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Molecular responses to drought and heat stress in durum wheat

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Abstract. Water scarcity and high temperature stress and their combination are the most important stresses experienced by durum wheat in the field. The responses induced by drought and heat stress at transcriptional level have been described, but much less is known about plant response to simultaneous drought and heat stress, although this is the most common event in field conditions. Several data indicate that in plants the molecular response to combined heat and drought stress activates networks which are different from that activated by the single heat or drought stress.

Keywords. Heat stress – Drought – Combined stress – Transcriptome – Microarray.

Réponses moléculaires à la sécheresse et au stress thermique chez le blé dur

Résumé. *Le déficit hydrique et le stress thermique et leur combinaison sont les contraintes les plus importantes auxquelles est exposé le blé dur au champ. Les réponses induites par la sécheresse et le stress thermique au niveau transcriptionnel ont été décrites, mais on n'a pas beaucoup d'informations sur la réponse des plantes au stress dû la sécheresse combinée à la chaleur, bien que celle-ci soit la condition la plus fréquente au champ. Plusieurs données indiquent que chez les plantes, la réponse moléculaire au stress combiné chaleur-sécheresse active des réseaux différents par rapport à ceux activés par l'une des deux contraintes seulement.*

Mots-clés. *Stress thermique – Sécheresse – Stress combiné – Transcriptome – Micropuces.*

I – Introduction

Wheat is one of the oldest crops and helped humans to develop their social communities, evolving itself (domestication processes) from the primitive form (emmer wheat) into the presently cultivated species (Peňa 2002). Selection and human habits have led mainly to the cultivation of two wheat species: *Triticum aestivum* L. (hexaploid bread wheat) and *T. turgidum* L. var. *durum* (tetraploid durum wheat). Durum wheat (*Triticum turgidum* L. ssp. *durum*) is a typical Mediterranean crop and is widely used to mill semolina for pasta production. Durum wheat is used also to make regional food (such as couscous). Although the main form of utilization of durum wheat is still represented by pasta, an increasing proportion of production is used for a wide range of baked goods and breads characterized by different flavors and shapes.

About 90 to 95 percent of the wheat produced in the world is common wheat, the rest is mostly durum wheat. Durum wheat adapts to all diverse climatic conditions and is cultivated all around the world. Even if Canada is the country with highest durum wheat production, the Mediterranean countries (Italy, Turkey, Syria, Spain, Algeria, Morocco, Tunisia and France) cover the 80% of world production.

In the Mediterranean region where durum wheat is grown under rainfed conditions, drought and heat are common abiotic stress factors. Heat and drought stress strongly affect grain quality enhancing or decreasing protein content, and often limit yield potential (Farooq *et al*., 2012).

In this review, we present the molecular responses of durum wheat to drought and elevated temperatures.

II – Impact of drought and heat stress

Drought and heat stresses strongly affect the cell physiology of plants because they interfere in photosynthesis and respiration, the two main cell processes. Chlorophyll and fluorescence parameters measured in the flag leaves of durum wheat genotypes, under control and heat-stress conditions in the grain-filling phase was measured by Dias *et al.* (2011), concluding that the chlorophyll reduction and the decrease in Fv/Fm probably resulted in an increasing energy dissipation (i.e., thermal energy) mediated by photoprotective mechanisms. Different studies indicate that loss of chlorophyll during grain-filling is associated with reduced yield (Reynolds *et al.* 1994).

Comparing their data to *T. aestivum*, Dias *et al*. (2010) concluded that the photosynthetic performance of durum wheat is better than bread wheat. Chlorophyll reduction was observed also by Akhkha *et al*. (2011) during drought stress.

A recent study (Li *et al*., 2013) reported the effects of drought and heat stress on yield and quality parameters of durum wheat grains, showing that protein content and SDS sedimentation volume increased under these stress conditions. Other quality parameters related to gluten-strength, were also significantly increased or decreased (Flagella *et al.*, 2010). Moreover drought and heat stress reduce grain yield but enhance flour yellowness and these differences are not equal among cultivars, suggesting that accurate comparative experiments should be done.

III Response mechanisms to drought and heat stress

The molecular mechanisms involved in drought and heat stress responses were well described in studies on *T. aestivum* (Qin *et al.*, 2008; Szucs *et al.*, 2010; Ge *et al*., 2012; Bowne *et al*., 2012; Ford *et al*., 2011), but less was done on *T. durum* wheat, both because *T. aestivum* is the most cultivated among wheat species and because *T aestivum* data are considered informative for all wheat species.

Aprile *et al*. 2009 have carried out a comparative transcriptomic study on drought stress in durum cultivar Creso and bread wheat cultivar Chinese Spring describing a series of molecular mechanisms activated by durum wheat in response to water deprivation.

1. ABA

Abscisic acid content is often associated to plant stress response; in particular as a result of increased water stress. Under intense water stress, the concentration of ABA in plants increases, which triggers a number of processes starting from decrease in turgor pressure, decline in cellular expansion, then stomatal closure to reduce water loss in leaves (Thompson *et al*., 1997). In durum wheat the levels of ABA in response to water stress were studied by Mahid *et al*. (2011) and Akhkha *et al*. (2011) and confirm the findings of other experiment in plants: ABA content is correlated to drought stress level.

The key enzyme in ABA biosynthesis is NCED (9-cis-epoxycarotenoid dioxygenase) and mRNA level, protein level, and ABA content are closely correlated in dehydrated leaves and roots, indicating a regulatory role of NCED in ABA biosynthesis (Qin and Zeevart 1999). In Creso durum wheat the expression level of the main genes involved in ABA pathway were investigated under drought stress and control conditions (Aprile *et al*., 2009), highlighting not only the *NCED* gene up-regulation, but a general up-regulation of the ABA pathway (Figure 1).

2. Osmolite accumulation

Proline accumulation in higher plants is a characteristic physiological response to osmotic stress. Proline is considered to play an important role in defense mechanisms of stressed cells and can work in protection against oxidative stress (Szekely, 2004).

In durum wheat the proline accumulation was also proposed as drought stress indicator (Dib *et al*., 1994) demonstrating the high correlation between proline content and drought stress level. In plants, proline can be synthesized starting from either glutamate or ornithine (Delauney *et al*., 1993; Kavi Kishor *et al*., 1995) and catalyzed, respectively, by P5CS and ornithine-d-aminotransferase. However, only *P5CS* gene was found differentially expressed in durum wheat in response to drought, whereas both genes are induced in bread wheat (Aprile *et al*., 2009). Metabolic data reported that proline tended to accumulate early, at the onset of the stress, while glycine betaine accumulation was observed during prolonged stress (Carillo *et al*., 2009). Glycine betaine pathway was found activated by drought stress in durum wheat also by Aprile *et al.*, 2009.

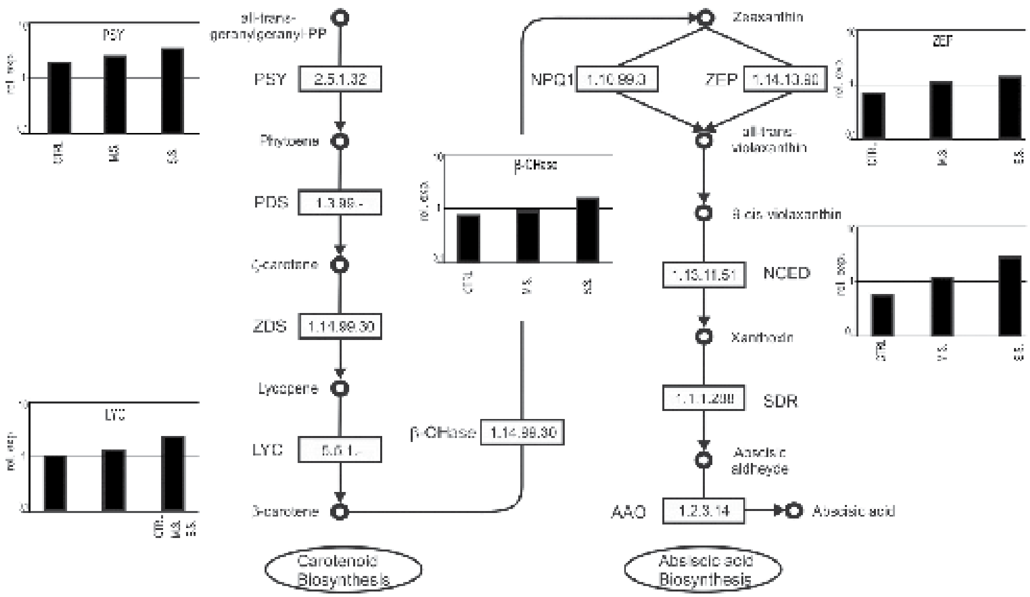

Figure 1. Brief overview of the ABA pathway (inferred by Aprile *et al*., 2009). On the left side the b-carotene biosynthesis steps. On the right the ABA-dedicated enzymatic reactions. Several probe sets related to ABA synthesis enzymes (PSY, LYC- b, β -OHase, NCED) were up-regulated by drought stress. Their expression levels based on array data are showed in the corresponding histograms. 2.5.1.32 = Phytoene synthase (PSY); 1.14.99.-= Phytoene desaturase (PDS); 1.14.99.30 = z-carotene desaturase (ZDS); 1.14.-.-= Lycopene β-cyclase (LYC-b); 1.14.13.- = β -carotene hydroxylase (β -OHase); 1.10.99.3 = Violaxanthin de-epoxidase (NPQ1); 1.14.13.90 = Zeaxanthin epoxidase (ZEP); 1.13.11.51 = 9-cis-epoxycarotenoid dioxygenase (NCED); 1.1.1.288 = xanthoxin dehydrogenase (SDR); 1.2.3.14 = Abscisic aldehyde oxidase (AAO).

3. Heat shock protein

The heat shock proteins (HSPs) were extensively studied among plant species. In durum wheat the HSP26 and HSP70 are activated by heat stress (Laino *et al*., 2010; Rampino *et al*., 2012). Moreover, differences in HSP transcripts accumulation were observed among durum wheat cultivars, and the HSP mRNA levels are related to the acquisition of thermotolerance (Rampino *et al*., 2009). *HSP101* gene was found differentially expressed also after water deprivation as well as the heat transcription factor HSF-C1 /Aprile *et al*., 2009).

4. Transcription factors

Transcription factors (TFs) are key molecular regulators that control genes and gene clusters (Nakashima *et al*., 2009). Many families of transcription factors have been demonstrated to play a role in stress responses in plants. Among them, the bZIP, WRKY, AP2, NAC and C2H2 zinc finger families comprise a high proportion of abiotic stress-responsive members (Rahaie *et al*., 2013).

The DREB proteins, known also as the C-repeat (CRT) binding factors (CBFs), regulate expression of drought/cold stress-related genes, while the ERFs are known to be involved in biotic and abiotic stress responses and both families of proteins contain the Apetala2 (AP2) domain. In durum wheat both DREB and ERF proteins regulate expression of the Cor410b gene, coding a dehydrin, a typical drought stress gene (Eini *et al*., 2013).

Two elements of the NAC family were studied by Baloglu *et al*. (2012), revealing that expression profiles *TaNAC69-1* and *TtNAMB-2* under drought, salt, cold, and heat stress conditions are strongly modulated. bZIP, MYB and WRKY drought-sensitive transcription factors were also found in durum wheat (Aprile *et al*., 2009).

The integration of information about durum wheat physiology and molecular mechanism with conventional or molecular assisted breeding will help to develop new durum wheat varieties with higher performances also if contrasted by drought and heat stress events.

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