National de Coordination et de la Planification de la Recherché Scientifique et Technique du Maroc). We thank the Director of SO.NA.CO.S (National Company for Seeds Marketing in Morocco) for a generous donation of the wheat seeds used in this study.

LITERATURE CITED

Asada, K. and M. Takahashi. 1987. Production and scavenging of active oxygen in photosynthesis. *In* D.J. Kyle, C.B. Osmond, and C.J. Amtzen (eds.), Photoinhibition. Elsevier, Amsterdam, pp. 227-287.

Bahl, J., B. Francke, and R. Monéger. 1976. Lipid composition of envelopes, prolamellar bodies and other plastid membranes in etiolated, green and greening wheat leaves. Planta **129:** 193-201.

Bartoli, G. C., F. Gómez, D. E. Martínez , and J. J. Guiamet. 2004. Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). J. Exp. Bot. **55:** 1663-1669.

Boyer, J.S. 1970. Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. Plant Physiol. **46:** 236-239.

Christie, W.W. 1982. Lipid Analysis. *In* A. Wheatson, (eds.), Pergamon Press, Oxford, pp. 107-134.

Dakhma, W.S., M. Zarrouk, and A. Cherif. 1995. Effects of drought-stress on lipids in rape leaves. Phytochemistry **40:** 1383-1386.

Delauney, A.J. and D.P.S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. Plant J. **4:** 215-223.

Del Rio, L.A., L.M., Sandalio, J.M. Palma, P. Bueno, and F.G. Corpas. 1992. Metabolism of oxygen radicals in peroxysomes and cellular implications. Free Rad. Biol. **13:** 557-580.

Deo, P.M. and B. Biswal. 2001. Response of senescence in cotyledons of clusterbean to water stress in moderate and low light: Possible photoprotective role of carotene. Physiol. Plant. **112:** 47-54.

Eastman, P.A.K., A Rashid, and E.L. Camm. 1997. Changes of the photosystem 2 activity and thylakoid proteins in spruce seedlings during water stress. Photosynthetica **34:** 201-210.

El Kaoua, M. and D. Laval-Martin. 1995. Evolution of PSIIα and PSIIβ centres during the greening of *Euglena gracilis* Z: Correlations with changes in lipid content. Photosynth. Res. **43:** 155-163.

El Kaoua, M., E. Duval, and D. Laval-Martin. 1991. Long-term effect of diuron on chlorophllous callus of *Bromus erectus*: Lipid composition. Z. Naturf. **46:** 7-12.

El-Hafid, L., A. Thu Pham, Y. Zuily-Fodil, and J. Viera da Silva. 1989. Enzymatic breakdown of polar lipids in cotton leaves under water stress. Degradation of monogalactosyldiacylglycerol. Plant Physiol. **27:** 495-502.

Ferrari Iliou, R., A. d'Arcy-Lamita, P. Iliou, A.T. Pham Thi, F. Monteiro de Paula, J. Vieira da Silva, and P. Mazliak. 1993. In vitro photodynamic lipid peroxidation of total lipolytic extracts from leaves of bean plants. Biochim. Biophys. Acta. **1166:** 48-54.

Foyer, C.H. and J. Harbinson. 1994. Oxygen metabolism and the regulation of photosynthetic electron transport. *In* C.H. Foyer and M.P. Mullineaux (eds.), Causes of Photooxydative Stress And Amelioration of defense System in Plant. CRC Press, Boca Raton F.L. pp. 1-42.

Foyer, C.H., P. Descourvières, and K.J. Kunert. 1994. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ. **17:** 507-523.

Hubac, C., D. Guerrier, J. Ferran, and A. Trémolières. 1989. Change of lipid composition during water stress on two genotypes resistant or susceptible to drought. Plant Physiol. Biochem. **27:** 737-744.

Huguet-Robert, V., R. Sulpice, C. Lefort, Maerskalck, N. Emery, and F.R. Larher. 2003. The suppression of osmoinduced proline response of *Brassica napus* L. var oleifera leaf discs by polyunsaturated fatty acids and methyl-jasmonate. Plant Sci. **164:** 119-127.

Iturbe Ormaetxe, I., P.R. Escuredo, C. Arrese-Igor, and M. Becana. 1998. Oxydative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. **116:** 173-181.

Kanituga, Z. and J. Gemel. 1984. Galactolipase activityand free fatty acid levels in chloroplasts. Novel approach to characterization of shilling sensitivity of plant. FEBS Lett. **171:** 55-58.

Kriedemann, P.E. 1986. Stomata and photosynthetic limitations to leaf growth. Aust. J. Plant Physiol. **13:** 15-31.

Laval-Martin, D. 1985. Spectrophotometric method of controlled pheophytinization for the determination of both chlorophylls and pheophytins in plant extracts. Anal. Biochem. **149:** 121-129.

Laval-Martin, D. and P. Mazliak. 1979. Physiologie Végétale TP et TD. Hermann, éditeurs des Sciences et des arts, Collection méthodes, Paris, pp. 25-34.

Levitt, J. 1972. Responses of Plants to Environmental Stresses. Academic Press. New York. NY. ISBN 0-12-445560-3.

Martin, B.A., J.B. Schoper, and R. Whinne. 1986. Change in soybean (*Glycin max* [L.] Merr.) Glycerolipids in response to water stress. Plant Physiol. **81:** 798-801.

Michel, B.E., O.K. Wiggins, and W.H. Jr. Outlaw. 1983. A guide to establishing water potential of aqueous two phase solutions (polyethylene glycol plus dextran) by amendment with mannitol. Plant Physiol. **72:** 60-65.

Monteiro de Paula, F., A.T. Pham Thi, J. Viera da Silva, A.M. Justin, C. Demandre, and P. Mazliak. 1990. Effects of drought stress on the molecular species composition of polar lipids from *Vigna unguiculata* L. leaves. Plant Sci. **66:** 185-193.

Monteiro de Paula, F., T. Pham Thi, Y. Zuily Fodil, R. Ferrari lliou, J. Viera da Silva, and P. Mazliak. 1993. Effects of drought stress on the biosynthesis and degradation of polyunsaturated lipid molecular species in leaves of *Vigna unguiculata* L. Plant Physiol. Biochem. **31:** 707-715.

O'Sullivan, J.N., N.W.M. Warwick, and M.J. Dalling. 1987. A galactolipase activity associated with the thylakoids of wheat leaves (*Triticum aestivum* L.). J. Plant Physiol. **131:** 393-404.

Paquin, R. and P. Lechasseur. 1979. Observations sur une méthode de dosage de la proline libre dans les extraits de plantes. Can. J. Bot. **57:** 1815-1854.

Parthier, B. 1991. Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions. Bot. Acta. **104:** 446-454.

Pham Thi, A.T., J. Vieira da Silva, and P. Mazliak. 1990. The role of membrane lipids in drought resistance of plants. Bull. Soc. Bot. Fr. **137:** 99-114.

Premachandra, G.S. and T. Shimada. 1988. Evaluation of polyethylene glycol test of measuring cell membrane stability as a drought tolerance test in wheat. J. Agr. **110:** 2000-2005.

Quartacci, M.F., C. Pinzino, C.L.M. Sgherri, and F. Navari-Izzo. 1995. Lipid composition and protein dynamics in thylakoids of two wheat cultivars differently sensitive to drought. Plant Physiol. **108:** 191-197.

Sahsah, Y., P. Campos, M. Gareil, Y. Zuily-Fodil, and A.T. Pham Thi. 1998. Enzymatic degradation of polar lipids in Vigna unguiculata leaves and influence of drought stress. Physiol. Plant. **104:** 577-586.

Sairam, R.K., D.S. Shukla, and D.C. Saxena. 1998. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. Biol. Plant. **40:** 357-364.

Sembdner, G. and B. Parthier. 1993. The biochemistry and the physiological and molecular actions of jasmonates. Ann Rev Plant Physiol. Plant Mol. Biol. **44:** 569-589.

Shalata, A. and M. Tal. 1998. The effect of salt stress on lipids peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicum pennellii.* Physiol. Plant. **104:** 169-174.

Shinitzky, M. 1984. Membrane fluidity and cellular functions. *In* M. Shinitzky (ed.), Physiology of Membrane Fluidity, CRC

Press, Boca, pp. 1-51.

Sieferman-Harms, D. 1987. The light-harvesting and protective function of carotenoids in photosynthetic membranes. Physiol. Plant. **69:** 561-568.

Singh, T.N., L.G. Paleg, and D. Aspinall. 1973. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant. Aust. J. Biol. Sci. **26:** 45-56.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. **125:** 27-58.

Svenningsson, H. and C. Liljenberg. 1986 Membranes lipid changes in root cells of rape (*Brassica napus*) as a function of water-deficit stress. Physiol. Plant. **68:** 53-58.

Vick, B.A. and D.C. Zimmerman. 1984. Biosynthesis of jasmonic acid by several plant species. Plant Physiol. **75:** 458-461.

Vieira da Silva, J., A.W. Naylor, and P.J. Kramer. 1974. Some ultrastructural and enzymatic effects of drought stress in cotton (*Gossypium hirsutum* L.) leaves. Proc Natl. Acad. Sci. USA **71:** 3243-324.

Vu, J.C.V, Jr. L.H.. Allen, and G. Bowes. 1987. Drought stress and elevated CO_2 effects on soybean ribulose biphosphate carboxylase activity and canopy photosynthetic rates. Plant Physiol. **83:** 573-578.

Yang, C.W., C.L. Peng, J. Duan, and Y.Z. Chen. 2002. Effects of β-carotene feeding on chlorophyll fluorescence, zeaxanthin content, and D1 protein turnover in rice (*Oryza sativa* L.) leaves exposed to high irradiance. Bot. Bull. Acad. Sin. **43:** 181-185.

Zadoks, J.C., T.T. Chang, and C.F. Konzok. 1974. A decimal code for the growth stages of cereals. Weed Res. **14:** 415-421.

Zuily-Fodil, Y., A.T. Pham Thi, A. Tashakorie, and J. Vieira da Silva. 1992. Effect of drought stress on membrane lipids of *Cajanus cajan*. *In* A. Chérif, D. Ben Miled- Daoud, B. Marzouk, A. Smaoui, and M. Zarrouk (eds.), Metabolism, Structure and Utilization of Plant Lipids. CNP Tunis, pp. 352-356.

Zuily-Fodil, Y., A. Vasquez Tello, and J. Viera da Silva. 1990. Effect of water deficit on cell permeability and on chloroplast integrity. Bull. Soc. Fr. Actual. Bot. **137:** 115-123.

兩種摩洛哥小麥對水逆境之不同敏感度：脂肪酸和脯氨酸累積量之相關性

Mimoun EL KAOUA[1], Rachid SERRAJ[2], Mohamed BENICHOU[3], and Driss HSISSOU[1]

[1]Laboratoire de Biotechnologie et Phytopathologie Moléculaire, Département de Biologie, Faculté des Sciences et Techniques Guéliz, Université Cadi Ayyad B.P. 549 - Av. Abd el karim El Khattabi, Marrakech, Maroc

[2]Crop Physiology Laboratory, International Crops Research Institute for the Semi-Arid Tropics, Po Patancheru 502 324, India, CGIAR-ICRISAT

[3]Department of Biology, Laboratory of Biochemistry Cadi Ayyad University, BP2390 Marrakech, Morocco

細胞膜脂，過氧化，乙型-胡蘿蔔素，脯氨酸累積，及光合作用都分別於兩種摩洛哥小麥 (*Triticum aestivum* L.) 品種，Nasma （適應於灌溉區）及 Tigre（適應於半乾旱區），測定。供試植物在實驗室分別於供水及乾旱條件下生長於塑膠盆內。缺水 30 天後（相當於產量潛能之 8%），葉中之脂肪酸含量（尤其是 18C3 雙鍵，即18:3 Δ9,12,15；此乃主要的不飽和脂肪酸）顯著地減少。減少程度 Nasma 比 Tigre明顯。半乳糖脂，磷脂，及乙型-胡蘿蔔素含量在乾旱條件下於兩種小麥品種中都減少，但中性脂肪含量却增加。脂質之過氧化（以 malondialdehyde 量評估）於乾旱時增加（增率於 Nasma 及 Tigre 分別為 41% 及 19%）。兩種小麥品種葉子於離體滲壓逆境時脯氨酸之累積量可添加 18C 脂肪酸及 18C2 雙鍵脂肪酸予以抑制。當培養基內添加上述兩種脂肪酸時，光合作用之受滲透逆境造成之抑制程度可減緩。脯氨酸之累積和膜安定度兩者間具正相關。本文討論這些生理反應和水逆境之關係。

關鍵詞：*Triticum aestivum*；水逆境；光合作用能力；脂質；脯氨酸。