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**Denis Tagu · Patricia Faivre Rampant**
**Frédéric Lapeyrie · Pascale Frey-Klett · Patrice Vion**
**Marc Villar**

# Variation in the ability to form ectomycorrhizas in the F1 progeny of an interspecific poplar (*Populus* spp.) cross

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**Abstract** The aim of this study was to determine the existence of a genetic basis for the ability to form ectomycorrhiza on a model angiosperm tree (*Populus*, poplar). Parental clones and 18 progeny from a controlled interspecific cross between *Populus deltoides* and *Populus trichocarpa* were grown in a glasshouse and inoculated with mycelium of the ectomycorrhizal fungus *Laccaria bicolor*. Three months after inoculation, the percentage of mycorrhizal root tips was determined for each inoculated plant. The data indicate variability in the ability to form ectomycorrhizas among the F1 progeny, including individual progeny which are different to either parent. This suggests a genetic basis for mycorrhiza formation.

**Keywords** Ectomycorrhiza · Fungus · Genetics · Heritability · Poplar

## Introduction

Ectomycorrhiza is a widespread type of symbiosis involving forest trees growing under boreal and temperate climates. The formation of this symbiosis is dependent on environmental factors (Smith and Read 1997), including both trophic and climatic factors. For example, nitrogen deposition in a soil can greatly influence mycorrhiza formation (Wallenda and Kottke 1998) and the presence of helper bacteria improves the colonization of tree roots (Garbaye 1994). However, there is increasing evidence that ectomycorrhiza formation has a genetic basis. Indications of specificity in the interaction come from *Lactarius deliciosus*, *Lactarius deterrimus* and *Lactarius salmonicolor*, which are specific to *Pinus sylvestris*, *Picea abies* and *Abies alba*, respectively (Trappe 1977; Giollant et al. 1993). Furthermore, gene expression and protein patterns change during in vitro formation of ectomycorrhizal tissues, indicating that genetic programmes in both partners are switched on or off during the interaction (Hilbert et al. 1991; Voiblet et al. in press). The hypothesis that ectomycorrhiza formation is governed partly by genetic traits has arisen from comparison of the capacity to form mycorrhizas by different progeny from a cross. The host-plant genotype influenced the number of mycorrhizal root tips and plantlet growth in progeny of *Pinus elliotii* or *Pinus taeda* inoculated with *Pisolithus tinctorius* (Marx and Bryan 1971; Dixon et al. 1987). Rosado et al. (1994) obtained a high value for heritability (0.81) of the percentage of ectomycorrhizal colonization on *Pinus elliotii*. These authors suggested that the ability to form ectomycorrhizas should be a criterion in selection schemes for tree breeding.

The genetic basis of mycorrhiza formation probably involves the combined action of several genes rather than the expression of a single gene. The differing allelic forms of these groups of genes may explain the variability in forming ectomycorrhizas observed between individuals. Furthermore, the segregation of these alleles may generate new genotypes favourable for ectomycorrhiza development. The molecular identity and the heritability of these putative genes and their alleles are still unknown.

The aim of the present study was to characterize the variability to form ectomycorrhizas generated by an interspecific cross in *Populus* spp. (poplar), comparing the full-sib progeny to the parental genotypes. Poplar was chosen as a model because of the availability of genetic maps. This allows future analyses of quantitative trait loci (QTL) involved in ectomycorrhiza formation.

D. Tagu (✉) · F. Lapeyrie · P. Frey-Klett · P. Vion
INRA–Nancy, Microbiologie Forestière, 54280 Champenoux, France
e-mail: tagu@nancy.inra.fr
Tel.: +33-383-394013, Fax: +33-383-394069

P. Faivre Rampant
Université Henri Poincaré, Nancy I, Faculté des Sciences, Laboratoire de Biologie Forestière, BP 239, 54506 Vandoeuvre cedex, France

M. Villar
INRA, Unité Amélioration, Génétique et Physiologie des Arbres Forestiers, BP 20619, 45160 Ardon, France

## Materials and methods

### Material

The plant material was an interspecific family (54B) from a controlled cross between *Populus deltoides* (female clone from Illinois, no. 73028-62) and *Populus trichocarpa* (male clone from Washington, no. 101-74) (Lefèvre et al. 1998). These two parents showed the largest difference in genotype in a screen of several poplar genotypes for ability to form ectomycorrhizas with several fungal species (unpublished data). The two parents and 18 progeny were tested for ectomycorrhiza formation after inoculation with the basidiomycete *Laccaria bicolor* S 238 N (Di Battista et al. 1996). The 18 progeny were chosen randomly from a population of 91 progeny for which mapping data were available (Faivre Rampant et al. 1999). The *L. bicolor* S 238 N fungal strain, obtained from the INRA–Nancy collection of ectomycorrhizal fungi, was chosen for its ability to form ectomycorrhizas with poplar and because it has been well characterized at both the ecological and molecular levels (see Kropp and Mueller 1999). The strain was maintained on Pachlewski medium (Pachlewski and Pachlevska 1974). For inoculum production, the mycelium was grown aseptically in a peat-vermiculite nutrient mixture (Duponnois and Garbaye 1991) in glass jars for 2 months in the dark at 25°C and kept at 4°C until use.

### Inoculation

Micro-cuttings (containing a single internode) of each of 18 poplar progeny and the two parents were each rooted without fungal inoculum in 80 ml of non-disinfected peat-vermiculite mix (1:1 vol/vol; pH 5.5) for 1 month, in a glasshouse during spring (glasshouse temperature 15–28°C). Rooting of 25 micro-cuttings per clone prior to fungal inoculation allowed the selection after 1 month of the 20 best-rooted plantlets of each clone. In 1998, 400 young rooted plantlets (20 of each clone) were transferred individually to 1-l pots containing non-disinfected peat-vermiculite (1:1 vol/vol; pH 5.5) mixed with a one-tenth volume of fungal inoculum. Plantlets were watered for 2 months and then received nutrient solution weekly (Frey-Klett et al. 1997). In the first experiment (1998), plants were randomly distributed in two blocks, each consisting of 10 ramets of each clone. The experiment was repeated in 1999 with eight of the 18 progeny clones previously tested and the parental clones. Two hundred plants (20 per clone) were randomly distributed without any block design.

### Measurements

At the end of the experiment, the percentage of mycorrhizal root tips was determined. Each root system was rinsed with tap water, cut into 1-cm pieces and analysed under a dissecting microscope. For each root system, 100 apices were randomly examined and assessed as mycorrhizal or non-mycorrhizal. The percentages of mycorrhizal root tips were arcsine (square root) transformed prior to analysis. The dry weights of the shoot and of the remaining micro-cutting were determined for each plantlet. Clonal variation was tested by a two-factor analysis of variance (ANOVA) according to $yij=\mu+gi+bj+eij$, where $yij$ is the observation value of the $i$th clone in the $j$th block, $\mu$ is the overall mean, $gi$ is the genetic effect of the $i$th clone, $bj$ the block effect of the $j$th block, $eij$ is the error term. The block component was absent from the second experiment. The clonal means were then compared by Bonferroni test. All statistical analyses were done at the probability threshold of 0.05 with Statwiew program 4.02 (Abascus Concepts). The variances were used to estimate broad sense heritability. Micro-cutting and shoot dry weights were submitted to variance analysis. Correlation between these traits and the percentage of mycorrhizal root tips was examined using a regression procedure.

## Results and discussion

Three months after fungal inoculation, some poplar clones were less adapted to growth conditions and dead plantlets were rejected. All surviving plantlets were mycorrhizal with *L. bicolor*. In a few pots, young fruit bodies of *L. bicolor* were observed with morphological characteristics of the inoculated S 238 N strain (Di Battista et al. 1996). No fungal contaminants were observed on roots.

In the 1998 experiment, the *Populus trichocarpa* parent had a clearly higher percentage of mycorrhizal root tips than *Populus deltoides* (Fig. 1A). In a preliminary study in which several poplar species and genotypes were screened for their ability to interact with *L. bicolor* S 238 N, a clone of *P. trichocarpa* (clone no. 36-134 from Oregon) showed a lower percentage of mycorrhizal roots (17.0% ±4.7) than that of the *P. trichocarpa* clone used in this work (35.0% ±8.5) (unpublished data). This indicates that the ability to form mycorrhiza is probably a clone effect. When tested for other plant–microbe interactions (mainly pathogenic), *P. trichocarpa* always appeared to be more sensitive to fungal or insect aggression than *P. deltoides* (Faivre Rampant et al. 1999). Whether or not this general sensitivity corresponds to linked QTLs is unknown.

Percentage of mycorrhizal roots of the 18 progeny clones varied from 39% to 68%. All progeny values were statistically different from those of the *P. deltoides* parent (Fig. 1A). Only progeny clones 54B095 and 54B027 (labelled * in Fig. 1A) were different from both parents. Whereas clone 54B095 was intermediate between the two parents, clone 54B027 had a higher percentage of mycorrhizal roots than its parents. Thus the ability to form ectomycorrhiza could be improved in poplar breeding. The genetic component of the mycorrhizal ability as assessed by comparison of clones through variance analysis was significant (Table 1). The mean of the progeny (56%) was not significantly different from that of the *P. trichocarpa* parent (54.3%). The *P. deltoides* parent had a significantly lower percentage of mycorrhizal roots. Thus, we suggest that the trait is dominant and inherited from the *P. trichocarpa* parent (Table 1). The broad sense heritability value was 0.49, indicating moderate genetic control of the trait. The clonal effect for dry weights of micro-cuttings and of shoots was also significant (Table 1). No correlation was found between either of these dry weight parameters and the percentage of mycorrhizal roots ($R^2$=0.015). This indicates that plant growth did not interfere with ability to form ectomycorrhizas. Thus in the conditions used, mycorrhizal status is probably partly independent of tree growth. The inoculation time was short enough (2 months) to discriminate between the mycorrhizal ability of the progeny without any interference from effects of the fungus on tree growth.

To test the robustness of these data, the same experiment was repeated in 1999 for eight of the 18 clones in the same conditions (Fig. 1B). As in 1998, all progeny

**Table 1** Analysis of variance for percentage of mycorrhizal roots and dry weights of shoots and cuttings of the F1 progeny from a cross between *Populus deltoides* and *Populus trichocarpa* (*df* degrees of freedom, *dry wt.* dry weight, *F* frequency, *n.a.* not applicable, *% myc* percentage of mycorrhizal roots, *** significantly different at $P<0.05$)

| | | 1998 | | | | 1999 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *df* | Mean of squares | Σ of squares | *F* | *df* | Mean of squares | Σ of squares | *F* |
| % myc | Clone | 17 | 0.089645 | 1.5239 | 9.20*** | 7 | 0.182612 | 1.2782 | 12.19*** |
| | Block | 1 | 0.022197 | 0.0221 | 2.28 | n.a. | n.a. | n.a. | n.a. |
| | Error | 284 | 0.009734 | 2.7646 | n.a. | 167 | 0.014978 | 2.5013 | n.a. |
| | Total | 302 | 0.014274 | 4.3108 | n.a. | 174 | 0.021722 | 3.7796 | n.a. |
| Dry wt. cuttings | Clone | 17 | 0.579980 | 9.8596 | 5.34*** | 7 | 9.307179 | 65.1502 | 49.90*** |
| | Block | 1 | 0.857158 | 0.8571 | 7.89*** | n.a. | n.a. | n.a. | n.a. |
| | Error | 284 | 0.108590 | 30.8396 | n.a. | 167 | 0.188382 | 31.4598 | n.a. |
| | Total | 302 | 0.137604 | 41.5564 | n.a. | 174 | 0.555230 | 96.6101 | n.a. |
| Dry wt. shoots | Clone | 17 | 1.1421 | 19.4172 | 7.91*** | 7 | 0.379650 | 2.6575 | 6.14*** |
| | Block | 1 | 1.0535 | 1.0535 | 7.30*** | n.a. | n.a. | n.a. | n.a. |
| | Error | 284 | 0.1443 | 40.9875 | n.a. | 167 | 0.061805 | 10.3214 | n.a. |
| | Total | 302 | 0.2035 | 61.4584 | n.a. | 174 | 0.074592 | 12.9790 | n.a. |

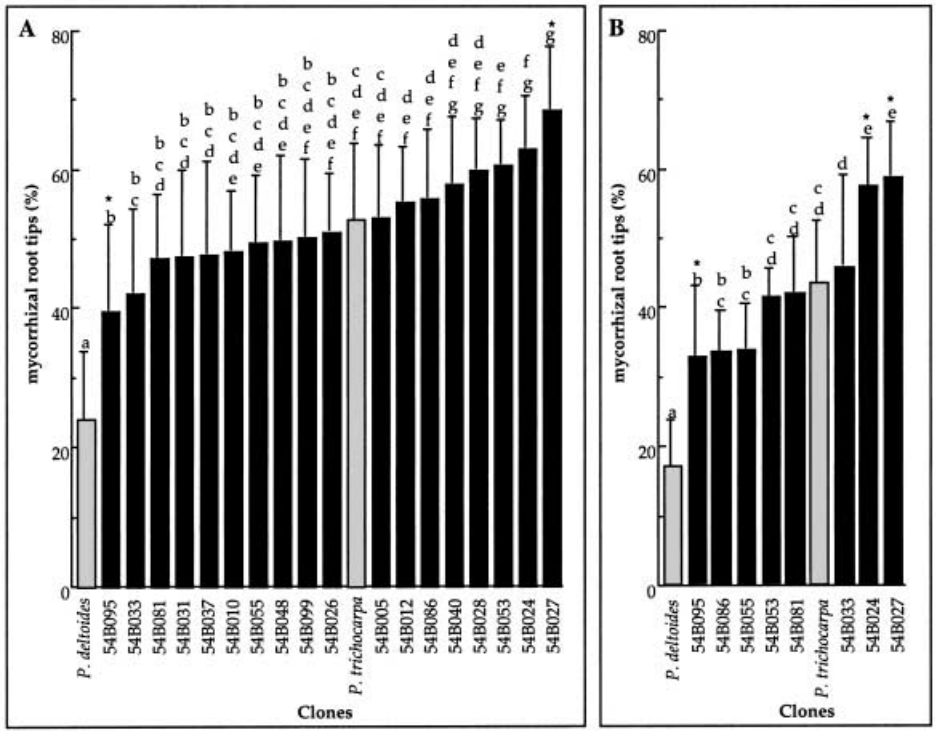


**Fig. 1A, B** Percentage of mycorrhizal root tips of clones of *Populus deltoides* and *Populus trichocarpa* (as parents) and their F1 progeny. The histograms compare the mean percentages of mycorrhizal tips for 9–20 replicates of each root system of each clone. *Bars* represent the standard error of the mean. *Letters* indicate statistically different values ($P<0.05$) (Bonferroni test) and * shows progeny clones statistically different from the parents. Data obtained in spring–summer 1998 are shown in **A** and in spring–summer 1999 in **B**

clones presented a higher percentage of mycorrhizal roots than the *P. deltoides* parent. Clone 54B095 was again intermediate between its parents and clone 54B027 had a higher proportion of mycorrhizal roots than the parents. Clone 54B024 was statistically different from the two parents in 1999 but not in 1998 (Fig. 1B). The value of the Spearman's rank correlation coefficient was 0.66 ($\alpha=0.05$), indicating that clone ranking was similar in both experiments. As in the 1998 experiment, no correlation was found between the shoot or micro-cutting dry weights and the percentage of mycorrhizal roots ($R^2=0.08$). Altogether, the two experiments indicate that there is a reproducible pattern in the percentage of mycorrhizal roots of the progeny clones in the conditions used for this study. This strongly suggests a genetic basis for mycorrhization in poplar in our conditions.

Similar observations have been made for other tree species, e.g. *Pinus elliotii* and *P. taeda* (Marx and Bryan 1971; Dixon et al. 1987; Rosado et al. 1994) but, to our knowledge, this is the first report involving an angiosperm tree. Currently, poplars are being genetically mapped (Bradshaw et al. 1994) and their genomes analysed (Sterky et al. 1998). Techniques for genetic transformation are available (Leplé et al. 1992). Hence, QTL analysis of mycorrhizal susceptibility could be used to localize parts of the poplar genome linked to the ability

to form ectomycorrhizas. Genetic maps of the two parents used in this study are being constructed (Faivre Rampant et al. 1999).

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