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Influence of Genotype, Explant, and Hormone Treatment on Agrobacterium-Transformation Success in Salix Callus Culture

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Abstract : Shrub willows (Salix spp.) have many characteristics which make them suitable for a variety of applications such as riparian zone buffers, environmental contaminant sequestration, living snow fences, and biofuel production. In some cases, these functions are limited due to physical or financial obstacles associated with the number of individuals needed to reasonably satisfy that purpose. One way to increase the efficiency of willows is to bioengineer them with the genetic improvements suitable for the desired use. To accomplish this goal, an optimized in vitro transformation protocol via Agrobacterium tumefaciens is necessary to reliably express genes of interest. Therefore, the aim of this study is to observe the influence of tissue culture with different willow cultivars, hormones, and explants on the percentage of calli expressing reporter gene green florescent protein (GFP) to find ideal transformation conditions. Each callus was produced from 1 month old open-pollinated seedlings of three Salix miyabeana cultivars (‘SX61’, ‘WT1’, and ‘WT2’) from three different explants (lamina, petiole, and internodes). Explants were cultured for 1 month on an MS media with different concentrations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) (No hormones, 1 $mg^{-1}L$ BAP only, 3 $mg^{-1}L$ NAA only, 1 $mg^{-1}L$ BAP and 3 $mg^{-1}L$ NAA, and 3 $mg^{-1}L$ BAP and 1 $mg^{-1}L$ NAA) to produce a callus. Samples were then treated with Agrobacterium tumefaciens at an OD600 of 0.6-0.8 to insert the transgene GFP for 30 minutes, co-cultivated for 72 hours, and selected on the same media type they were cultured on with added 7.5 $mg^{-1}L$ of Hygromycin for 1 week before GFP visualization under a UV dissecting scope. Percentage of GFP expressing calli as well as the average number of fluorescing GFP units per callus were recorded and results were evaluated through an ANOVA test ($\alpha = 0.05$). The WT1 internode-derived calli on media with 3 mg-1L NAA+1 $mg^{-1}L$ BAP and $mg^{-1}L$ BAP alone produced a significantly higher percentage of GFP expressing calli than each other group (19.1% and 19.4%, respectively). Additionally, The WT1 internode group cultured with 3 $mg^{-1}L$ NAA+1 $mg^{-1}L$ BAP produced an average of 2.89 GFP units per callus while the group cultivated with 1 $mg^{-1}L$ BAP produced an average of 0.84 GFP units per callus. In conclusion, genotype, explant choice, and hormones all play a significant role in increasing successful transformation in willows. Future studies to produce whole callus GFP expression and subsequent plantlet regeneration are necessary for a complete willow transformation protocol.

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