

BRIEF COMMUNICATION

Changes of dehydrin profiles induced by drought in winter wheat at different developmental stages

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Abstract

Two cultivars of winter wheat (*Triticum aestivum* L.) differing in their drought tolerance (KTC86211 and ND7532) were subjected to a progressive soil water stress and recovery at four developmental stages. Dehydrins with molecular masses of 45 and 37 kDa were constitutively accumulated during all stages in both genotypes. The 28 kDa dehydrin accumulated exclusively at seedling stage in both genotypes. The 49 and 40 kDa dehydrins accumulated at both tillering and jointing stages but showed a genotype-specific pattern. The content of most dehydrins increased with decreased soil moisture and then decreased during recovery. These results suggest that accumulation pattern of dehydrins during water stress was related to the genotype and developmental stage.

Additional key words: anthesis, drought resistance, jointing stage, seedling stage, soil moisture, tillering stage, *Triticum aestivum*.

Drought is particularly damaging to wheat crops during both vegetative and reproductive stages (Vassileva *et al.* 2009). Dehydrins are a family of hydrophilic proteins that are expressed in many plants in response to harsh environmental conditions with a dehydrative component. Because a number of studies have established a positive correlation between drought stress tolerance and dehydrin accumulation in different plant species, dehydrins are considered a potentially useful marker in selection for drought tolerance in crops (Lopez *et al.* 2001, 2003, Olave-Concha *et al.* 2005, Samarah *et al.* 2006). Most reports on dehydrins focus on the impact at a certain development stage, *e.g.*, Lopez *et al.* (2002) reported that 24 kDa dehydrin is accumulated in wheat in response to drought during anthesis. Winter wheat exposed to low temperature in a field accumulates 209, 196, 66, 50, and 41 kDa dehydrins at different developmental phases (Stupnikova *et al.* 2002). Nevertheless, only fragmentary and incomplete data are available about the accumulation

of dehydrins during drought acclimation at individual developmental stages.

The objectives of this study were to determine whether accumulation of dehydrins is dependent on the genotype, its developmental stage, and level of water stress.

Two winter wheat (*Triticum aestivum* L.) genotypes were used in this study. Genotype KTC86211, an ABA-insensitive somaclone, was selected from tissue culture. Another genotype, ND7532, is its parent line. Lu *et al.* (1989) verified that KTC86211 was more tolerant to drought than ND7532 by comparison of some physiological parameters. An experimental model was set up as described in Demirevska *et al.* (2008), with slight modifications. Seeds of each genotype were placed in plastic pots (30 cm diameter and 50 cm depth) holding nine plants each. Five pots of each genotype were randomly assigned to control and drought treatments in seedling stage, tillering stage, jointing stage, and anthesis.

Received 8 March 2013, accepted 2 May 2013.

Abbreviations: DOR - days of rehydration; FC - field capacity.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (31071349) and the Research Fund for the Doctoral Program of Higher Education of China (20120204110033). Seeds of wheat were generously provided by the Department of Botany, National University of Washington, USA.

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A randomized complete block design with two replications was used. The plants were grown in the pots with a mixture of soil, sand, and clay (8:1:1, m/m/m) in a glasshouse under a 16-h photoperiod, photosynthetically active radiation (PAR) of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 21 - 25 °C, and relative humidity of 40 %. The seedlings were fully irrigated until they grew to predetermined stages. For the drought treatment, the plants were subjected to gradually increasing water deficit by withholding watering for 17 - 20 d leading to decrease in soil moisture from 70 to 20 % of full field capacity (FC). When FC decreased to 20 %, the plants were irrigated to FC of 70 % and recovered for 3 d. All the plant material was harvested at different levels of water stress (70, 60, 50, 40, 30, and 20 % FC) and 1, 2, and 3 d after recovery (DOR), frozen immediately in liquid nitrogen, and stored at -70 °C for further analyses.

Total soluble proteins were extracted according to Patton *et al.* (2007) with modifications. Prior to the analysis, pelleted proteins extracted from 300 mg of fresh tissue were dissolved in 0.3 cm³ of a sample buffer, from

which 40 μg was taken as a sample for 12 % (m/v) SDS-PAGE separation. Protein measurement was performed according to Bradford (1976) using bovine serum albumin as a standard. Total soluble proteins were detected by Coomassie brilliant blue (CBB) staining and dehydrins by Western blot analysis according to Klima *et al.* (2012).

The pattern of dehydrins in leaves of the wheat cultivars KTC86211 and ND7532 acquired from the plants at four developmental stages is shown in Fig. 1. Five dehydrins (Mr of 28, 37, 40, 45, and 49 kDa) were identified in the leaves from the wheat plants exposed to drought and each protein exhibited unique features. The 45- and 37-kDa dehydrin peptides were detected at all four stages in the two genotypes. The 28-kDa peptide was only detected at seedling stage in both genotypes (Fig. 1). Other two dehydrins, 49- and 40-kDa, accumulated in KTC86211 and ND7532 at tillering and jointing stages, respectively (Fig. 1). These findings confirm the viewpoint that accumulation pattern of a dehydrin is strongly affected by plant developmental stage as well as ambient growth conditions including air/soil temperature

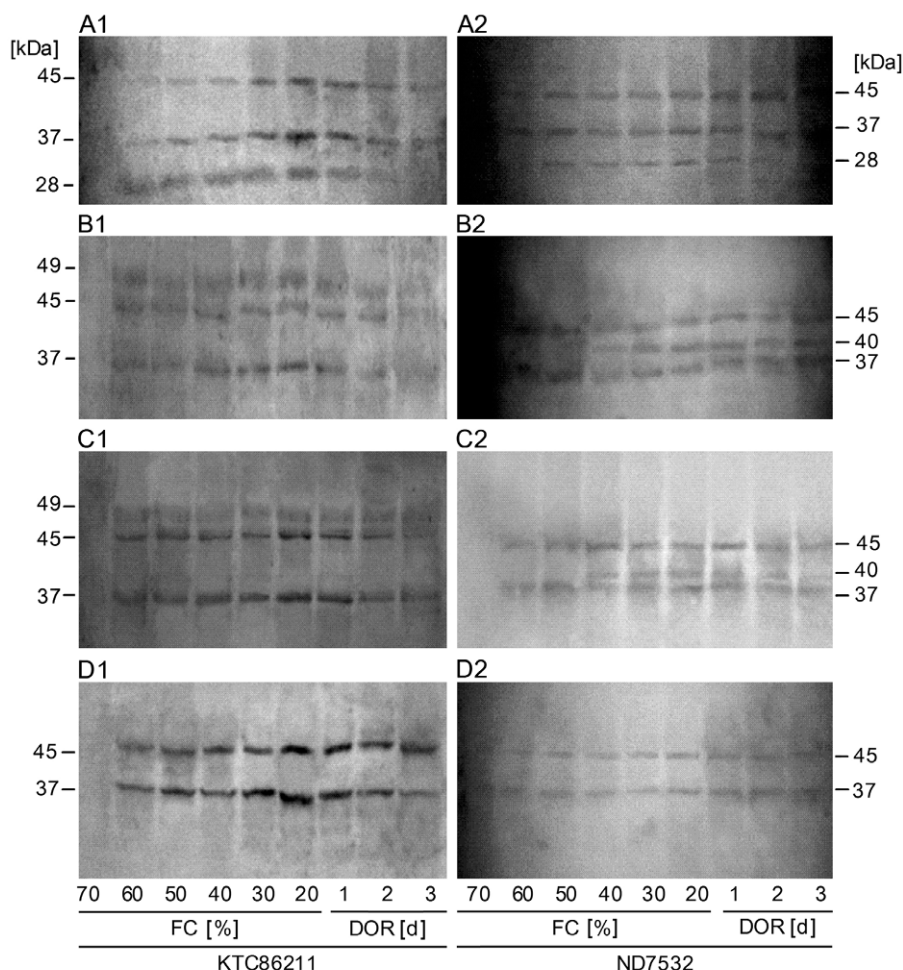


Fig. 1. Effects of dehydration and rehydration on the profiles of dehydrins at different developmental stages. A1, B1, C1, and D1 represent anti-dehydrin immunoblot profiles of leaves of KTC86211 at seedling stage, tillering stage, jointing stage, and anthesis, respectively. A2, B2, C2, and D2 represent anti-dehydrin immunoblot profiles of leaves of ND7532 at the same stages.

and precipitation (Kosová *et al.* 2011). Notably, amount of these dehydrins (28, 40, and 49 kDa) increased very rapidly and peaked already at FC of 50 or 40 % which was different from the 45- and 37-kDa dehydrins. These results suggest that the expression of these three dehydrins was regulated by a developmental phase and might act as an efficient supplement to drought resistance. Moreover, these dehydrins appeared at the vegetative stages rather than at the reproductive stage which was probably related to the characteristics of those developmental stages.

No dehydrin was observed in the leaves from the well-watered plants (Fig. 1) which is in accordance with other researches (Lopez *et al.* 2003, Samarah *et al.* 2006, Hu *et al.* 2010). During acclimation, five dehydrin proteins in both genotypes showed a very similar trend in accumulation. With water loss, the amount of the dehydrins gradually increased, maximum accumulation was observed at 20 % FC with an exception of the 28-kDa peptide whose amount was close to its peak at 40 % FC. After rehydration, the amount of the dehydrins decreased more rapidly in KTC86211 than in ND7532 (Fig. 1). This trend is consistent with previous report (Eichenberger 2009).

Up to now, extensive studies have reported the presence of dehydrins (37- and 45-kDa) in different species. Han *et al.* (1997) reported that a 37-kDa peptide was detected in seedlings of *Castanospermum australe* under water deficit. Lopez *et al.* (2002) also found a 37-kDa dehydrin in wheat grains in response to drought stress during anthesis. Korol and Klein (2002) described that a 37-kDa dehydrin was induced by ABA in wheat seeds. In addition, Arora *et al.* (1998) identified a 45-kDa dehydrin that was induced by water stress in *Geranium* leaves. Kalembe and Pukacka (2012) demonstrated a 46-kDa dehydrin in seeds of three *Acer* species. In the present study, two dehydrins (45- and 37-kDa) were induced by the water deficit in both genotypes at four development stages (Fig. 1). This suggests that the two dehydrins detected here were probably constitutive

components of the desiccation phase in wheat developmental program. Although both proteins accumulated in response to drought, their accumulation at the same level of the water stress was different between the two genotypes. The amount of the 37-kDa peptide was higher than that of 45-kDa in the drought-tolerant genotype KTC86211 at both seedling and jointing stages whereas the drought-sensitive genotype ND7532 had an opposite result (Fig. 1). This implied that the 37-kDa dehydrin probably contributed more to drought resistance than the 45-kDa peptide did. Although the present study could not elucidate the function of both dehydrins in the entire life cycle, they probably played an important role in avoiding impairment upon dehydration in the wheat species.

Interestingly, two dehydrins (40- and 49-kDa) displayed genotype-specific accumulation in the winter wheat. Similar results were reported by Cooper *et al.* (1986). As described previously, the genotype KTC86211 is somaclone of the line ND7532, thus there are at least two possible explanations for these results. First, the 49-kDa protein may be the result of a mutation within the structural gene that codes for the 40-kDa protein producing an altered transcript that now codes for the 49-kDa protein. Second, there could be a mutation in a regulatory gene which represses the synthesis of the 40-kDa protein and promotes synthesis of the 49-kDa protein. Mapping the purified 40-kDa and 49-kDa proteins with HPLC or two-dimensional electrophoresis would show whether there is any homology between the two proteins. Considerable homology would favour the hypothesis that a mutation occurred in the structural gene for the 40-kDa protein, and a lack of homology would favour the regulatory gene theory.

In summary, the patterns of dehydrins varied with different developmental stages, genotypes, and a level of water stress. Nevertheless, extended studies are needed to understand a precise mechanism how these dehydrins participate in stress tolerance at different developmental stages.

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