

## GENETIC TRANSFORMATION OF CREEPING BENTGRASS WITH *IPT* GENE CONTROLLING CYTOKININ SYNTHESIS

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### ABSTRACT

Leaf yellowing and reduced plant density are major problems associated with heat stress injury in cool-season turfgrasses. Our previous research indicates that high soil temperatures reduce the production of cytokinins, the hormone that controls leaf senescence, tillering and root formation, which are the principal physiological processes underlying this problem. Genetic modification, through transformation with genes that may delay or suppress leaf senescence, offers a promising approach to both identifying key physiological factors controlling leaf senescence and underlying heat stress tolerance in cool-season turfgrasses. The objectives of this study were to develop creeping bentgrass (*Agrostis stolonifera*) with increased cytokinin synthesis and to determine whether transformation of plants with *ipt* gene would help delay or suppress leaf senescence and improve heat tolerance of creeping bentgrass.

We created two types of transgenic bentgrass with a bacterial gene (*ipt*) encoding the enzyme adenine isopentenyl phosphotransferase. The *ipt* gene was ligated to two stress-activated promoters. The first plasmid contains the *SAG12* promoter from *Arabidopsis* that is activated at the start of leaf senescence (*Psag12-ipt*). The second plasmid contains the *ipt* gene with the *Arabidopsis HSP18* heat shock promoter (*Phsp18-ipt*). This promoter is activated by exposing the plants to temperatures >35 C. Each of the plasmids contains the hygromycin resistance gene (*hyg*) for transgenic plant tissue selection, and the GUS reporter gene (*uid*) for easy identification of transgenic plants. Creeping bentgrass callus derived from stolons was transformed with each of the plasmids using the *Agrobacterium tumefaciens* (agro) infection technique.

A total of 142 senescence-activated plants and 66 heat-shock plants were confirmed as transgenic using PCR. Over 40 *Psag12-ipt* and *Phsp18-ipt* clones have been confirmed positive with Northern blotting. *Psag12-ipt* plants were screened for chlorophyll content and maintenance of green color after the activation of senescence using an excised leaf bioassay. Leaves from some *Psag12-ipt* plants remained green longer in the dark and had higher chlorophyll content than controls one and three weeks after excision and after 19 d of growth in the dark (growth chamber without light). Some *Psag12-ipt* plants had increased number of tillers and roots, and higher root dry weight than non-transgenic plants. Plants containing the *Phsp18-ipt* construct were also tested in an excised leaf bioassay after treatment at 40°C for one day. Leaves from transgenic plants maintained green color and turgidity one week after treatment. Some transgenic plants from both groups had higher cytokinin in the leaves and the roots after stress treatments, suggesting a role for this hormone in delaying leaf senescence and improving heat stress tolerance in mature turfgrass leaves.

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