

**PROLINE BIOSYNTHESIS IN
TRANSGENIC SOYBEAN PLANTS**

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ABSTRACT

Plants have evolved numerous strategies for the adaptation to drought. Although many investigations reported on the potential value of proline accumulation during environmental stress, it is still unknown whether or not a constitutive higher level of proline accumulation enhances plant tolerance. Thus, it was investigated if underproduction and overproduction of proline will influence the susceptibility to drought stress in soybean plants. This was made possible with the transformation of soybean plants with an L- Δ^1 -pyrroline-5-carboxylate reductase (*P5CR*) gene.

First, an *Agrobacterium*-mediated vacuum infiltration transformation system, using partially germinating Carnia 2233 soybean seed, was established through the assessment of several conditions that can affect transformation efficiency with the use of β -glucuronidase reporter genes. Transformation was confirmed with PCR and Southern blot analysis and results indicated that stable transgenic soybean plants were obtained within one generation with a transformation rate of $\pm 30\%$. This technique was used in the transformation of Carnia 2233 soybean seed with the *P5CR* gene in the antisense orientation under the control of an inducible heat shock gene promoter (IHSP). It was confirmed that the *P5CR*-IHSP gene construct was integrated into the soybean cells and was conserved over three generations. Physiological screening of the antisense *P5CR* transgenic plants in the greenhouse proved that, with activation of the promoter, an under-expression of the *P5CR* gene and subsequent inhibition of the accumulation of proline were experienced during drought and osmotic stress. The decline of the viability of the transgenics with prolonged drought stress, as monitored with a woodenbox screening test, is an indication that proline is needed for survival

of soybean plants under drought stress conditions. The transgenic plants demonstrated a sensitive reaction in contrast to the control plants that displayed a tolerant reaction to osmotic stress in a TTC assay. The underexpression of the *P5CR* gene resulted in a decline protein synthesis due to proline shortage as was observed with the evaluation of the efficiency of protein synthesis. All these results suggest that a decrease in the proline level due to the antisense *P5CR* gene, yielded plants that are more osmotic and drought stress sensitive.

Subsequently, the soybean cultivar Ibis was successfully transformed with the *P5CR*-IHSP construct in the sense and antisense directions in order to test the reproducibility of the transformation process and to assessed the link between the biochemical traits involved in the drought stress mechanism. Three different experiments were conducted: a mild heat and drought stress on "To" transgenic plants exploring changes in chlorophyll fluorescence transients, a mild heat stress on "T1" transgenic plants comparing proline accumulation and chlorophyll fluorescence transients and a severe drought and heat stress on the "T1" transgenic plants comparing proline accumulation, NADP⁺ synthesis and chlorophyll fluorescence transients. Chlorophyll fluorescence transients were successfully used as a screening method for transgenic soybean plants during this study. The sense transgenics responded to the mild stresses with a significant decrease in their electron transport, trapping and absorption compared to the antisense plants that displayed significant increases in electron transport and trapping. During the severe stress, the antisense transgenics experienced total photoinhibition indicated by the enormous loss of electron transport but the sense plants had the ability to overcome the stress as is revealed in the increase in the electron transport.

It was demonstrated that although proline accumulation yielded no significant differences during the mild heat stress, the sense plants accumulated substantially more proline than the control and antisense plants during the severe heat and drought stress. It was demonstrated that proline plays an important role in the plant's response to a drought stress as well as in the recovery phase after drought, as the sense plants also had the ability to reduce the accumulated proline during the recovery period in contrast to the antisense transgenics that experienced protein degradation. The transgenics responded to a period of heat and drought stress with a reduction in NADP^+ levels in the antisense plants and increasing levels in the sense plants. The sense plants were able to fully recover after the stress period, thus adaptation to drought may depend on different mechanisms, including the capacity to maintain high levels of proline and to regenerate them through the "reduction" of NADP^+ .

It was possible to alter the drought tolerance of Ibis by transformation with antisense and sense *P5CR* gene constructs, which resulted in respectively more sensitive and more tolerant Ibis plants. It can be concluded that over-expression of *P5CR* during a drought stress resulted in higher proline levels, better photosynthetic efficiency, higher NADP^+ production and thus a more drought tolerant plant. This study gave additional proof that a constitutively higher level of proline accumulation enhances drought tolerance in soybean.