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Within-population variation in susceptibility to *Agrobacterium tumefaciens* A281 in *Picea abies* (L.) Karst

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Summary. The purpose of this study was to investigate the genetic variation of susceptibility to *Agrobacterium* within a *Picea abies* population. Tumor formation was studied in 16 open-pollinated families belonging to a central Swedish population of *Picea abies*. Strain A281 of *Agrobacterium tumefaciens* was used to infect 3-month-old seedlings in a five-block greenhouse experiment. The analysis of variance showed strong significance for the between-family variation of tumor-formation percentages, varying from 28% to 73%. The most susceptible material will be suitable for experiments on the production of transgenic plants in vitro using disarmed *Agrobacterium* strains.

Key words: *Picea abies* – *Agrobacterium tumefaciens* – Tumors – In vivo – Within-population variation

Introduction

Several gymnosperms can be transformed by *Agrobacterium tumefaciens* (reviews: DeCleene and DeLey 1976; Ahuja 1988; Stomp et al. 1988; for more recent references see Morris et al. 1989; Hood et al. 1990). The percentage of susceptible seedlings varies from 0% to 100%, depending on bacterial strain and host species. We are not aware of any studies of the genetic variation within conifer populations of *A. tumefaciens* susceptibility.

Within-species genetic variation in susceptibility is of interest for several reasons. For the production of transgenic plants using an agrobacterial system, it may be essential to choose especially susceptible genotypes. For investigations aimed at optimizing such a system, it can be valuable to have contrasting seed lots of more and less susceptible plants, and to have a base for comparison of various agrobacterial strains. In addition, the study of variation in susceptibility within a population can help us to understand general disease resistance mechanisms, even if crown galls are not reported in conifer plantations.

In a previous study (Hood et al. 1990), we have shown that *Picea abies* tumors induced by *A. tumefaciens* A281 can be cultured without plant hormones, that they form the characteristic compounds agropine and mannopine, and that the tumors contain T-DNA sequences from the bacterial tumor-inducing plasmid, all in contrast to untransformed tissue. This indicates that the tumors are not just a wound response.

The purpose of this investigation was to study the genetic variation in tumor formation following infection with *A. tumefaciens* strain A281 in one population of *Picea abies*.

Materials and methods

Plant material, experimental design, and infection procedure

Sixteen families, open-pollinated from one population at latitude 59° 59′ in eastern Sweden, were grown in boxes in a greenhouse with continuous light to maintain vigorous growth. We used five blocks, with the families in randomized rows within each block, 16 seedlings to a row. However, owing to reduced germinability and seedling mortality, there were fewer than 16 plants in the row plots. The number of remaining plants for family i in block j is denoted by n_{ij} and the frequencies of occurring values of n_{ij} are presented in Table 1. Three-month-old seedlings were infected at the cotyledonary node and at a site on the stem with a sterile 0.9 mm injection needle that had been dipped in a 48 h agar plate culture of *A. tumefaciens* strain A281. Infectors were assigned one or two blocks each to control for variation in technique. Seedlings were examined for tumor formation after 2 and 3 months. A seedling was scored as positive if one of the infected sites developed a tumor. There was little change from 2 to 3 months, and we present results for tumor formation after 3 months.

Table 1. Occurring values of n_{ij}, $i=1,\ldots,16$, $j=1,\ldots,5$

n_{ij}	9	10	11	12	13	14	15	16
Frequency	1	0	3	5	7	7	17	40

Table 2. Analysis of variance according to model (1)

Effect	Sum of squares	Degrees of freedom	Observed level of significance
Families (α_i)	81.17	15	0.000
Blocks (β_j)	10.27	4	0.036
Interaction ($(\alpha\beta)_{ij}$)	75.63	60	0.084

Table 3. Ranking of families with respect to estimated infection percentages ($\hat{p}_i \times 100$). Range of ranks at simultaneous confidence level 0.95

Rank	Family	$\hat{\mu}+\hat{\alpha}_i$	Percent	Range of ranks
1	451	2.052	73.1	1– 8
2	413	1.941	68.1	1–11
3	432	1.777	60.2	1–14
4	459	1.720	57.4	1–15
5	416	1.705	56.7	1–15
6	450	1.670	54.9	1–16
7	425	1.661	54.5	1–16
8	455	1.489	45.9	1–16
9	424	1.467	44.8	2–16
10	442	1.421	42.5	2–16
11	410	1.412	42.1	2–16
12	446	1.363	39.7	3–16
13	456	1.334	38.3	3–16
14	401	1.319	37.5	3–16
15	449	1.174	30.7	4–16
16	443	1.123	28.3	6–16

Statistical model for the ANOVA

The number of infected plants from family i in block j is denoted by $\varkappa_{ij}$. This random variable is assumed to be binomially distributed with the parameters n_{ij} and p_{ij}, where the latter denotes the probability of infection for a single plant.

In order to remove the influence of p_{ij} on the variance of the relative frequencies $\varkappa_{ij}/n_{ij}$, the transformation

$$y_{ij} = \arcsin\left(\frac{\varkappa_{ij}}{n_{ij}+1}\right)^{1/2} + \arcsin\left(\frac{\varkappa_{ij}+1}{n_{ij}+1}\right)^{1/2}$$

is applied (Freeman and Tukey 1950). It can be shown that y_{ij} is approximately normally distributed with the mean $\arcsin p_{ij}^{1/2}$ and the variance $(n_{ij}+1/2)^{-1}$. The family and block effects on the tranformed infection frequencies are expressed by a two-way analysis of variance model, i.e.,

$$y_{ij} = \mu + \alpha_i + \beta_i + (\alpha\beta)_{ij} + e_{ij}, \quad (1)$$

$$\Sigma_i \alpha_i = \Sigma_j \beta_j = \Sigma_i (\alpha\beta)_{ij} = \Sigma_j (\alpha\beta)_{ij} = 0$$

where μ is the overall mean, α_i, β_j, $(\alpha\beta)_{ij}$ are the effects of family i, block j and their interaction and, finally, the e_{ij} are the independent and approximately normally distributed errors with the mean zero and the variance $(n_{ij}+1/2)^{-1}$. Weighting y_{ij} by $n_{ij}+1/2$, the sums of squares for the various effects are calculated by PROC GLM of the SAS (1985) statistical package. Since the variance of e_{ij} is known in this case, any F-ratios need not be calculated. Instead, it can be shown that the sums of squares are χ^2-distributed. If a factor has no effect on the variation, then the corresponding χ^2 distribution is central; a fact that can be used for testing the hypotheses of no family, block, and interaction effects.

If there are no interaction effects, i.e., all $(\alpha\beta)_{ij}=0$ in model (1), then it is reasonable to rank the families in terms of the values of α_i. In order to guarantee the ranking at simultaneous confidence level 0.95, the confidence interval for each of the $\binom{16}{2}=120$ pair-wise differences $\alpha_i-\alpha_k$ is calculated at level $1-0.05/120=0.99958$. The average probability of infection for family i is estimated by the retransformation $\hat{p}_i=\sin^2(\hat{\mu}+\hat{\alpha}_i)$, where $\hat{\mu}+\hat{\alpha}_i$ is obtained by model (1).

Results

The results from the analysis of variance for model (1) is summarized in Table 2 and indicates that model (1) can be simplified by removing the interaction effects. The result of ranking the families under the model without such effects is given in Table 3. As seen from Tables 2 and 3, there was a large and significant variation between the families.

Discussion

The large variation in tumor-formation percentage between the open-pollinated families was somewhat unexpected. For characters of adaptive value, a large genetic variation within populations is expected (Stern and Roche 1974). The resistance to *Agrobacterium* infection can hardly be of adaptive significance in *Picea abies*, since no infections in natural populations have been reported as far as we know. However, if the resistance to *Agrobacterium* in our material reflects a general mechanism of resistance against bacterial or fungal infections, one would expect a large genetic variation in *Agrobacterium* infection percentage. Variation as well as absence of variation between genetic entries have been reported for other species (e.g., Ooms et al. 1985; Byrne et al. 1987; De Block 1988; Mackay et al. 1988; Vahala et al. 1989; Hobbs et al. 1989; Puonti-Kaerlas et al. 1989). However, the main purpose of these studies was not to disclose any genetic variation within and between populations, but to obtain general information as to the possibility of inducing tumors by *Agrobacterium* infection.

The large between-family variation suggests that the reported species differences with *Agrobacterium* infections in conifers should be treated with care. The differ-

ences might be attributed to random selection of materials with varying sensivities within each species. On the other hand, the differences between species reported by Morris et al. (1989) following infection with several *Agrobacterium* strains can be hardly be attributed to random selection of research material, since some species were consistently more sensitive than others. The significance for block effects (Table 2) can partly be attributed to the difference in infection technique between the infectors. It must be stressed that the infections of all plants in our investigation could not be done by one person without risk of a possible bias owing to time variation in infection response. Great care was taken to use the same infection technique among the infectors. Our data clearly show a genetic variation in tumor induction in vivo in *Picea abies*.

To obtain transgenic plants, one has to use in vitro infection with disarmed strains of *Agrobacterium*. Since tissue culture is a laborious technique, it is advisable to use host material with known high susceptibility. Such an approach was used by Byrne et al. (1987) and Hinchee et al. (1988) to obtain transformed plants in *Glycine max*. Supervirulent strains of *A. tumefaciens* are continually being constructed mainly for gene tranfer in angiosperms (Jin et al. 1987). We are currently interested in testing such strains on *Picea abies*. In this context it is useful to have a genetically defined material with known reaction to strain A281 as a basis for developing a transformation system.

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