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DISSERTATION SUMMARY

Characterisation of the ABA sensitivity influencing *Arabidopsis* PPR gene

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T-DNA insertion mutagenesis is a recently developed technique, allowing the identification and easy isolation of genes from higher plants, in this case from *Arabidopsis thaliana*. We initiated a mutagenesis program by generating a collection of T-DNA insertion mutants (Szabados et al. 2000). Using this technique, we are trying to genetically dissect signal transduction pathways mediating cellular and organ differentiation, developmental regulation and stress responses. In order to identify tagged genes, T-DNA insertions sites are characterized by random sequencing the flanking genomic plant DNA (Szabados et al. 2002). During this mutagenesis program, we identified a transgenic line with different behaviour possessing for osmotic and hormonally stress response, where the T-DNA insertion is in the exon of *At3g16890*. The tagged gene encodes a pentatricopeptide repeat-containing protein.

The pentatricopeptide repeat (PPR) is a degenerate 35-amino acid repeating motif, which was first described by Small and Peeters (2000). The PPR proteins have been identified in animal, fungi, and plant, but not in the procaryote and in *Archea*. This family is particularly large in plants, where the majority of family members are predicted to be targeted to mitochondria or chloroplasts. Most observations (Williams and Barkan 2003) suggested that PPR domains can function as nucleic-acid-binding domains (tipically as RNA-binding domains), but some protein-binding motifs are also known. The biological function of PPR proteins could be connected with organellar RNA metabolism, but the directed substrates are not known yet.

In the course of morphological examination a semidwarf mutant was identified from the studied transgenic line, and the segregation analysis suggested that it contains only one T-DNA insertion. We confirmed the linkage between the T-DNA insertion and phenotype by molecular analysis (Southern blotting, PCR). The semidwarf mutant is homozygous for the T-DNA-tagged locus. The homozygous plants showed an increased environmental stress sensivity such as salt and cold. ABA and glucose treatments greatly prevented the mutant germination and its growing. In order to test several stress-related gene expression in this transgenig line, we used quantitative and RT PCR technique. It was verified that there is no *At3g16890* mRNA production in the homozygous mutant plants. We observed that the *AtP5CS1* and *AtRD22* RNA levels were augmented in mutant plants compared to wild type *Arabidopsis* under normal conditions as well as after ABA and NaCl treatments. We concluded, that the *At3g16890* gene can play a role in an ABA-dependent stress signaling pathway.

To further analyze the biological function and localization of this protein, we cloned the full-lenght cDNA in a HA-epitope tagging binary vector, and transformated into the homozygous and wild type plants. We confirmed the transformation by Northern hybridization and Western analysing. The phenotype of the complemented mutants were similary to the wild type under stress (ABA, NaCl, glucose) and normal conditions. The overexpressing transgenic lines showed insensitivity to toxic concentrations of NaCl, glucose, and ABA treatments at germination level. To locate the protein by Western analysis, we isolated intact chloroplast and mitochondria from overexpressing plants, and compared the recombinant protein level in the two organelles and whole plant. The protein showed mitochondrial localization. Experiments are in progress to further characterize this protein and its RNA interacting partners.

References

Szabados L, Kovács I, Ábrahám E, Oberschall A, Nagy R, Zsigmond L, Krasovskaja I, Kerekes I, Ben-Haim A, Koncz Cs (2000) Arabidopsis genome project in Hungary: generating T-DNA tagged Arabidopsis genes. Plant Physiol Biochem 38:S98.

Szabados L, Kovács I, Oberschall A, Ábrahám E, Kerekes I, Zsigmond L, Nagy R, Alvarado M, Krasovskaja I, Gál M, Berente A, Rédei GP, Ben-Haim A, Koncz Cs (2002) Distibution of 1000 sequenced T-DNA tags in the Arabidopsis genome. Plant J 32:233-242.

Small I, Peeters N (2000) The PPR motif – a TPR-related motif prevalent in plant organellar proteins. Trends Biochem Sci 25:46-47.

Williams PM, Barkan A (2003) A chloroplast-localized PPR protein required for plastid ribosome accumulation. Plant J 36:675-686.

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