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DISSERTATION SUMMARY

The role of proline in *Arabidopsis thaliana* osmotic stress response

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Plants have evolved different ways of adapting to osmotic stress. Proline accumulation in higher plants is a characteristic physiological response to osmotic stress. Proline is considered to play an important role in defense mechanisms of stressed cells which can ameliorate shifts in redox potential by replenishment of NADP+ supply as well. Proline degradation can provide carbon, nitrogen and energy source after stress (Hare et al. 1999). In higher plants prolin is synthesized from glutamate or ornithine. Proline biosynthesis in the glutamate pathway is controlled by the first enzyme, the bifunctional 1-pyrroline-5-carboxylate (P5CS), which phosphorylates glutamic acid and reduces it to glutamyl-5-semialdehyde (G5SA). Proline is synthesized from G5SA via pyrroline-5-carboxylate (P5C), by the Δ1-pyrroline-5-carboxylate reductase (P5CR) enzyme (Delauney and Verma 1993). The rate-limiting step in this pathway is the γ–glutamyl kinase activity of the P5CS enzyme, which is controlled by feedback inhibition in plants as well as in bacteria (Zhang et al. 1995). Degradation of proline takes place via oxidation to P5C by proline dehydrogenase (ProDH) and subsequently to L-Glu by P5C dehydrogenase (P5CDH; Kiyoshue et al. 1996). Proline accumulation in salt stressed *Arabidopsis* plants follows the activation of P5CS1 and P5CS2 genes and repression of the ProDH gene, suggesting that transcriptional regulation is the main control of proline biosynthesis (Verbruggen et al. 1996; Strichov et al. 1997).

To study the role of P5CS1 gene function, we used *Arabidopsis* knock out mutants, *p5cs1,* with T-DNA insertion in the promoter or coding region of AtP5CS1 gene. The *p5cs1* mutants showed hypersensitivity to exogenous application of 200 mM NaCl. Decreased proline content was observed in *p5cs1* mutants during external NaCl supply, as compared with wild type plants. These results prove the role of P5CS1 in adapting plants to osmotic stress. NaCl treatment greatly increased *AtP5CS1* RNA levels in wild type plants. The transcript level of *AtP5CS1* was significantly reduced in mutants; this result was confirmed by Northern hybridization.

It was reported that the elevation of proline as an osmoprotectant in the mechanism of salt stress adaptation led to the enhancement of the enzymes scavenging the reactive oxygen species (ROS).

To further analyze the defense mechanisms of *p5cs1* mutants we measured the activity of ROS scavenging enzymes. Reactive oxygen species are produced in both unstressed and stressed cells. Even under optimal conditions, ROS, including superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen, are metabolic by-products of plant cells. These ROS affect lipid peroxidation, protein denaturation and DNA structure. To remove ROS, plant cells possess an antioxidant system consisting of low-molecular-weight antioxidants, such as ascorbate, α–tocopherol, glutathione and carotenoids, as well as antioxidant enzymes. These include superoxide dismutase for scavenging the superoxide radicals and other key enzymes, ascorbate peroxidase and glutathione reductase, detoxifying hydrogen peroxide in the ascorbate-glutathione cycle. Under normal conditions, the production and destruction of ROS is well regulated in plant cells. Under environmental stress, the balance between the production of reactive oxygen species and the quenching activity of the antioxidant system is upset.

After the measurement of the activity of glutathione S-transferase, glutathione reductase, guaiacol peroxidase, ascorbate peroxidase, catalase and superoxide dismutase, we observed that the level of the mentioned enzymes, excepting superoxide, are elevated by salinity, due to the imbalance in the production and destruction of reactive oxygen species. Compared the wild type plants with the *p5cs1* mutants, we realized that the level of the measured enzymes was decreased, except guaiacol peroxidase, which enzyme level was increased in mutants. These results suggest that the defense mechanisms of *p5cs1* mutants are less effective, which is in accordance with the hypersensitivity of *p5cs1* mutants to salt.

References

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