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ORIGINAL PAPER

Identification and expression profiling of drought-regulated genes in mulberry (*Morus* sp.) by suppression subtractive hybridization of susceptible and tolerant cultivars

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Abstract Mulberry, the backbone of sericulture industry, is a rainfed crop, and its biomass production is affected adversely under drought conditions. In this study, genes expressed differentially during drought stress response have been examined by PCR-Select subtractive hybridization. The sensitive and tolerant genotypes were identified based on physiological evaluation by determination of proline content, electrolyte leakage, and measurement of relative water content. In total, 1,920 clones were sequenced, representing 208 contigs and 151 singletons. The expressed sequence tags generated from this subtracted cDNA library comprises a broad repertoire of stress-responsive genes, which contribute to the process of drought tolerance in mulberry. Additionally, 23% of the cDNA library is represented by transcripts of unknown function. The expression of a select number of these drought-inducible genes was studied based on cDNA macroarray and Northern blot analyses. In order to unravel the crosstalk with other abiotic stresses, expression profile of *Arabidopsis* homologs of selected genes in response to a wide range of different stresses was studied using Genevestigator as a reference expression database. The results of this study show that subtractive hybridization coupled with validation steps for differential screening is an effective method for identification of stress/drought-induced genes in plants with limited sequence information available.

Keywords Mulberry · Subtractive hybridization · Transcriptome analysis · Water stress

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Introduction

Mulberry belongs to the fig family, Moraceae, under the order Rosales. Mulberry is an important component of combined pastoral system (Talamucci et al. 2000) as its leaves are used as a protein-rich forage supplement for animal husbandry (Benavides 2000). However, the most important utility of mulberry is that its leaves are used for feeding the monophagus silkworm (*Bombyx mori*). India is the second largest producer of raw silk accounting for more than 18% of global raw silk production. In spite of the gradual increase in silk production in the country, the quality and quantity of silk produced is suboptimal. Since *B. mori* feeds solely on mulberry leaves, therefore, mulberry varieties with higher leaf yield are a pre-requisite in sustaining the sericulture industry.

Abiotic stresses significantly reduce crop yields and restrict the latitudes and soils on which commercially important species, including mulberry, can be cultivated. Plants overcome stressful environments by altering gene expression leading to a new equilibrium between plant growth, development, and survival. The response of plants to every environmental stress is unique and imparts to plants the ability to survive under stress conditions (Mahalingam et al. 2003). Drought tolerance is a complex trait and is controlled by multiple genes (Zhu 2002). In recent years, considerable attention has been directed