

# Genetic Analysis of Growth and Susceptibility to Bacterial Wilt (*Ralstonia solanacearum*) in *Eucalyptus* by Interspecific Factorial Crossing

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## Summary

Factorial mating crosses of *Eucalyptus urophylla* × *E. tereticornis* (3 × 5), *E. urophylla* × *E. camaldulensis* (3 × 3) and *E. urophylla* × *E. exserta* (3 × 3) were used for genetic analysis of growth traits and susceptibility to bacterial wilt (*Ralstonia solanacearum*). Genetic effects including female, male and female × male interaction were examined for height (H) and diameter at breast height (DBH) at age five as well as final bacterial wilt index (BWI) in both nursery inoculation and field assessment by five years of age. Female, male and female × male variances appeared to have a significant role in growth traits H and DBH though their magnitude varied for the factorials studied. For the trait BWI additive (male) and dominant (female × male) effects were both involved in the genetics of bacterial wilt susceptibility, and additive was the major. Estimates of narrow-sense heritability ( $h^2$ ) for H, DBH and BWI ranged from  $0.11 \pm 0.06$  to  $0.70 \pm 0.09$ , varying with either trait or factorial. Growth traits (H and DBH) had low and non-significant phenotypic and genetic correlations with BWI in all the three factorials, ranging from  $-0.10 \pm 0.08$  to  $0.17 \pm 0.14$  in coefficient of correlation. This indicates that it may be possible to select superior trees with both fast growth and high resistance to bacterial wilt in eucalypt hybrid populations in operational breeding programs.

**Key words:** *Eucalyptus*, growth, bacterial wilt (*Ralstonia solanacearum*), genetic parameters.

## Introduction

*Eucalyptus* species are important for commercial plantation forestry both in China and internationally. Genetic parameters are backbone for designing optimal breeding strategy in tree improvement programs. Up to date genetic parameters have been reported for a number of *Eucalyptus* species in such traits as growth, wood properties and insect resistance (BOUVET and VIGNERON, 1995, 1996; HARDNER and POTTS, 1995; HODGE *et al.*, 1996; ARAÚJO *et al.*, 1996; GREVAVES *et al.*, 1997; SORIA and BORRALHO, 1997; WEI and BORRALHO, 1998; OSORIO *et al.*, 2001; JORDAN *et al.*, 2002; JONES *et al.*, 2002; LOPEZ *et al.*, 2003). However, little information was available on the genetic structure of disease susceptibility or resistance in the genus. In addition, there were few reports comparing genetic parameters over different interspecific hybrid populations and/or within populations of interspecific hybrids in *Eucalyptus*. Most studies reported to date have used half- and/or full-sib families of pure species.

Bacterial wilt caused by *Ralstonia solanacearum* (synonym *Pseudomonas solanacearum* E. F. Smith) (YABUCHI *et al.*, 1995) is one of the most significant, widespread and lethal diseases of plants in tropical and subtropical countries (BUDDENHAGEN, 1964). The disease was not observed in eucalypt plantations until 1980s when infection cases were found in China (CHAO, 1982) and Brazil (SUDO *et al.*, 1983). There have been

thereafter a number of reports on its occurrence on eucalypts in Australia, Venezuela, South Africa and Uganda (COUNTINHO *et al.*, 2000; ROUX *et al.*, 2001). In China, yield losses of 20–60% have been observed in stands planted with susceptible clones. The infection of the bacterium is constraining further expansion of eucalypt plantation worldwide. Few measures have so far proved adequate control of the disease, and growing resistant varieties might provide a partial solution (SATHYANARAYANA and ANAND, 1993). In *Eucalyptus* screening for resistance to bacterial wilt has been conducted in a range of pure species (WU and LIANG, 1988a; DIANESE *et al.*, 1990), hybrids (GAN *et al.*, 1998) and clones (COUNTINHO *et al.*, 2000). To our knowledge, however, no studies have been carried out to estimate the genetic variation and genetic parameters of bacterial wilt susceptibility in *Eucalyptus*.

In this study we present genetic analysis of height (H), diameter at breast-height (DBH) and bacterial wilt index (BWI) in *Eucalyptus* using three factorial mating hybrid populations. The maternal species is *E. urophylla*, and the paternal species include *E. tereticornis*, *E. camaldulensis* and *E. exserta*. The objectives are to examine the genetic variation and estimate the genetic parameters in growth traits and bacterial wilt susceptibility in eucalypts.

## Materials and Methods

### Plant material and mating design

During 1990–1993 three maternal trees of *E. urophylla* were control pollinated with five paternal trees of *E. tereticornis* and three paternal trees of each of *E. camaldulensis* and *E. exserta* in a factorial mating design (Table 1). All the parents were raised from randomly selected seedlings in earlier provenance trials in mid-1980s. Unfortunately, a few of crosses were missed due to some reasons in hybridization and seedling rais-

Table 1. – Mating designs for the three interspecific factorials used in this study.

Factorial	Male parent	Female parent		
		<i>E. urophylla</i> 9001	<i>E. urophylla</i> 3138	<i>E. urophylla</i> 3134
I	<i>E. tereticornis</i> 6001	×	×	
	<i>E. tereticornis</i> 4301	×	×	
	<i>E. tereticornis</i> 1801	×	×	
	<i>E. tereticornis</i> 4403	×	×	×
	<i>E. tereticornis</i> 2001		×	×
II	<i>E. camaldulensis</i> 3802	×	×	×
	<i>E. camaldulensis</i> 0603	×	×	×
	<i>E. camaldulensis</i> 1503	×	×	
III	<i>E. exserta</i> 9101	×	×	×
	<i>E. exserta</i> 9201	×	×	×
	<i>E. exserta</i> 9001		×	×

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ing procedures, and the final number of crosses available for experiment was eleven, eight and eight for *E. urophylla* × *E. tereticornis* (Factorial I), *E. urophylla* × *E. camaldulensis* (Factorial II) and *E. urophylla* × *E. exserta* (Factorial III), respectively, with each parent representing at least two crosses (Table 1).

In May 1994 35 grains of seed of each cross and each maternal open-pollinated family were sown into heat-sterilized medium (20% vermiculite, 79% soil and 1% compound fertilizer) contained in a 10 × 14 × 4 cm plastic tray in greenhouse. After a month 25–30 seedlings per entry were transplanted into 10-cm-diameter polyethylene bags in nursery.

#### Nursery inoculation and symptom assessment

A strain of *R. solanacearum* was isolated from a diseased tree in an *E. urophylla* plantation in Xinhui County, Guangdong Province, China, and its virulence was tested both on the tetrazolium chloride (TZC) medium (KELMAN, 1954) and through an inoculation procedure (WU and LIANG, 1988b). A virulent colony was re-streaked on the same medium without TZC and then cultured for 48 hours at 30°C, and cells were then harvested by flooding the plates with sterilized distilled water. The concentration of the inoculum suspension was adjusted to  $1.5 \times 10^9$  cells/ml spectrophotometrically.

Before inoculation 12 seedlings per entry were arranged in a randomized complete block design with four seedlings per plot and three replicates. Seedlings with six to eight true leaf pairs were inoculated artificially in nursery in August 1994 with a modified top-inoculation procedure based on the method of WU and LIANG (1988b). Briefly, a small cotton ball was placed on each seedling around a cut made above the highest pair of true leaves, which were truncated with a clean blade, and then two drops of bacterium suspension were applied, following the wrapping of the cotton-covered top with tape. The environmental conditions were 70–80% in humidity, 28–32°C in day temperature and 24–28°C in night temperature.

Plant responses were assessed in 5, 10, 15, 20, 25, 30 and 35 days after inoculation. The symptom scores were recorded as: 0, no symptom; 1, no wilt symptom but top stained darkly; 2, part of leaves wilted; and 3, dead. The scores were converted to bacterial wilt indices (BWI) following the simplified equation based on WINSTEAD and KELMAN (1952):

$$D = X_i / 3 \quad (1)$$

where,  $D$  is the disease index;  $X_i$  the symptom score; and 3 the highest grade of symptom score.

#### Field trial and trait measurement

The field trial was planted at a site previously occupied by a plantation of an *E. urophylla* × *grandis* hybrid clone in Gaoyao County (112°34'E and 23°01'N), Guangdong Province, China in April 1995, where more than half of the cuttings died of evident bacterial wilt symptoms by age five years. The site was cleared in blocks to retain the slightly infested patches. The field experimental design was same as nursery arrangement, namely, four-seedling row plot and three replicates. Seedlings that died during the nursery phase due to bacterial wilt infection were treated as missing.

Measurements were made in the five consecutive years after planting, and the traits measured were height (H), diameter at breast height (DBH) and wilt symptoms. The symptom scores were assessed using the subjective visual scores as used to assess symptoms in the nursery. The highest wilt symptom was recorded as the final performance and converted to a similar BWI as nursery manipulation. All trees that died during the observation years were excluded from analyses of H and DBH.

#### Statistical analyses

The highest wilt symptom in both nursery and field investigations was recorded as the final performance and converted to BWI as described in the equation (1) above. For both growth traits (H and DBH) and BWI, the following linear model on individual basis was employed to estimate the genetic components of variance:

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + B_k + E_{ijk} \quad (2)$$

where,  $Y_{ijk}$  is the phenotypic value of the individual between the  $i$ th male parent and the  $j$ th female parent in the  $k$ th replicate;  $\mu$  an overall mean;  $M_i$  the genetic effect of the  $i$ th male parent;  $F_j$  the genetic effect of the  $j$ th female parent;  $MF_{ij}$  the interaction effect between the  $i$ th male and the  $j$ th female;  $B_k$  the effect of the  $k$ th block (fixed); and  $E_{ijk}$  the residual error. The analysis of variance (ANOVA) was performed using SAS Proc GLM SS1 and the variance components were estimated using SAS Proc VARCOMP method REML (SAS INSTITUTE, 1997). Standard errors of variance components were estimated according to BECKER (1984, p. 44–45).

Narrow-sense heritability based on all individual trees ( $h^2$ ) was estimated using the following formula:

$$h^2 = 2(\sigma_F^2 + \sigma_M^2) / (\sigma_F^2 + \sigma_M^2 + \sigma_{MF}^2 + \sigma_E^2) \quad (3)$$

assuming an inbreeding coefficient of zero between the parents. Standard errors of  $h^2$  (approximation) were calculated according to BECKER (1984, p. 82–87).

Phenotypic correlation coefficients ( $r_p$ ) and genetic correlation coefficients ( $r_g$ ) between growth traits and BWI were estimated on plot mean basis by MINQUE (0/1) method (Zhu, 1992). Both phenotypic and genetic correlation analyses were conducted through computerized software QGA Station 1.0 (<http://www.cab.zju.edu.cn/english/ics/faculty/zhujun.htm>).

#### Results

Growth of the field trial was moderate at age five years as no tending and fertilization were applied after planting, totally averaging  $12.16 \pm 2.71$  m in H and  $9.19 \pm 2.10$  cm in DBH. Mean performance varied slightly among factorials (Table 2). Factorial I demonstrated the best performance in survival and average growth traits (H and DBH) while Factorial III had the lowest BWI. Standard errors were relatively higher in BWI than growth traits for all the three factorials, suggesting a higher coefficient of variation in the disease susceptibility.

Variance components due to female, male and female × male effects varied for the traits and factorials studied (Table 3). For both growth traits H and DBH, female variance was statistically significant at 0.1, 0.05 or 0.01 level in factorials I and II while male and female × male variances were both significant at 0.1, 0.05 or 0.01 level in factorials II and III. Thus, it appeared that female, male and female × male effects had a significant role in H and DBH. Also, the percentage explained by female, male or female × male of the total variance varied with either trait or factorial. For example, female × male vari-

Table 2. – Average performance of the field trial for different interspecific factorials by age five.

Mating design	Average			
	Survival	H (m)	DBH(cm)	BWI
Factorial I ( <i>E. urophylla</i> × <i>E. tereticornis</i> )	68.2%	12.98±2.18	9.60±2.09	0.1437±0.1728
Factorial II ( <i>E. urophylla</i> × <i>E. camaldulensis</i> )	64.6%	12.17±3.87	8.96±2.52	0.1561±0.1456
Factorial III ( <i>E. urophylla</i> × <i>E. exserta</i> )	66.7%	11.57±2.16	8.55±1.65	0.1284±0.1193

Survival was calculated by taking into account of trees that died of both bacterial wilt and other reasons.

Table 3. – Analysis of variance of the hybrid traits.

Source	d.f.	Variance component for each trait (%)		
		H	DBH	BWI
Factorial I ( <i>E. urophylla</i> × <i>E. tereticornis</i> )				
Female	2	0.8829±0.3321 (12.3)*	0.9596±0.2869 (14.9)**	0.0±0.0 (0.0) <sup>ns</sup>
Male	4	0.3368±0.3066 (4.7) <sup>ns</sup>	0.2124±0.1320 (3.3) <sup>ns</sup>	0.0111±0.0020 (19.0)***
Female×Male	8	0.0±0.0 (0.0) <sup>ns</sup>	0.4351±0.3832 (6.8) <sup>ns</sup>	0.0126±0.0092 (21.6)***
Residual	99	5.9764±0.5526 (83.0)***	4.8234±0.5022 (75.0)***	0.0347±0.0026 (59.4)***
Factorial II ( <i>E. urophylla</i> × <i>E. camaldulensis</i> )				
Female	2	1.6419±0.2021 (9.8) *	1.2785±0.2120 (14.0)***	0.0±0.0 (0.0) <sup>ns</sup>
Male	2	1.7348±0.1124 (10.4) *	1.9121±0.1354 (20.9)***	0.0029±0.0032 (5.6)*
Female×Male	4	2.3029±1.2343 (13.8) *	1.0124±0.8876 (11.1)**	0.0±0.0 (0.0) <sup>ns</sup>
Residual	72	10.9997±0.9934 (66.0)***	4.9469±0.3284 (54.0)***	0.0490±0.0054 (94.4)***
Factorial III ( <i>E. urophylla</i> × <i>E. exserta</i> )				
Female	2	0.2356±0.2544 (2.3) <sup>ns</sup>	0.4632±0.6023 (5.7) <sup>ns</sup>	0.0±0.0 (0.0) <sup>ns</sup>
Male	2	0.4814±0.2069 (4.8)*	1.1883±1.1122 (14.6)*	0.0052±0.0078 (12.4)**
Female×Male	4	1.5586±1.4478 (15.4)**	1.6878±2.0461 (20.8)**	0.0±0.0 (0.0) <sup>ns</sup>
Residual	63	7.8208±0.8266 (77.5)***	4.7895±0.6876 (58.9)***	0.0366±0.0048 (87.6)***

\*\*\*, \*\* and \*: significant at 0.01, 0.05, and 0.1 levels, respectively; ns: non-significant at 0.1 level.

ance made up 20.8% in DBH of the total variance in Factorial III, whilst the ratios were 0.0% in H and 6.8% in DBH in Factorial I.

For the trait BWI, female variance was consistently zero, male variance was, on the contrary, significant at 0.1, 0.05 or 0.01 level over the three factorials, and female × male interaction variance was significant only at 0.01 level in factorial I, which suggested both additive (male) and dominant (female × male) effects were involved in the genetics of bacterial wilt susceptibility in eucalypts, and additive was the major.

The narrow-sense heritability ( $h^2$ ) estimates for the growth traits on individual basis ranged between  $0.14 \pm 0.12$  and  $0.70 \pm 0.09$  over the three factorials (Table 4), suggesting that growth of the hybrids was under a low to high level of additive genetic control in all the factorials. The estimates of  $h^2$  varied strongly with either trait or factorial. For instance, the heritability was found low for H in Factorial III ( $0.14 \pm 0.12$ ) while moderately high in factorials I and II ( $0.34 \pm 0.18$  and  $0.40 \pm 0.08$ , respectively), and the trait DBH generally had the highest value of  $h^2$  across all the factorials ( $0.36 \pm 0.12$ – $0.70 \pm 0.09$ ) (Table 4).

The  $h^2$  estimates for BWI varied also with factorial (Table 4). Bacterial wilt susceptibility appeared to be a moderately inheritable trait in all three factorials ( $h^2$  ranging from  $0.11 \pm 0.06$  to  $0.38 \pm 0.17$ ).

Growth traits had low and non-significant correlations with BWI in both phenotypic and genetic terms for all three factorials ( $-0.10 \pm 0.08$ – $0.17 \pm 0.14$ ), negative in factorials I and II, but positive in Factorial III (Table 5). Meanwhile, both phenotypic and genetic correlation coefficients between H and DBH were at 0.01 or 0.05 significance level (data not shown).

Table 4. – Individual-based narrow-sense heritability ( $h^2$ ) for each trait over the three factorials.

Mating design	$h^2$		
	H	DBH	BWI
Factorial I ( <i>E. urophylla</i> × <i>E. tereticornis</i> )	0.34±0.18	0.36±0.12	0.38±0.17
Factorial II ( <i>E. urophylla</i> × <i>E. camaldulensis</i> )	0.40±0.08	0.70±0.09	0.11±0.06
Factorial III ( <i>E. urophylla</i> × <i>E. exserta</i> )	0.14±0.12	0.41±0.11	0.25±0.10

Table 5. – Phenotypic and genetic correlations between growth traits (H and DBH) and BWI.

Mating design	$r_p$ with BWI		$r_g$ with BWI	
	H	DBH	H	DBH
Factorial I ( <i>E. urophylla</i> × <i>E. tereticornis</i> )	-0.03±0.07 <sup>ns</sup>	-0.01±0.08 <sup>ns</sup>	-0.05±0.03 <sup>ns</sup>	-0.01±0.05 <sup>ns</sup>
Factorial II ( <i>E. urophylla</i> × <i>E. camaldulensis</i> )	-0.09±0.14 <sup>ns</sup>	-0.05±0.13 <sup>ns</sup>	-0.10±0.08 <sup>ns</sup>	-0.09±0.08 <sup>ns</sup>
Factorial III ( <i>E. urophylla</i> × <i>E. exserta</i> )	0.15±0.13 <sup>ns</sup>	0.17±0.14 <sup>ns</sup>	0.11±0.09 <sup>ns</sup>	0.13±0.06 <sup>ns</sup>

ns: non-significant at 0.1 level.

## Discussion

Both bacterial wilt resistance and growth are traits of major economic significance for tropical and subtropical eucalypt plantations worldwide. In this study we have examined the genetic variation and evaluated the genetic parameters for these traits in *Eucalyptus* hybrids. Even though the results presented suffer to some extent from the limited number of parents employed in each of the interspecific factorials, they have nonetheless important implications for operational breeding of eucalypts.

Variance components due to female, male and female × male effects differ markedly between traits and factorials. Such variation is common in a number of previous reports, e.g. in eucalypts (BOUVET and VIGNERON, 1996) and pines (DIETERS *et al.*, 1997), and may be caused by the limited number of either male or female parents (PAUL *et al.*, 1997; KUSNANDAR *et al.*, 1998; ISIK *et al.*, 2003) or progeny analysed for each mating design (GRIFFIN and COTTERILL, 1988). In this experiment only three or five parents per sex, along with 12 sibs per cross, were tested for each factorial mating, and thus a larger number of parents would be preferable for each factorial in order to obtain more reliable genetic parameter estimates.

For each hybrid population in this study, the residual variances obtained in the analysis of variance were fairly large. These large residual variances could be due to substantial levels of non-additive genetic variance, environmental variation, error or combination of these factors. If a significant proportion of the residual variance was in fact due to non-additive genetic variance, there would be the possibility of exploiting this type of genetic effects via mass vegetative propagation of clones selected for superior growth and disease resistance (YU *et al.*, 2003).

The mode of inheritance of bacterial wilt resistance, or susceptibility, is still controversial in most plant species. The presence of a single dominant gene controlling resistance to bacterial wilt has been reported in tomato (TIKOO *et al.*, 1983; MONMA and SAKATA, 1993), eggplant (CHAUDHARY and SHARMA, 1999) and *Arabidopsis thaliana* (HO and YANG, 1999). However, YUAN *et al.* (1999) reported that the inheritance of resistance to bac-



terial wilt (pathotype II) in sweet potato was controlled by multiple genes. The results reported here indicate that susceptibility to bacterial wilt, at least in the eucalypt hybrids examined, is related with both additive (male) and dominant (female  $\times$  male) genetic effects. This supports an "additive-dominant" model as proposed by FENG *et al.* (2003). Thus, both additive and non-additive gene actions appear to be important for bacterial wilt resistance in eucalypts, as has been reported for tomato by ANAND *et al.* (1993). Nonetheless, it is still appropriate to select parents with high general combining ability in terms of bacterial wilt resistance.

Though the individual-based narrow-sense heritability estimates obtained in this study for growth traits differed among the factorials, they were within the range of those reported previously in other eucalypt crosses except for one case where heritability of  $0.70 \pm 0.09$  was estimated in DBH in Factorial II (see ELDRIDGE *et al.*, 1993). For instance, HODGE *et al.* (1996) reported heritabilities of 0.04 to 0.23 for two-year-old volume over different intraspecific crosses between *E. globulus* and *E. nitens*, and BOUNET and VIGNERON (1995) reported heritabilities of 0.25 to 0.45 for 39-month-old growth traits (H, DBH and V) for *E. urophylla*  $\times$  *E. pellita* crosses. The heritability obtained for DBH in Factorial II ( $0.70 \pm 0.09$ ) in our study was markedly higher than those published from other eucalypt work, which might be attributed to small family  $\times$  replication interaction and within-family variances in comparison with the family variances, regarding the small size of progeny studied.

It is common in forest trees for  $h^2$  estimates of the same trait to vary among different populations and/or trial sites, especially when the number of parents tested is relatively small. For instance, BOUVET and VIGNERON (1995) found that heritability and its trend with age varied with mating design even in the same hybrid population of *E. urophylla*  $\times$  *E. pellita*, and HODGE *et al.* (1996) found that heritability differed with site in the same control-pollinated population in *E. globulus* and *E. nitens*. Similarly, DIETERS *et al.* (1997) found heritability estimates were substantially different between *Pinus caribaea*  $\times$  *P. oocarpa* and *P. caribaea*  $\times$  *P. tecunumanii* populations and between trial sites.

In current study growth traits show weak correlations with BWI in both phenotypic and genetic terms. The results obtained were generally in agreement with experiments conducted previously in other crops. For example, MONMA and SAKATA (1992) found no correlation between bacterial wilt resistance and fruit weight in tomato. This indicates that it may be possible to select superior trees with both fast growth and high resistance to bacterial wilt in eucalypt hybrid populations in operational breeding programs.

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## Patterns of Pollen Flow and Genetic Differentiation Among Pollen Pools in *Quercus salicina* in a Warm Temperate Old-growth Evergreen Broad-leaved Forest

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### Abstract

Paternity analysis and analysis of molecular variance were used to determine patterns of pollen flow and genetic differentiation among pollen pools in *Quercus salicina* in an 11.56-ha plot in a temperate old-growth evergreen broad-leaved forest.

The genotypes at seven microsatellite loci were determined for 111 adult trees and 276 seeds collected from under eight seed parents. The proportion of pollen flow from outside the plot (further than 100 m) was 52.2%, indicating that long-distance pollen flow occurred frequently in this species, as observed in other *Quercus* species. The pollen pools from inside and outside the plot differed genetically, and genetic structure was detected in the population of adult trees within the plot. Therefore, long-distance pollen flow from outside the plot may introduce new or low-frequent alleles, and increase genetic diversity in this population. However, the actual average distance of pollen flow within the plot was significantly shorter than the average potential distance, and negative exponential curves explained well the frequencies of matings as functions of the distance

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