Agrobacterium as a Vector for the Genetic Transformation of Trees in the Family Casuarinaceae

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Abstract

The combination of the proper tissue culture protocol with a suitable genetic transformation method is essential for tree species and recovery of transgenic trees and introduction of genes involved in important silvicultural traits. Organogenesis is the method of choice in species such as *Casuarina glauca* and *Allocasuarina verticillata*. It consists of plant regeneration through bud formation on excised epicotyl fragments grown on the proper tissue culture medium.

Whereas both microprojectile bombardment and *Agrobacterium* were first tested in Casuarinaceae, *Agrobacterium tumefaciens*-vector system is currently employed for genetic transformation of epicotyl fragments excised from 45-day-old plantlets, and regeneration of stable transformed plants. The best agrobacterial strain is C58C1(pGV2260) carrying derivatives of the binary vector BIN19. Selection of transformed cells is achieved on kanamycin (50 to 100 mg L-1 depending upon the tree species). Large scale propagation of the transgenic plants can then be obtained by using cuttings of transformed shoots.

Response of *A. rhizogenes* was also exploited to develop a rapid method to generate so-called composite plants in *C. glauca*, in which transformed root system is grown on a non-transgenic aerial shoot. Generally nine months are necessary to obtain a rooted

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transformed *C. glauca* plant with *A. tumefaciens*, whereas the expression of chimeric genes in composite plants can be studied in less than four months.

To ensure a commercial use of transgenic Casuarinaceae trees, the evaluation of potential risks resulting from their release into the environment need to be studied.