Monitoring of Gene Expression Profiles and Identification of Candidate Genes Involved in Drought Tolerance in Festuca Mairei with cDNA-AFLP

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Introduction Drought stress is one of the most complex environmental constraints on plants. Response of plant to drought stress is manifested by various changes in physiological and metabolic processes, which are reflected at the molecular level. cDNA-amplified fragment length polymorphism (cDNA-AFLP) is a high-throughput transcript profiling technique for temporal and spatial gene expression analysis (Bachem *et al*., 1996). To understand the molecular genetic basis of drought tolerance of grasses, we applied the cDNA-AFLP procedure to identify the genes responding to drought stress of *Festuca mairei* (Fm), which showed a xeriphytic adaptation (Marlatt *et al*., 1997).

Materials and methods Five Fm plants were deprived of water until they were severely stressed and the other five plants as treatment control were watered daily throughout the drought stress period. During the drought stress treatment, leaf samples from both stressed and control Fm plants were detached for RNA isolation. 128 primer combinations for cDNA-AFLP were conducted on nine time-points of Fm during drought stress. Differentially expressed fragments were identified and recovered from polyacrylamide gel. Reverse northern hybridization was conducted to confirm the differential expression pattern.

Results cDNA-AFLP revealed 11,346 TDFs with fragment size distributed from 50 to 1000bp. 464 fragments were identified as differentially expressed across the nine time-points, and 434 (94%) were recovered from acrylamide gel. The expression patterns of these differentially expressed fragments included up-regulated (29.7%), down-regulated (54.3%), transient-expressed (12.1%) and up- then- down- regulated (3.7%). 406 TDFs (> 100 bp) were subjected to reverse northern analysis. 172 (42.4%) TDFs showed a consistent differential expression pattern with the cDNA-AFLP analysis. The sequences of these confirmed TDFs were compared to the Arabidopsis protein database with BLASTN to target the potential function of these gene fragments. About 10% of them were novel transcripts, while about 70% could be assigned putative function. The putative function of the differentially expressed genes could be classified as: 1) defence related or protective proteins, 2) transport facilitation, 3) amino acid and carbohydrate metabolism, and 4) signal transduction and transcriptional and translational control.

Conclusions The results of the present study demonstrate that cDNA-AFLP is an efficient high through-put transcript profiling technique for gene discovery. Fm responses to drought at a comprehensive molecular regulation level involving signal transduction, transport facilitation, metabolism regulation and protective defence.

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