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Experimental system for studying long-term drought stress adaptation of wheat cultivars

Maria Secenji, Ágnes Lendvai, Zsuzsa Hajósné, Dénes Dudits, János Györgyey*

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

ABSTRACT Water limitation is a well-known problem for plants. Lack of water affects their biomass, their yield that is the most conspicuous in case of crops causing severe uncertainty of agricultural productivity. Under drought stress, plants generally display many physiological responses such as stomata closure, decreased/stopped photosynthetic activity, increased root/shoot ratio, reduced growth of vegetative parts. Many of the physiological changes are caused by underlying transcriptional alterations of high number of genes in many cases. One of the most studied phenomenon is the accumulation of proline as an osmoprotectant. Proline biosynthesis is increased by water deficit due to increased expression of the key enzyme, namely Δ^1-pyrroline-5-carboxylate synthetase (P5CS). In our experiments, we applied P5CS as a positive control to evaluate our new experimental system, which will allow to follow transcriptional changes in shoots, as well as in roots during drought adaptation. Our alternative approach allows greenhouse or growth chamber experiments that are more similar to natural conditions than the widely used experimental systems based on osmotic agents such as polyethylene glycol (PEG).
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KEY WORDS

wheat
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Water deficit can cause different changes in plants effectuating their responses to drought stress. There is a strong connection between these modifications that happen at molecular and cellular levels as well as physiological level (Yamaguchi-Shinozaki et al. 2002). There are several drought-stress inducible genes and their expression can be affected through either abscisic acid (ABA)–dependent or ABA–independent signal transduction pathways. Functionally, these genes` products are mostly osmoprotectant (proline, glycine betaine) synthases (Chen and Murata 2002), protection factors of macromolecules (chaperons, LEA proteins), proteinases, membrane proteins (aquaporin, transporters), detoxification enzymes (GST, SOD). Nevertheless, genes of regulatory proteins such as transcription factors (MYC, MYB, bZIP), protein kinases (MAPK, CDPK), protein phosphatases are induced by drought as well (Wang et al. 2003). Physiological answers such as stomata closure (Papp et al. 2004), decreased/stopped photosynthetic activity, increased root/shoot ratio (Wu and Cosgrove 2000) are the consequences of processes conducted by these gene products.

Materials and Methods

Plant material

Wheat (*Triticum aestivum* L.) seeds were germinated in water at 18ºC. Four genotypes were used to examine their various adaptation patterns to drought. These are: "Capelle Desprez", a drought-sensitive genotype; "Öthalom", a Hungarian genotype with an average drought tolerance; "Plainsman", which tolerates moderate drought well while delivering good yield; and "Kobomugi", an Asian landrace, which can set seeds under extreme arid conditions.

Three-day-old seedlings were planted into pots containing 3 liters of wet perlite, fifteen plantlets in each pot. Seedlings were irrigated with Hoagland nutrient solution (Gamborg and Wetter 1975) twice per week for two weeks. After two weeks the quantity of the irrigating solution was reduced from 300ml to 50ml to imitate drought conditions, except the controls: they were further irrigated with 300 ml solution. In the case of the control, irrigation solution contained the same amount of nutrients as in the case of treated samples but the amount of water was increased. Shoots and roots were collected separately and they were stored at -80ºC.

RNA isolation

RNAs were extracted according to the AGPC (acid guanidinium thiocyanate-phenol chloroform) method (Chomczynski and Sacchi 1987) using TRI reagent with slight modifications, namely temperature during extraction was increased to 65ºC and a purification step using chloroform/isoamylalcohol was included.

DNase treatment and first-strand cDNA synthesis

DNase treatment was done for preparation of DNA-free RNA prior to RT-PCR. First-strand cDNAs were synthesized by using random hexamers. Both procedures were carried out according to the protocols of Fermentas.

*Corresponding author. E-mail: arthur@brc.hu

Real-time PCR

Real time quantitative PCR analysis was carried out using ABI Prism 7000 Sequence Detection System. Primers for real-time PCR were designed by software Premier Express developed by Applied Biosystems. The length of the product was 131bp.

Results and Discussion

The aim of our work was to find an alternative experimental system that is free of osmotic agents, allows following transcriptional changes in roots during long-term adaptation to drought stress. As a candidate artificial soil, perlite was chosen for the experiments with an advantage that root can be collected from it for RNA isolation. The progress of the experiment was followed by measurement of weights of root and shoot tissues. In this system, growth of these plant parts exhibits a decrease compared to the control in addition, the root/shoot ratio was increased at all four genotypes. In parallel, cDNAs derived from collected samples were analyzed by real-time PCR using P5CS specific primers as molecular marker of drought stress. Relative transcript levels of P5CS in shoot samples originated from all four genotypes display similar expression patterns showing maximum around three weeks. Nevertheless, in Capelle Desprez, the drought sensitive cultivar, this increase is not so remarkable than in case of other three genotypes. Similar analyses in root tissues are in progress. Expression data of P5CS prove that such an artificial soil based system can be used successfully to study the molecular background of abiotic stress adaptation.

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