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Molecular and biochemical characterization of transgenic rice plants over-expressing the Datura *s-adenosylmethionine decarboxylase* (*SAMDC*) cDNA

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SUMMARY – We have been investigating the potential protective role of polyamines (PAs) against drought stress in transgenic rice plants. Rice (*Oryza sativa* L. cv EYI105) plants transformed with the Datura *S-adenosylmethionine decarboxylase* (*samdc*) cDNA driven by the constitutive maize ubiquitin-1 (*Ubi-1*) promoter have been regenerated. Each independent transformant was characterized molecularly and biochemically. Plants with higher spermidine (Spd) and spermine (Spm) content were selected for drought stress experiments. Expression of endogenous PA pathway genes has also been analysed. Preliminary results indicate that recovery of the drought tolerant phenotype is linked to transgene expression and concomitant accumulation of Spd and Spm. We will present a detailed analysis of the drought tolerant phenotype in these plants and in the process, we will define crucial factors that are implicated in the response of cereal crops to abiotic stress tolerance such as drought and salinity.

Introduction

Polyamines are small, ubiquitous, nitrogenous compounds that have been implicated in a variety of stress responses in plants (Bagni, 1989). The polyamine pathway comprises an anabolic phase leading to the elaboration of Spd and Spm from putrescine (Put). Ornithine decarboxylase (ODC) catalyses the removal of the carboxyl group from ornithine (orn) to yield Put, while *S*-adenosyl-L-methionine decarboxylase (SAMDC), introduces S-adenosyl-L-methionine (SAM) into the pathway which is then used in its decarboxylated form (dcSAM) as an aminopropyl donor in the conversion of Put to Spd and subsequently to Spm (Bagni, 1989). The actual transfer of the aminopropyl moiety is catalysed by two separate and distinct enzymes, spermidine (SPD SYN) and spermine synthase (SPM SYN). In bacteria and also in plants, two alternative pathways lead to putrescine formation. In addition to the ODC pathway, decarboxylation of arginine by arginine decarboxylase (ADC) also results in putrescine formation via two intermediate steps (Bagni, 1989). Arginine decarboxylase is a single or low copy number nuclear gene with a coding region of approximately 2,100 nucleotides. No introns have been found to date in any *adc* gene. Full or partial *adc* sequences have been reported in the GenBank database from eight eudicots (Galloway *et al*., 2000) and two *adc* loci have been identified in taxa from the Brasicae family (Galloway *et al*., 2000). The oat (*Avena sativa*; Bell and Malmberg, 1990) and rice [Sasaki and Minobe, unpublished (D15966)] *adc* sequences are the only two monocot sequences deposed in the GenBank. The study of plants transformed with genes involved in PA biosynthesis may shed light on the importance of PAs, their role in the acquisition of stress tolerance and relevant stress tolerance mechanisms. In this report, we describe the molecular characterization of progeny plants expressing the Datura *adc* and the Datura *samdc* cDNAs.

Material and methods

Plasmid construction, rice transformation and plant regeneration

The 2916 bp *D. stramonium adc* cDNA (AJ251898) and the 1839 bp *D. stramonium samdc* cDNA (Y07768) were excised from the pBluescript plasmid and subcloned into pAL76 (Christensen and Quail, 1996) which contains the *Ubi-1* promoter and first intron and an *Agrobacterium tumefaciens nos* transcriptional terminator. These plasmids were subsequently referred to as *Ubi:Dadc* and *Ubi:Dsamdc*, respectively. A plasmid containing the hygromycin phosphotransferase (*hpt*) gene was used as the selectable marker gene. Rice transformation, selection and plant regeneration procedures were performed as previously described (Sudhakar *et al*., 1998).

DNA and RNA gel blot analysis

Rice genomic DNA was extracted from leaf tissue according to the method of Edwards *et al*., (1991). Following *Kpn*I digestion and electrophoresis on a 0.8 % TBE agarose gel (Sambrook *et al*., 1989), DNA (13 μg) was transferred to a positively charged nylon membrane. Nucleic acids were fixed by UV crosslinking. Labeling of the Datura *adc*, Datura *samdc* and rice *adc* probes, as well as the hybridization and relative washes, were performed as previosly described in Bassie *et al*., (2000). Chemiluminescent detection was carried out according to the manufacturer's instructions. Re-probing of the membranes was performed as described in Lahaye *et al*., (1996). Total RNA for RNA gel blot analysis, was extracted from leaves from one month-old plants using Trizol®. Denatured RNA (30 μg) was subjected to electhophoresis on 1.2% agarose-formaldehyde gel using 1X MOPS buffer (Sambrook *et al*., 1989). Hybridization of the RNA gel blots was as described for DNA analysis.

Results and discussion

Recovery of primary transformants containing Datura adc and Datura samdc genes

Mature rice embryos were co-bombarded with plasmids *Ubi:Dadc* and *Ubi:Dsamdc* (in two independent transformation experiments), all driven by the *Ubi-1* promoter, and a plasmid containing the *hpt* gene as a selectable marker (Sudhakar *et al*. 1998). We analyzed 18 independent primary transgenic rice plants for each construct. Genomic DNA gel blot analysis confirmed integration of the transgene(s) within the genome of the 36 plants that were studied. Digestion was carried out with *Kpn*I, which cuts once within the backbone sequence of the transforming plasmids. Twenty progeny plants from clone 86, containing the *Ubi:Dadc* and twenty progeny plants from clone 98 containing the *Ubi:Dsamdc* construct were selected for further analysis. Figs 1A and 1B show that each progeny exhibited the same integration pattern confirming that all plants originated from the same primary transformant. Segregation analyses confirmed a Mendelian segregation of the transgenes.

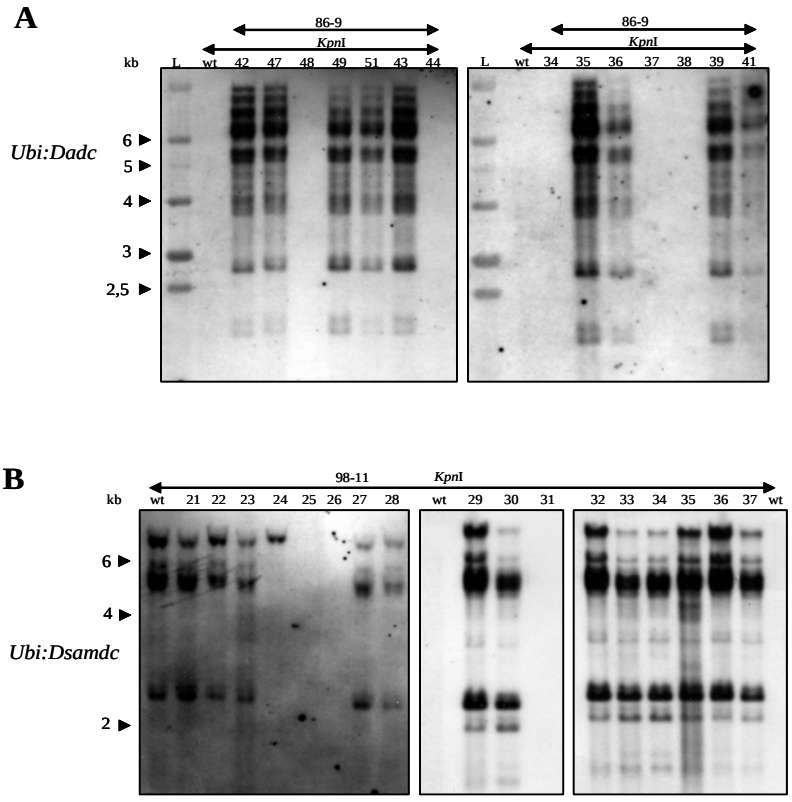

Fig. 1. Genomic DNA gel blot analysis. Genomic DNA (25 mg) was digested with *KpnI* (single restriction site in construct). A. Progeny plants from clone 86 containing the *Ubi:Dadc* plasmid. B. Progeny plants from clone 98 containing the *Ubi:Dsamdc* plasmid. wt: wild-type; numbers represent putative transgenic plants; L: molecular size marker.

Datura adc or *Datura samdc* steady-state mRNA level was detected in all respective progeny plants (Figs 2 and 3). Re-probing of the membrane (plants expressing the *Ubi:Dadc* gene) with the sequence of the endogenous rice *samdc,* indicated that no changes in the steady-state rice *samdc* mRNA took place (Fig. 2). Similar results were obtained with plants expressing the *Ubi:Dsamdc* plasmid (membranes were re-probed with the endogenous rice *samdc* gene) (Fig. 3). This clearly demonstrates that the heterologous transgene operates independently of its rice orthologue. These plants are currently been evaluated for drought stress responses.

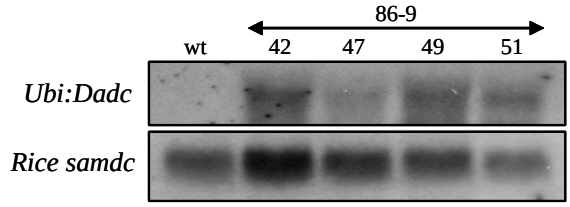

Fig. 2. RNA gel blot analysis of progeny plants from clone 86. Membrane was probed with the *Ubi:Dadc* plasmid and the endogenous rice *samdc*.

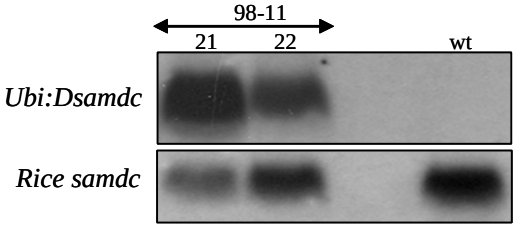

Fig.3. RNA gel blot analysis of progeny plants from clone 98. Membrane was probed with the *Ubi:Dsamdc* plasmid and the endogenous rice *samdc*.

Acknowledgements

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