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Expression analysis of genes involved in response to drought stress in wheat

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Introduction

Plant growth is greatly influenced by environmental stresses including water deficit, salinity and extreme temperatures. The study of molecular and biochemical responses of plants to abiotic stress is very complex depending not only on the severity and duration of the stress event, but also on the developmental stage and morphological/anatomical parameters of the plants (Rizhsky *et al*., 2002; Bartels *et al*., 2004). Soon after the perception and recognition of external changes, different signalling pathways are activated in order to convert a physical stress into a biochemical response, each of them promoting the expression of a set of stress-responsive genes; the full activation of all signal cascades induced by a given stress event promotes acclimation and leads to stress tolerance.

Many families of transcription factors have been shown to be involved in stress-induced signalling cascade in plants. Among them, bZIP proteins (ABRE-binding factors – Uno *et al*., 2000), MYC, MYC-like bHLH and MYB proteins (Abe *et al*., 2003; Yamaguchi-Shinazaki and K. Shinozaki, 2005), WRKY proteins (Marè *et al*., 2004), *Cbf/DREB1* (C-repeat binding factor/dehydration-responsive element-binding factor1) and DREB2 (Novillo *et al*., 2004; Sakuma *et al*., 2006) are the ones with the most clear roles in drought and cold response. About twenty *Cbf/DREB* genes have been identified in barley, ten in rice (Skinner *et al*., 2005) and thirteen in *Triticum monococcum*, eleven of which are clustered at the *Fr-A^{m}2* frost tolerance locus on chromosome 5A (Miller *et al*., 2006).

The analysis of the signalling pathways pointed to an extensive cross talk between drought and cold responses (Seki *et al*., 2003; Shinozaki *et al*., 2003; Chinnusamy *et al*., 2004) and many genes commonly are induced or repressed in the two stress conditions, a conclusion supported by the presence of common physiological components in the two stress events, cellular dehydration and accumulation of reactive oxidative species (Mittler, 2002; Apel and Hirt, 2004). It is therefore not surprising that the ectopical expression of some transcription factors such as *Cbfs* improved resistance to both stress conditions (Bajaj *et al*., 1999; Hsieh *et al*., 2002a; Hsieh *et al*., 2002b; Oh *et al*., 2005).

The plant response to a stress event can greatly change relative to the stress conditions or the life stage. In a recent paper Bray (2004) compared three independent array experiments dedicated to the *Arabidopsis* water stress response; the experiments differed for plant age (3, 4, 7 weeks), substrate of growth (agar, liquid culture, soil) and stress applications (drying on filter paper, 200 mM mannitol, soil dehydration till the leaves reached 65% of RWC) (Seki *et al*., 2002; Kreps *et al*., 2002; Kawaguchi *et al*., 2004). Comparative analysis of the array data showed that only a small set of genes were commonly induced or repressed (1.4% and 0.2%, of all the genes induced and repressed in all three experiments, respectively). This report concludes that a single study on gene expression, in laboratory conditions is not sufficient to offer a set of individual genes that will be important in a crop response to soil-water deficit although it provides a starting point to develop a clear picture of the molecular mechanisms that control adaptation to soil-water-deficit stress.

Different factors influence the expression profile of a gene in response to abiotic stresses

In a work performed recently, the expression profiles of a set of ten genes (isolated from durum wheat as early induced in response to cold and light, and characterised by low expression levels), and ten *Cbf* genes (Miller *et al*., 2006) were tested in a range of drought stressed samples representing different varieties, different developmental stages and different dehydration conditions (De Leonardis *et al*., 2007).

Cbf3 and *6H8* genes, the latter one putatively coding for a transmembrane protein of unknown function, represent examples of genes undergoing different regulation by water stress depending on different factors. In Fig. 1 the expression profiles of these genes following a fast and a slow dehydration treatment are represented. The two protocols of water stress application caused contrasting expression of the genes: the genes were down-regulated or up-regulated in case of fast or slow dehydration respectively.

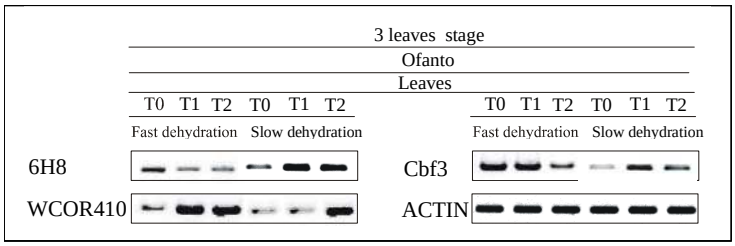


Fig. 1. Reverse transcription PCR (RT-PCR) analysis of *6H8* and *Cbf3* genes in the durum wheat cultivar 'Ofanto' following fast and slow dehydration. The expression of the dehydrin gene *WCOR410* was assessed to verify the correct application of the drought treatment, while the expression of *actin* was measured to normalize the RT-PCR analysis.

When the expression levels were assessed in different developmental stages and tissues, the 6H8 gene was up-regulated by dehydration in all conditions, while the effect of life stage was particularly evident for the *Cbf3* genes, being up-regulated by water stress at booting (leaves) and dough stage (seeds), but down-regulated at three leaf stage (roots) (Fig. 2).

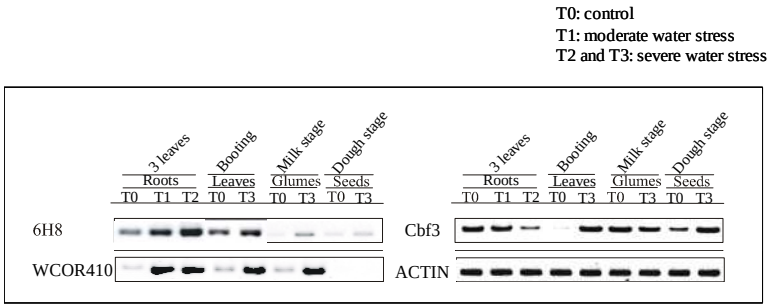
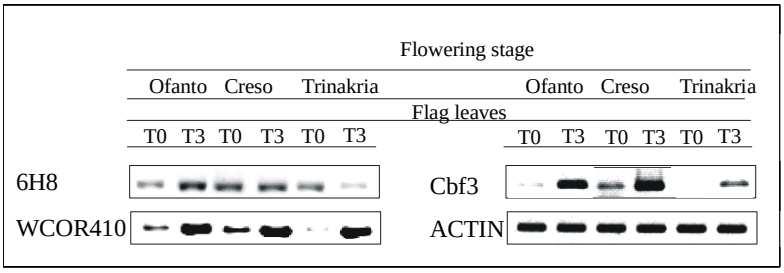


Fig. 2. RT-PCR analysis of *6H8* and Cbf3 genes in the durum wheat cultivar 'Ofanto' in different developmental stages and tissues. The expression of the dehydrin gene *WCOR410* was assessed to verify the correct application of the drought treatment, while the expression of *actin* was measured to normalize the RT-PCR analysis.

Since all genes assessed in this study were up-regulated by water stress at flowering, the analysis at this stage was extended to other two durum wheat cultivars, characterised by a lower water stress tolerance degree with respect to 'Ofanto'.

Only few genes, as *Cbf3*, showed similar expression profiles in all varieties. In other cases the effect of water stress on transcript accumulation was dependent on genotype, as reported for *6H8* gene, whose transcript amount increased following water stress in Ofanto, decreased in Trinakria, and remained unchanged in Creso (Fig. 3).



T0: control
T1: moderate water stress
T2 and T3: severe water stress

Fig. 3. RT-PCR analysis of *6H8* and Cbf3 genes in the durum wheat cultivars 'Ofanto', 'Creso' and 'Trinakria' at flowering stage. The expression of the dehydrin gene *WCOR410* was assessed to verify the correct application of the drought treatment, while the expression of *actin* was measured run to normalize the RT-PCR analysis.

Overall, expression data at flowering stage showed a higher number of up-regulated genes in the tolerant cultivar 'Ofanto' compared to the more susceptible varieties 'Trinakria' and 'Creso'.

Microarray-based expression profiling in wheat

The comparison between tolerant and susceptible varieties can be extended to a global level by using microarray-based approaches. The transcriptome analysis of three wheat genotypes (the hexaploid wheat 'Chinese Spring', a 5A ditelosomic deletion line and the tetraploid durum wheat 'Creso') under different levels of water availability at the grain filling stage, is in progress to provide data on genome-wide molecular changes associated with drought stress.

Comparison between the bread wheat variety 'Chinese Spring' and the ditelosomic deletion line for a region on chromosome 5A carrying *loci* with a role in abiotic stress response may allow the identification of 5A located genes or regulatory factors for their expression.

In a similar way, the comparison between tetraploid and hexaploid wheats may lead to the identification of genes putatively located on D genome, or genes whose expression depends on trans-acting factors located on the D genome.

Conclusions

The expression profiles of many genes change in response to water stress treatment, depending on developmental phase, tissue and stress protocols. When subjected to stress, the plants adjust their cellular metabolism to a new condition in coordination with the developmental program of the specific tissues and cell types. For this reason, it is not so simple defining a gene as "water stress related", and a gene described as "water stress responsive" in one particular condition may not behave the same way in another condition.

Furthermore, data on transcript accumulation in different conditions are not necessarily informative on amount and activity of proteins encoded by those genes. Nevertheless, experiments aimed to study changes of expression profile of genes, and in particular using approaches based on global analysis such as microarrays, can be strategic in giving indications on functional meaning of biological processes that can be verified by designing *ad hoc* experiments.

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