

Genetic transformation of the lettuce cultivar Grand Rapids (*Lactuca sativa* L.) by *Agrobacterium tumefaciens* to improve osmotic stress tolerance
(Transformação genética de alface (*Lactuca sativa* L.), cultivar “Grand Rapids”, via *Agrobacterium tumefaciens*, para melhoramento de tolerância ao estresse hídrico)

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Proline is an osmoprotector that can protect plants against decreased water availability in the environment (osmotic stress). The *P5CS* gene codifies the enzyme Δ^1 -pyrroline-5-carboxylate synthetase (P5CS), which takes part in proline synthesis and is inhibited by the final product. A mutation in this gene changes the allosteric site of this enzyme, which then becomes insensitive to feedback inhibition. Consequently, genetically modified plants accumulate proline because constant synthesis of this amino acid, without inhibition, overcomes enzyme degradation. Our objective was to evaluate the tolerance of transformed lettuce strains to three types of osmotic stress: high temperature, freezing, and high saline concentration. We adapted *in vitro* culture and transformation protocols for lettuce (*Lactuca sativa* L.) c.v. Grand Rapids. The transformation system used was co-culture with *Agrobacterium tumefaciens* harboring the recombinant plasmid pBI-P5CS. This plasmid also contains the genes *nptII* (resistance to kanamycin) and *uidA* (β -glucuronidase). Fifteen transgenic lettuce strains were obtained. Spectrophotometric methods were used to quantify proline. Some strains were more tolerant to stress than the control, probably due to a lower osmotic potential, which helps the plant to maintain and acquire water. Proline accumulation could be important for recovery from stress. Evaluations by PCR, using specific primers, indicated that the *P5CS* transgene was present in transformed lettuce strains. There was greater proline accumulation in these strains, when compared to non-transformed plants.