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Molecular aspects of abiotic stress resistance in durum wheat

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SUMMARY – Physiological parameters, proline accumulation and expression levels of drought related genes were analysed in durum wheat plants at flowering stage, comparing two varieties with different drought tolerance capacity. The imposed drought stress induced a lowering of water potential and of relative water content, a strong increase in proline and expression of drought related genes. The results showed differences in water potential and gene expression, being, the former, more negative and, the latter, higher in the drought tolerant variety Capeity 8 than in the susceptible one Creso. No differences were found in relative water content and in proline accumulation.

Key words: Durum wheat, drought stress, drought related genes, proline accumulation.

RESUME – *"Aspects moléculaires de la résistance aux stress abiotiques chez le blé dur". Les paramètres physiologiques, l'accumulation de proline et les niveaux d'expression des gènes associés à la sécheresse ont été analysés sur des plantes de blé dur qui présentent différents degrés de tolérance à la sécheresse. Le stress hydrique imposé a provoqué une diminution du potentiel hydrique ainsi que du contenu hydrique relatif, une forte augmentation de la quantité de proline ainsi que l'expression des gènes associés à la sécheresse. Les résultats ont montré des différences en ce qui concerne le potentiel hydrique et l'expression génique. En effet, le premier est plus négatif et le second plus fort pour Capeiti 8 (variété tolérante à la sécheresse) que pour Creso (variété susceptible). Au contraire, aucune différence n'a été observée pour le contenu hydrique relatif et pour la quantité de proline.*

Mots-clés : *Blé dur, sécheresse, expression génique, proline.*

Introduction

Water is among abiotic stresses the most widespread and limiting durum wheat distribution and productivity in Mediterranean environment. For many years breeding for drought tolerance has been an important task to increase crop productivity in dry environments, although this issue has not been completely successful, being the drought tolerance a polygenic trait characterised by low heritability and high genotype x environment interaction. A different strategy to obtain plants with higher performance under water stress conditions is to identify and modify the molecular mechanisms that take place when the water availability becomes limiting. Under water stress conditions the lowering of the cellular water potential and the ABA accumulation give rise to a reorganisation of the cellular metabolism and of the gene expression, allowing the plant to adapt itself to the stress. The molecular response to water stress can be divided in three main aspects:

(i) Accumulation of osmolytes, such as proline, glycine betaine, polyols, polyamines and ions (i.e. potassium), which act both by contributing to osmotic adjustment and by protecting proteins and cellular membranes. Many genes involved in the biosynthesis of osmolytes have been cloned: choline monooxygenase and betaine aldehyde dehydrogenase control the biosynthesis of glycine betaine, while the Δ^1-pyrroline-5-carboxylate synthetase and the corresponding reductase are responsible for proline accumulation in many plant species like spinach, sorghum, and soybean (Delauney and Verma, 1990; Burnet *et al.,* 1995; Wood *et al.,* 1996). Recent results in durum wheat have showed a correlation between the degree of water stress and Δ^1-pyrroline-5-carboxylate reductase activity (Mattioni *et al.,* 1997).

(ii) Accumulation of Late Embryogenesys Abundant (LEA) proteins. It has been widely demonstrated that drought and desiccation cause changes in gene expression, and many genes induced under these conditions have been isolated and characterised. Many, although not all of these genes are ABA-regulated and encode for a class of protein similar to the LEA proteins originally found during embryo

development. LEA are highly hydrophilic proteins expressed during dehydration both in physiological and environmental-related stress situations, being abundant during the desiccation phase of seed maturation as well as under drought, salinity and low temperature (Skriver and Mundy, 1990). The genes coding for the group 2 LEA proteins, also called dehydrins, are particularly expressed upon environmental stress (Close *et al.,* 1989). They are characterised by a 15 amino acid consensus sequence at or near the carboxy-terminous that is repeated one or more times, and by a stretch of 6-9 contiguous lysine residues. It has been suggested that LEA proteins act by binding water and/or by protecting the cellular membranes. Clones encoding dehydrins have been identified in several species including durum wheat (Labhilili *et al.,* 1995). The gene *af93* encodes for a dehydrin expressed upon either cold or drought treatment, although its induction is independent from ABA (Cattivelli and Bartels, 1990; Grossi *et al.,* 1995). In durum wheat plants the accumulation of *af93* corresponding mRNAs during the grain filling stage was linked to a reduction in water potential. In drought course experiments non-irrigated plants exhibited a generally higher level of *af93* expression than the irrigated ones, although even in the latter the progressive water potential decline was associated with a parallel rise in the level of induction of messengers homologous to *af93*. When the same plants were tested with other dehydrin clones such as *dhn1* (Close *et al.,* 1989) no expression was detected suggesting that different dehydrin genes may be differently regulated (Lacerenza *et al.,* 1995).

(iii) Modifications of the expression of the genes coding for the enzymes having a key role in the cell metabolism under water stress conditions for instance: the sucrose synthetase and the sucrose-P-synthase (Bianchi *et al.,* 1991) involved in the sugar metabolism; the superoxide dismutase and the glutathione reductase which remove the toxic compounds produced during oxygenic metabolism (Mittler and Zilinskas, 1994). When the cell undergoes water deficit cellular proteins can be damaged and, consequently, degraded by proteases (Koizumi *et al.,* 1993) or repaired. The L-isoaspartyl methyltransferase has been proposed to be involved in the repairing of spontaneously damaged proteins by facilitating the conversion of abnormal L-isoaspartyl residues to normal L-aspartyl residues (Mudgett and Clarke, 1994).

There are two main evidences supporting the role of these mechanisms in the protection of the plant exposed to water stress conditions. First, the expression of genes involved in the response of the plant to drought is higher in stressed plants than in the control, and often is higher in resistant genotypes than in susceptible ones. Second, mutants have been characterised in which the repression of drought-induced genes resulted in stress susceptibility: mutants of *Escherichia coli* lacking the induced L-isoaspartyl methyltransferase survive poorly in stationary phase or under thermal and oxidative stresses (Li and Clarke, 1992). Progress in understanding the role of ABA in desiccation tolerance has been achieved by characterising mutants for production or sensitiveness to ABA in tomato, potato, and Arabidopsis (Quarrie, 1982; Cohen and Bray, 1990; McCarty, 1995).

In the present work the mechanisms involved in the response to drought were investigated in two durum wheat cultivars with contrasting drought tolerance capacity, in order to study the relationship between the molecular basis of drought response and the plant stress tolerance.

Materials and methods

Plant materials and growing conditions

Two durum wheat (*Triticum durum* Desf.) cultivars, Creso and Capeiti 8, susceptible and tolerant to water stress respectively (Flagella *et al.,* 1990, 1996), were grown in a greenhouse. After vernalization at 4ºC for 1 week, they were sown in pots (17 cm diameter, 14 cm deep; 5 seeds each) containing 2000 g of medium-textured soil, sand and peat (6:3:1 v/v). A completely randomised design with three replications and three treatments was used. Soil water content (SWC) was maintained close to field capacity until plants were exposed to the drought treatments. At flowering stage well watered plants (T_0) continued to be watered as described above, while plants of the two water stress treatments (T_1 and T_2) were allowed to reach 18.6 and 12.5% of SWC respectively, expressed on a fresh weight basis. These values of SWC were maintained for 10 days, during which plants were watered daily restoring the amount of water lost during the previous 24 hours.

Water relations

To evaluate the plant water status during the stress period, the youngest fully expanded leaves were selected at random from one plant of each pot and the leaf water potential was measured using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA) according to Scholander *et al.* (1965). Relative water content (RWC, %) was determined using the method of Barrs and Weatherley (1962), and calculated using the formula: (fresh weight – dry weight)/(turgor weight – dry weight) x 100. Turgor weight was determined after imbibition of the tissue in distilled water for 3 hours.

Proline determination

Proline was determined in fully expanded leaves according to Pesci and Beffagna (1984). The samples (50 mg dry weight) were extracted with 10 ml of 3% sulphosalicylic acid solution for 1 hour at room temperature and filtered on Whatman fibre glass paper. A part of extract was added to 4 ml of ninhydrin reactive (1.25% w/v ninhydrin, 2.4 M phosphoric acid in acetic acid) and 4 ml of acetic acid and incubated in boiling water for 1 hour. After fast cooling in ice, the samples were added to 5 ml of toluene and strongly shaken. The toluene phase, containing the coloured complex was used to measure the absorbance at 515 nm versus toluene. From obtained absorbance values it has been calculated the proline amount of each sample by means of a calibration curve, made by starting from known amounts of proline.

RNA extraction and Northern analysis

Total RNA was extracted from leaves by using Trizol Reagent (Life Technologies) following the manufacturer's instructions. Equal amounts (20 μg) of total RNA for each sample were separated on 1% agarose/formaldehyde gel, visualised by staining with ethidium bromide to verify the equal loading of all RNA samples, and transferred to a positively charged nylon membrane (Amersham Life Science). Three drought-related clones were used in northern experiments to test the expression of durum wheat homologous mRNAs under water deficit. The cDNA clones *af93* isolated in barley in response to cold and drought stress encode for a LEA group 2 protein (Cattivelli and Bartels, 1990; Grossi *et al.,* 1995). The wheat *MBM1* clone codes for L-isoaspartyl methyltransferase, an enzyme involved in the pathway of repairing of damaged proteins (Mudgett and Clarke, 1994). The sorghum clone *BADH15* encodes for the betaine aldehyde dehydrogenase which catalyses the oxidation of betaine aldehyde to glycine betaine (Wood *et al.,* 1996). All cDNA clones were labeled with [α-^{32}P] dCTP in a randomly primed reaction and used as probe. The hybridization was performed at 65ºC in 6× SSC, 2× Denhardt's solution, 0.1% SDS, and 100 g mL^{-1} of denatured herring-sperm DNA. The filter was then washed at 65ºC two times with 2× SSC, 0.1% SDS, and one time with 1× SSC, 0.1% SDS for 20 min.

Results and discussion

Two wheat genotypes chosen on the basis of their different drought tolerance were grown in greenhouse until flowering stage and subjected to drought. Two levels of stress were applied, corresponding to 18.6 and 12.5% of SWC. Highly significant differences were detected between watered and stressed plants for water potential and RWC in both cultivars (Fig. 1) proving that the treatment applied leads to a real water deficit. The two cultivars showed the same behavior for RWC values, although they were different for water potential at 18.6% SWC, being the water potential value more negative in the drought tolerant cultivar Capeiti 8 than in the susceptible one Creso (Fig. 1).

An important component of the plant response to drought is the accumulation of solutes compatible with cellular metabolism, which are thought to play a central role in the osmotic adjustment. Proline is, with glycine betaine, the most common osmolyte accumulated in water stress conditions and since many years the accumulation of these compounds is thought to represent an important adaptive response to drought stress (Hanson *et al.,* 1979). The accumulation of osmolytes was also investigated during drought stress in durum wheat. Proline was strongly up-regulated by water stress, the proline content increased about twenty times in plants stressed at 12.5% SWC (Fig. 2).

These results, obtained using plants at flowering stage, confirm previous works on durum wheat seedlings desiccated in Petri dishes (Mattioni *et al.,* 1997), suggesting the important role of proline in the osmotic adjustment in durum wheat plants. Nevertheless, no difference was observed between Creso and

Capeiti 8, neither in the control nor in stressed plants: proline accumulation seemed to have the same weight on osmotic adjustment in both genotypes and cannot explain the different behaviour of the two cultivars under stress.

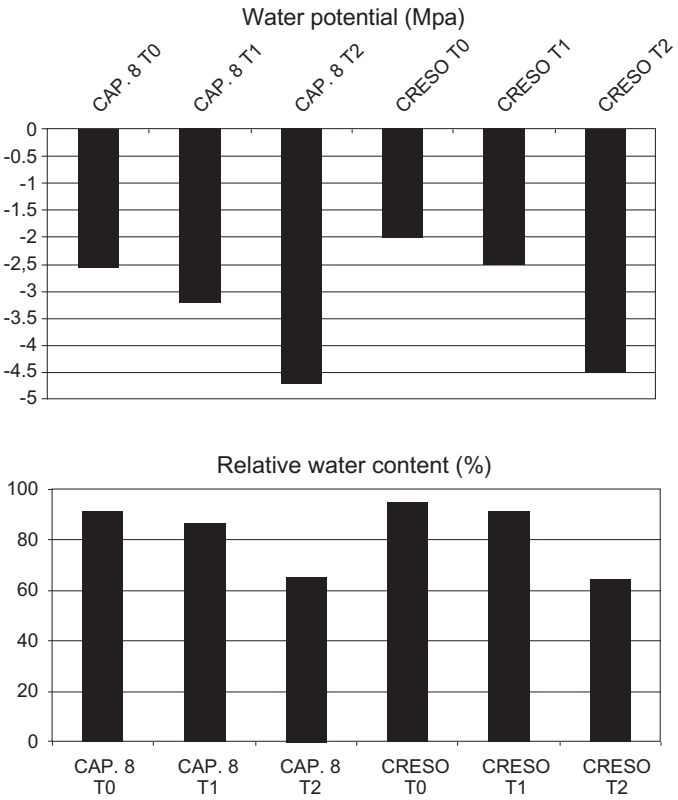

Fig. 1. Changes in water potential and relative water content in the durum wheat cultivars Capeiti 8 (CAP 8) and Creso at flowering stage exposed to different level of water availability. Soil water content was maintained close to field capacity (sample T_0) or lowered till 18.6% (sample T_1) or till 12.5% (sample T_2). Water parameters were measured after 10 days at the indicated level of soil water content. Water potential $LSD_{(00.5)}$ = 0.4 Relative water content $LSD_{(00.5)}$ = 6.7.

The expression of a number of drought-related genes takes place in water deficit conditions. In our experiments three probes were used to determine the expression of homologous mRNAs in durum wheat in response to drought stress. *Af93*, a dehydrin sequence originally selected as cold-regulated gene (Cattivelli and Bartels, 1990), can also be induced by drought stress in an ABA independent manner (Grossi *et al.,* 1995). The *MBM1* sequenced from bread wheat codes for L-isoaspartyl methyltransferase, an enzyme involved in the pathway of repairing of damaged proteins (Mudgett and Clarke, 1994); it can be considered representative of the class of genes coding for enzymes which play a key role in the reorganisation of the cellular metabolism. The sorghum clone *BADH15* encodes for the betaine aldehyde dehydrogenase which catalyses the oxidation of betaine aldehyde to glycine betaine (Wood *et al.,* 1996). All three clones detected homologous mRNAs in durum wheat (Fig. 3). mRNAs corresponding to a*f93* were present in all tested samples and their amount was related to the degree of water deficit (higher in plants treated at 12.5% SWC than in those exposed to 18.6% SWC). *Af93* was more expressed in the drought tolerant cultivar (Capeiti 8) than in the susceptible one (Creso). The induction of *MBM1* related gene was detected only in plants stressed till 12.5% SWC, under these conditions *MBM1* expression was higher in Capeiti 8 than in Creso. Northern analysis performed with the *BADH15* clone revealed that the corresponding mRNA has a basal level of expression which is up-regulated only in plants of the cultivar Capeiti 8 treated at 12.5% SWC.

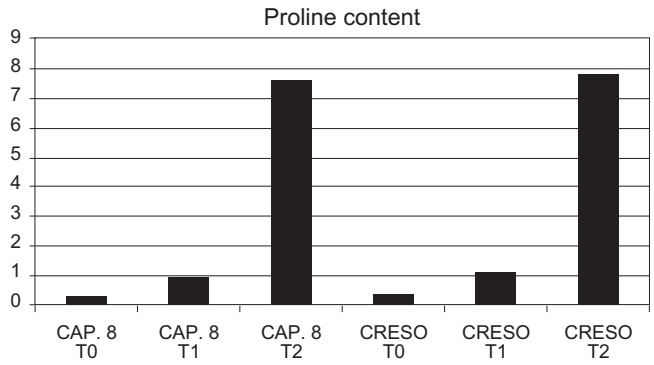

Fig. 2. Proline accumulation in the durum wheat cultivars Capeiti 8 (CAP 8) and Creso at flowering stage exposed to different level of water availability. Soil water content was maintained close to field capacity (sample T_0) or lowered till 18.6% (sample T_1) or till 12.5% (sample T_2). Plants were collected after 10 days at the indicated level of soil water content. Proline is expressed as µg/mg dried weight. $LSD_{(00.5)}$ = 1.9.

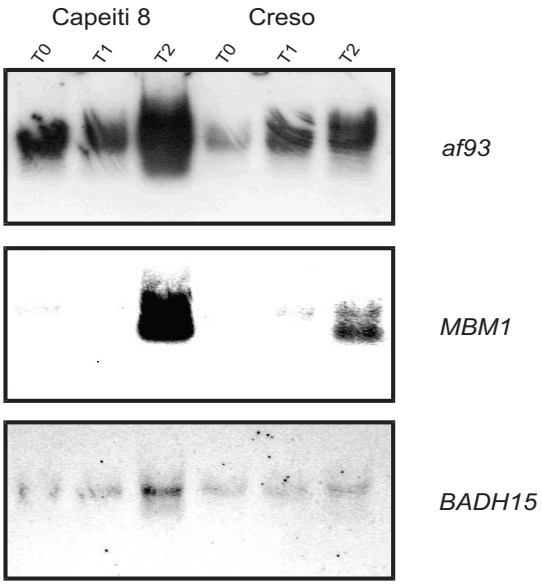

Fig. 3. Expression of drought-related mRNAs in the durum wheat cultivars Capeiti 8 (CAP 8) and Creso at flowering stage exposed to different level of water availability. Soil water content was maintained close to field capacity (sample T_0) or lowered till 18.6% (sample T_1) or till 12.5% (sample T_2). Total RNA was extracted from plants collected after 10 days at the indicated level of soil water content. The following cDNA clones were used: *af93* cold and drought induced dehydrin from barley (Grossi *et al.,* 1995); *MBM1* coding for wheat L-isoaspartyl methyltransferase (Mudgett and Clarke, 1994); *BADH15* coding for the sorghum betaine aldehyde dehydrogenase (Wood *et al.,* 1996).

Conclusions

All the drought-related genes considered in this experiment showed a higher expression in Capeiti 8 than in Creso particularly in drought stress conditions proving that the two cultivars have a different ability to induce the drought molecular response. This observation could be explained considering the lower water potential of Capeiti 8 respect to Creso. The changes in water potential could be the early signal that activates the drought response cascade (Shinozaki and Yamaguchi-Shinozaki, 1997). The different ability of Capeiti 8 and Creso to tolerate drought can therefore be linked to their different capacity to sense the drought stimulus and to trigger an appropriate molecular response. No differences were found for RWC values and proline accumulation. However recent studies have shown that at low SWC Capeiti 8 had

greater water affinity, measured as differential water sorption values, than Creso (Rascio, 1997). So, although the relative water content is the same in the two cultivars, the water distribution is quite different, being the bound water fraction higher in the drought tolerant cultivar Capeiti 8 than in the susceptible one Creso.

The results here presented give a general overview of the cell re-organization under drought stress conditions. The development of new genetic materials such as segregant population for QTL analysis, drought resistant/susceptible mutants and transgenic plants over-expressing single components of drought response will allow the investigation of the role of the mechanisms involved in drought tolerance.

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