Differential expression of water stress- regulated genes in drought tolerant or sensitive sunflower genotypes

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Abstract

we have identified and characterized water stress- and abscisic acid- regulated genes from a drought tolerant sunflower genotype. We have used two lines of sunflower (*Helianthus annuus* L.) selected in field as drought tolerant (R1 genotype) or drought sensitive (S1 genotype). R1 tolerance is characterized by a delay of both wilting and decrease of leaf water potential. To analyse R1 tolerance at a molecular level, we have isolated different cDNAs (named *sdi* for Sunflower Drought induced) corresponding to transcripts accumulated in water-stressed R1 leaves, by subtractive hybridization. Analysis of transcripts accumulation in both genotypes upon drought stress suggests a differential expression of the *sdi* genes. Abscisic acid- mediated induction in the tolerant genotype is observed for four of the *sdi* genes, and differs among these genes. Sequence analysis of SDi clones shows high identity with known proteins including nsLTPs, Elips or dehydrin, predicted to be involved in various physiological processes.

Introduction

Plants respond to water stress through modifications of their morphological, physiological and metabolic processes. Depending on their level of tolerance, many plant species can withstand more or less longer period of drought. It is now well established that drought induced plant responses are largely the result of changes in gene expression. Some responses may be correlated to cell damage while others may result of an adaptative response (reviewed in Bray. 1994). During the last few years a large number of drought-induced genes were identified in a wide range of plant species, however direct evidences of the implication of a given gene in adaptative response remain yet to be established. Therefore, linking the expression of a gene to a higher degree of tolerance within a genotype might provide arguments for its role in adaptation (Galvez et al. 1993; Moons et al. 1995). Endogenous abscisic acid concentrations increase in different plant tissues during environmental stress, and this is proposed to mediate molecular responses. In some tolerant species the stress-induced endogenous ABA level was shown to be correlated with the varietal differences in tolerance (Lee et al. 1993; Moons et al. 1995). ABA-regulated gene products might then be correlated with stress tolerance (Chandler and Robertson. 1994).

Results and discussion

R1 and S1 sunflower genotypes display different levels of tolerance upon progressive drought-stress

R1 and S1 sunflower genotypes were selected in field based on their ability or not to withstand long periods of drought. Their respective behaviour upon water-stress was characterized by monitoring the evolution of soil and leaf water status during experimental progressive drought (Fig. 1). Our results indicate that, when submitted to a similar decrease in soil water availability (Fig. 1A), R1 tolerance is characterized by a delay of both wilting appearance (data not shown) and decrease of leaf water potential (Fig. 1B). Furthermore, we observe a similar decrease in osmotic potential for both

genotypes (Fig. 3C). To identify genes potentially involved in drought tolerance, we have isolated and characterized cDNAs clones from mRNA accumulated in leaves of R1 sunflowers submitted to water stress before they were affected by water deficit (ie between days 4 and 6).

Fig. 1: Soil and leaf water potential and osmotic potential of R1 and S1 *Helianthus annuus* genotypes during drought stress treatment initiated by withholding watering (start point is considered day 0). Water potentials were determined with a thermocouple psychrometer on daily irrigated control plants (open circle) and drought-stressed R1 (black square) or S1 (black triangle). Leaf water potential was determined on leaf discs, osmotic potential was determined on the same leaf discs after freezing and thawing. Data are means of four to six replicates +/- standard deviation.

Drought-stress induced accumulation of SDi mRNA in R1 and S1 sunflower

We have isolated different cDNAs (named *sdi*-) corresponding to transcripts accumulated in drought-stressed leaves of R1, by subtractive hybridization. Sequences homologous to each *sdi* clones are present in the S1 genome. Homologies of four SDi deduced proteins with known proteins are reported in table 1.

Table 1: Homology of SDi deduced proteins. ELIP (Early Light Induced Protein), nsLTP (non specific Lipid Transfer Protein).

Clone	SDi-1	SDi-5	SDi-8	SDi-9
Homology	ELIP	dehydrin	dehydrin	nsLTP

Each one of these *sdi* clone corresponds to drought-induced transcripts in both genotypes (Fig. 2). We observe differences in the relative amount of *sdi* transcripts accumulated in water stressed R1 and S1 leaves, that are particularly significant for *sdi*-1 and *sdi*-5. The pattern of transcripts accumulation suggests then a differential expression between R1 and S1 for the *sdi*i genes. These results indicate that our system can be used to link the expression of a given gene with the evolution of leaves hydric status and therefore to determine in which extent water stress- induced R1 genes are involved in tolerance. To accumulate evidences for *sdi* genes to be involved in tolerance, we are now analysing more precisely *sdi* mRNAs accumulation in both genotypes as a function of various physiological parameters during water stress.

Fig. 2: Drought-stress induced accumulation of *sdi* transcripts. Total RNA were purified from water stressed leaves of R1 0 or S1 0 sunflowers collected 3, 4 and 6 days after the imposition of drought, or from daily irrigated control R1 0 and S1 0, and analysed by northern hybridization with labelled cDNA insert of each clone as indicated. The intensity of each hybridization signal was quantified, the strongest hybridization signal was was set at 10 and the other intensities were quantified on the basis of this signal.

ABA- induced expression of SDi genes in R1 sunflower leaves

Fig. 3: ABA-induced accumulation of *sdi* transcripts in R1 leaves. Total RNA was purified from leaves of R1 sunflowers cultivated in hydroponic medium supplemented (+) or not (-) with 10 µM of ABA for the times indicated, or from R1 leaves collected four days after the onset of stress (D), and analysed by nothern hybridization with the indicated probes. The intensity of each hybridization signal was quantified as described in Fig. 2.

To determine in which extent ABA is involved in the induction of *sdi* genes, we have analysed the effect of applied ABA on the *sdi* mRNAs accumulation in R1 sunflower. ABA induces accumulation of SDi-5, SDi-8 and SDi-9 transcripts in R1 leaves (Fig. 3), while increase in *sdi*-1 transcripts level was never detected upon ABA treatment. The response observed to exogenous ABA treatment suggests that this hormone could mediate the water stress induction of the dehydrin- and nsLTP-related in R1 genotype. Interestingly, although *sdi*-5 and *sdi*-8 belong to the same family of proteins, differences are observed in their ABA- induced expression, as differences was observed in their drought- induced expression (Fig. 2). This might indicate that they have somewhat different roles in drought response.

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