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Osmotic stress responses of wheat species and cultivars differing in drought tolerance: some interesting genes (advices for gene hunting)

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ABSTRACT The aim of the present work is to provide information for the establishment of gene bank and to obtain comparative data for the new transgenic lines to be established in a later stage of this project. For revealing traits for drought tolerance, wheat species of different ploidy levels and hexaploid cultivars of different stress tolerance were chosen. For gene isolation the osmotic stress resistant *Triticum aestivum* L. cv. Kobomugi, and the sensitive cv. Öthalom were chosen. Osmotic treatment was administrated using PEG 6000 at a final concentration of 400 mOsm (19.0%). Significant differences were found between the two cultivars in carbohydrate accumulation, in changes in water relation and chlorophyll fluorescence parameters measured *in vivo*. It is suggested that cv. Kobomugi may be a useful source for isolation of drought tolerance-related genes. **Acta Biol Szeged 46(3-4):63-65 (2002)**

KEY WORDS

carbohydrate accumulation
drought tolerance
Triticum species
wheat cultivars

Under continental climate, drought is one of the most frequent abiotic stressor for wheat. Drought tolerance, however, is a multigenic trait that manifests at different levels of organisation and different stages of development. Selection for drought tolerance therefore, must involve molecular biological, biochemical and physiological approaches using provocative induction treatments. Genes expressed under osmotic and salt stress conditions in young seedlings and during the grain filling period may be related with special responses to these treatments and to defence responses in general.

In former investigations we have characterised several wheat varieties which differed in drought tolerance (Erdei and Trivedi 1989; Erdei et al. 1990; Trivedi et al. 1991; Salama et al. 1994; Szegletes et al. 2000). Results have revealed several physiological traits, like polyamine and cation accumulation, maintenance of chloroplast membrane structure and function, in favour of osmotic and salt tolerant cultivars. At that time, however, molecular biological work has not been initiated. The aim of the present work is to provide preliminary information for the establishment of gene bank and to obtain comparative data for the new transgenic lines obtained from the investigated cultivars and land races.

Materials and Methods

A comparative study

For experimental survey, wheat species of different ploidy levels and hexaploid cultivars of different stress tolerance were chosen (Tables 1 and 2).

Caryopses were imbibed in distilled water and after 24 h the viable germs were placed into complete 0.5x diluted Hoagland solution complemented with 100 mM (200 mOsm) NaCl or 200 mOsm (14.3% polyethylene glycol).The nutrient solution without addition served as control. Plants were grown under controlled conditions in phytotron (Conviron, type EF7) in 14/10 day /night period with 200 μmol m^{-2} s^{-1} irradiance. Day/night temperature was 24/18°C). The containers were aerated and the nutrient solution was exchanged weakly. For experiments plants were harvest-ed at day 21.

Growth, cation concentrations and total carbohydrates in shoots and roots were determined.

Plant material for gene isolation

Based on recent and previous (Cseuz and Erdei 1994) results, for gene isolation the osmotic stress resistant cv. Kobomugi, and the sensitive cv. Öthalom were chosen. Osmotic treatment was administrated using PEG 6000 at a final concentration of 400 mOsm (19.0%), built up gradually (Fig. 1).

Table 1.

Species/cultivars	Ploidy	Genom
Triticum monococcum	2n=2x=14	AA
T. dicoccum cv. Betadur	2n=4x=28	AABB
T. durum	2n=4x=28	AABB
T. spelta	2n=6x=42	AABBDD
T.aestivum cvs.	2n=6x=42	AABBDD

Table 2.

Species/cultivars	Tolerance	Origin
T aestivum cv. Kharchia	Salt tolerant	India, land race
cv. Sakha	Salt tolerant	Egypt, breeded
cv. Kobomugi	Drought tolerant	Inner Asia, land race
cv. Regina	Sensitive	Europe, breeded

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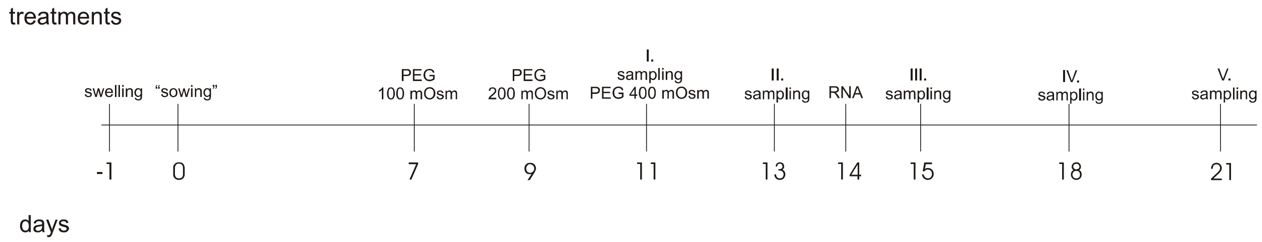

Figure 1. Experimental design for treatment of plant material for gene isolation.

Sampling for RNA isolation was carried out 84 hours after the onset of the 400 mOsm treatment. Growth, cation concentrations and total carbohydrates were also determined according to the protocol shown by Figure 1.

Results

Growth. As characterised by fresh and dry mass, growth decreased under salt- and osmotic stresses for most of the species and cultivars, however, in case of *T. monococcum, T. dicoccum* , *T. aestivum* cvs. Kobomugi and Sakha no decrease or slight increase was observed. Probably as an adaptive trait, root length increased under osmotic stress for *T. dicoccum* and cvs Kobomugi and Sakha.

Ion concentrations. Under osmotic stress, in the tetraploid lines K^+ concentration increased in the leaves or in the root, while in the rest of lines it was decreased. Calcium concentration also increased, to the highest level in the roots of Kobomugi whereas *T. durum*, Sakha and Regina showed no changes. Under salinity stress, in most of the lines higher Na^+ concentrations were accumulated in the roots than in the shoots, with the exception of the tetraploide lines where the opposite trend appeared. The salt tolerant Kharchia evenly distributed Na in both roots and shoots. Calcium levels, as expected decreased under salt stress except in Kobomugi and Regina, where they did not change.

Total carbohydrate. In the comparative experiments, under both osmotic and salt treatments carbohydrate accumulation was high in all the wheat lines except Sakha and Regina. The highest total carbohydrate level was found in Kobomugi.

In the experiment for RNA isolation, Kobomugi and a known drought sensitive line, cv. Öthalom were chosen . Eighty hours after the administration of the final osmotic concentration (400 mOsm PEG), the concentration of total carbohydrates was twice as high in Kobomugi compared to the negative control (Fig. 2). Later on this difference decreased since cv. Öthalom gradually recovered.

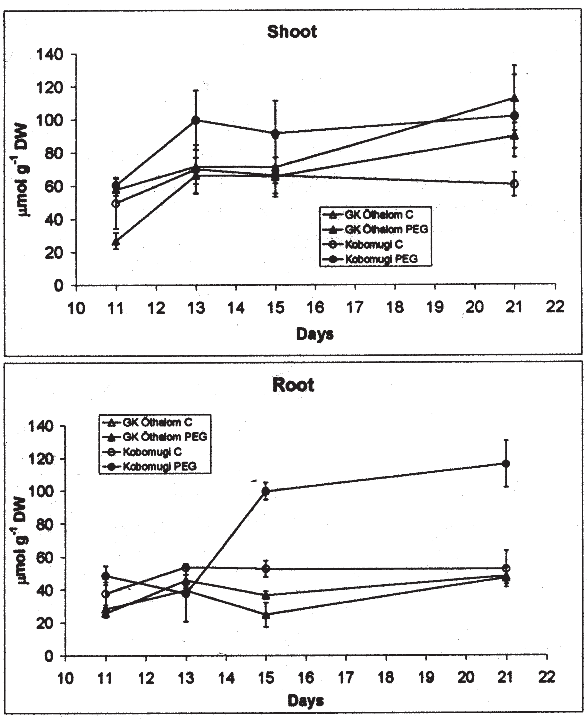

Figure 2. Accumulation of total carbohydrates expressed as sucrose units under polyethylene glycol (PEG) treatment in shoots and root of *T.aestivum* cvs Öthalom and Kobomugi. C, control; PEG, PEG treatment. Samples for RNA isolation were taken 84 hours after the onset of the 400 mOsm treatment, at day 14 (see Fig. 1).

Discussion

Since drought tolerance is a multigenic trait and manifests at different levels of organisation and different stages of development, present results suggest that at least in the young vegetative stage of growth and development, an increased carbohydrate synthesis can be considered as a promise for drought tolerance. High carbohydrate concentration, beside its role in decreasing water potential, contributes in preventing oxidative damage and maintaining the structure of proteins and membranes under moderate dehydration during drought period (Hoekstra et al. 2001). Carbohydrates also serve as signalling molecules for sugar-responsive genes which leading to different physiological responses like defense responses and turgor-driven cell expansion (Koch 1996; Sturm and Tang 1999). This is in good agreement with our results that in contrast to most of the species and cultivars investigated, root carbohydrate concentration was increased and root growth was stimulated by osmotic stress in the cv. Kobomugi. Sugar accumulation difference between Kobomugi and Öthalom, however, has ceased in time, with a delayed response by Öthalom. This acclimatization process was in positive correlation with changes in chlorophyll fluorescence parameters measured *in vivo* (Gallé et al. 2002). In addition, a high sugar accumulation capacity in cv. Kobomugi was still maintained in flag leaves in the late developmental phase of heading (Cseuz et al. 2002). It is suggested that cv. Kobomugi may be a useful source for isolation of drought tolerance-related genes (Zhiponova et al. 2002).

Acknowledgments

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