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DISSERTATION SUMMARY

Oxidative stress tolerance and plant development: the functional characterization of the "oxprot" gene

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Reactive oxygen species (ROS) are toxic compounds produced by normal metabolic processes. Their reactivity with cellular components is a major stress for aerobic cells that results in lipid, protein, and DNA damage. ROS-mediated DNA damage contributes to spontaneous mutagenesis, and cells deficient in repair and protective mechanisms have elevated levels of spontaneous mutations. The human OXR1 (oxidation resistance) gene was described to be involved in the prevention of oxidative DNA damage. OXR1 is a member of a conserved family of genes found in eukaryotes but not in prokaryotes (Volkert et al. 2000). The most highly conserved region of the gene is its carboxyl-terminal half, which contains a TLDc domain (unknown function), furthermore it has a calcium binding EF-hand motif and a mitochondrial localization signal (Volkert and Elliott 2004). Homologues are present in many eukaryotic organisms from yeast to humans, so thus in *Arabidopsis thaliana* (At) and *Medicago truncatula* (Mt*)* as well. The MtOxprot gene was identified from an alfalfa cDNA library and analized in several ways. It has a relatively high level of expression in the stem, leaf and cell suspension culture as detected by Real-time PCR. Its transcription was increased by various stress treatments as wounding, drought and paraquat (a widely used herbicide generating ROS in plants).

Deletion of the *OXR1* gene in *oxr1* mutant haploid *Saccharomyces cerevisiae* (*scOXR1*) resulted in 10- fold more sensitivity to hydrogen peroxide damage than in wild-type strains. Therefore the MtOxprot cDNA was cloned into a yeast expression vector to check whether it can complement the peroxide sensitivity of the yeast *oxr1_* mutant strain. The results obtained indicates a succesful complementation as the yeast strain expressing the MtOxprot cDNA was approximately 5-fold less sensitive to hydrogen peroxide in a lethality assay than was the *oxr1_* mutant strain.

The human OXR1 gene has two homologues in *Arabidopsis thaliana* (At4g34070, At5g06260), and knock out *Arabidopsis* mutants with T-DNA insertions in the coding regions of the two homologous genes were available (SALK mutants). Neither mutants show hypersensitivity to stress treatments as salinity, paraquat or osmotic stress in seed germination assays, for the possible reason that the homologous genes could complement each other. After crossing the two mutants, double mutant lines will be the subject of further analysis.

Transgenic *Arabidopsis* plants overexpressing the AtOxprot and MtOxprot genes were also generated by an Agrobacterium-mediated in-planta transformation method. Molecular and physiological characterization of the transgenic lines is in progress.

Rice plants overexpressing one of the two AtOxprot cDNA-s (At5g06260; kindly supplied by CropDesign, Gent, Belgium) are also available for the investigations. In preliminary tests the plants showed some kined of tolerance to paraquat treatment indicating that the "oxprot" protein may protect the plant cells from oxidative damage.

In order to produce antibody and characterize biochemically the activity and function of the „oxprot" protein, 6xHis-tagged cDNA clones were overexpressed in *E. coli,* and the proteins were purified from bacterial extracts under native conditions. The *in planta* cellular localization of the oxprot genes will also be determined using fluorescently tagged proteins and the produced antibody.

References

Volkert MR, Elliott NA, Housman DE (2000) Functional genomics reveals a family of eukaryotic oxidation protection genes. Proc Natl Acad Sci 97:14530-14535.

Elliott NA, Volkert MR (2004) Stress Induction and Mitochondrial Localization of Oxr1 Proteins in Yeast and Humans. Mol Cel Biol 24:3180-3187.

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