CLONE INFLUENCE ON AGROBACTERIUM-MEDIATED DNA TRANSFER TO PINUS RADIATA

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Research in the last five years has shown that the host range of *Agrobacterium tumefaciens* extends to many species in the genus *Pinus (1).* This observation provides the basis for developing DNA transfer methods in pine species using *Agrobacterium* as the gene vector. Successful DNA transfer depends on a specific host-pathogen interaction involving genes of both the *Agrobacterium* strain (1) and the host plant (2). In crop plants where sufficient breeding has occurred to produce inbred lines or cultivars, the genetics of the host-pathogen relationship can be explored using cultivar seedlings. In pines, cultivars or inbred lines do not exist. Therefore, we have examined the influence of host genotype on *Agrobacterium-mediated* DNA transfer in *Pinus radiata* using clones produced by tissue culture.

In this study, we compared the frequencies of gall formation in clonal *P. radiata* shoots produced by the method of Aitken-Christie (3). Shoots of identical clonal lines or a clonal mix were either inoculated *in vitro* or after establishment in the greenhouse. Seedlings of the same seed lot used to produce the tissue culture clonal plantlets were grown and inoculated at the same time as the clonal plants. *Agrobacterium* virulent strains C2/74, wild type 542, wild type 542 carrying pEND4K (confering kanamycin resistance), and the avirulent strain A136 were used for inoculation. Inoculation consisted of stabbing the shoot or stem several times close to the apex with a scalpel blade dipped in freshly grown *Agrobacterium* cultures. Wound-only controls were maintained to compare the hypertrophy around the wound area with and without the presence of bacteria. As verification of foreign gene transfer, *in vitro* galls (inoculated with 542/pEND4K) were placed onto medium containing kanamycin (resistance from the npt-II gene), and galls produced *ex vitro* were analysed for agropine/mannopine synthesis.

Frequency of gall formation differed significantly among clones *in vitro* and ranged from 0 and 39%. Strains 542 and C2/74 did not differ in their ability to induce galls in clonal plantlets inoculated *in vitro.* Although clones varied significantly in the number of shoots produced per original embryo and shoot rootability, no correlation was found between these parameters and the frequency of gall formation *in vitro.* Frequency of gall formation differed significantly among clones inoculated under greenhouse conditions and ranged from 26 to 41%. Gall formation frequency on greenhouse clonal trees was positively correlated with growth rate. Across all clones, gall formation was lower in clonal shoots inoculated *in vitro* (24%) compared to trees inoculated in the greenhouse (38%). Rankings of clones by the frequency of gall formation were different when comparing *in vitro* inoculation to greenhouse inoculation. No difference in the frequency of gall formation was observed between seedlings and a 21-clone mixed population of trees produced through tissue culture.

The major finding of this work is that clonal genotype does significantly influence transfer of foreign DNA via direct stem inoculation with *Agrobacterium,* but differing cultural conditions *(in vitro* versus greenhouse) and tree growth rates are of at least equal importance.

[1] Stomp, A-M *et al.* 1990. P1 Physiol 92:1226-1232.
[2] Hinchee, MAW *et al.* 1988. Bio/technology 6:915-922.
[3] Aitken-Christie, J *et al.* 1988. In: Genetic Manipulation of Woody Plants. Plenum Press, New York: 413-432.