

DEVELOPMENT OF SALT-TOLERANT *E. CAMALDULENSIS* × *E. GRANDIS* HYBRID CLONES USING PHENOTYPIC SELECTION AND GENETIC MAPPING

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Soil salinity caused by rising ground water tables is a major environmental problem throughout both the western and developing worlds. Currently, more arable land is lost through salinity than new agricultural land is gained through clearing of valuable forests (Frommer et al. 1999). Fast growing trees that are also tolerant of salt offer one strategy for addressing the problem of rising salinity. Large scale and strategic tree plantations have the capacity to lower local water-tables by drawing water from deep in the soil profile and releasing it into the atmosphere through evapotranspiration. However, many species desirable for their growth and timber characteristics are sensitive to salt and inappropriate for use in saline areas.

Within the genus *Eucalyptus*, there is considerable variation within and between species for salt tolerance. Six inter-specific hybrid families were constructed between field tested salt tolerant clones of *E. camaldulensis*, and lines of the more salt sensitive *E. grandis* selected for growth and form traits, with the objective of combining salt tolerance and commercial production traits. Genetic mapping and quantitative trait locus (QTL) analysis based on glasshouse screening trials has identified six chromosomal segments, three from each parental species, for reduced foliar chloride, an indicator of salt tolerance. Clonally replicated field trials using large families provide the basis for verifying initial QTL analysis results, and for identifying additional QTL for salinity tolerance and growth traits under field conditions. The successful identification of genomic regions for salt tolerance within *E. camaldulensis* would allow these to be introgressed into *E. grandis* in the first backcross generation using marker guided selection. The putative occurrence of cryptic genetic variation, with genes for salt tolerance being contributed to the hybrid by the salt sensitive phenotype, should allow selection of transgressive segregants in both the F₁ and BC₁ generation that exceed the salt tolerance of either parent. Such selections would be deployed through clonal propagation.

INTRODUCTION

Salinity has been an important historical factor in the decline of ancient agrarian societies (Frommer et al. 1999). Even today, rising groundwater salinity represents one of the most severe environmental problems in Australia, and is a major problem in both irrigated and dryland areas in many other parts of the world. Around 9×10⁸ ha are affected by salinity worldwide, with around one third of all irrigated lands being unsuitable for crop production due to high levels of salt (Frommer et al. 1999; Lambert and Turner 2000). In Australia alone, about 2.5 million hectares are affected by salinity, and there is potential for this to increase six fold to 15 million ha in the next 20 to 50 years (MDBC 1999).

There are three broad options for dealing with salinity: engineering works (groundwater pumping, surface drainage etc); biological control using vegetation to pump water from the soil into the atmosphere through transpiration; and adapting to more saline conditions. The strategic application of trees in the rural landscape is a potential tool in the biological control

of salinity. Trees that are fast growing, possess desirable timber characteristics and are tolerant of salt will have a valuable and unique application in the array of measures for biological control of salinity through their capacity to generate economic returns while addressing the environmental issue. They will also have the advantage of being efficient in the task of lowering water tables, as fast growth is correlated with high water use.

Excessive salinity has multiple physiological effects on plants. However, the observation of significant variation for salt tolerance within typically salt sensitive species, and single gene differences between salt tolerant and non-salt tolerant species within the same genus, indicates that only a small number of genes may be involved in conferring salt tolerance (Frommer et al. 1999). Within the genus *Eucalyptus*, there is both considerable variation within and between species for salt tolerance (Lambert and Turner *in press*). On this basis, inter-specific hybrids were constructed between established salt tolerant genotypes of *E. camaldulensis*, and lines of the more salt sensitive *E. grandis* selected for growth and form traits, with the objective of combining salt tolerance with commercial production traits in F₁ hybrids, and using genetic mapping to identify genomic regions within *E. camaldulensis* that could be introgressed into *E. grandis* in the first backcross generation through marker guided selection. The early results of this work are reported in this paper.

MATERIALS AND METHOD

Pedigrees and plant material

Six full-sib, F₁ hybrid families were constructed, comprising all combinations of three *E. camaldulensis* and two *E. grandis* parents. *E. camaldulensis* parents were selected on the basis of proven salt tolerance and relatively good growth and form in replicated clonal trials in Victoria and Western Australia. *E. grandis* parents were selected on the basis of early growth and form in an irrigated provenance trial at Shepparton in northern Victoria. The matrix of families, detailing individual full-sib family size is illustrated in Table 1. Families within columns or rows of Table 1 comprise half-sib arrays of two or three families respectively.

Table 1: Matrix of *E. camaldulensis* x *E. grandis* families constructed for genetic mapping and field trial establishment. Tabulated numbers indicate total number of genotypes in each family, numbers in brackets indicate number of unique genotypes included in field trials.

		<i>E. camaldulensis</i> mothers		
		E.Cam-1	E.Cam-2	E.Cam-3
<i>E. grandis</i> fathers	E.Gra-1	148 (41)	123 (26)	223 (215)
	E.Gra-2	59 (20)	107 (45)	476 (176)

Cuttings motherstock plants were established from each seed line, and clonally propagated. A selection of these were subsequently used in glasshouse screening and field trials.

Glasshouse screening for foliar chloride

Five ramets of 192 genotypes of family E.Cam-3 x E.Gra-1 were randomly selected for glasshouse screening. Cuttings were set in 13cc plug trays, and once rooted, were transferred to pots of washed fine sand and continuously sub-irrigated with a dilute nutrient solution in a hydroponic system within a climate-controlled glasshouse. When the plants reached an average height of approximately 40 cm, the nutrient medium was salinised by addition of NaCl at a concentration of 2g/l (34mM). After two weeks of saline irrigation the plants were harvested. The shoot fresh weight of each plant was recorded, and a sample of four expanded

leaves from the upper stem was collected. Fresh weight, oven dry weight and chloride content were measured on the leaf sample from each plant. Plants were screened in two batches of 109 and 93 genotypes, with an overlap of 10 genotypes between screenings. Results from each screening were expressed as standard deviations from the mean in order to adjust for differences in the mean and variance between screening batches.

Field trials

Two clonal field trials have been established to date. Data presented herein are from Mt Scobie near Kyabram in northern Victoria, established in October 1998. A second trial, planted near Deniliquin in southern NSW in October 1999, will provide future data for QTL analysis. Both trials are incomplete block designs with single tree plots and 5 replicates per treatment. The Mt Scobie trial incorporated 217 genotypes, including 73 genotypes of family E.Cam-3 x E.Gra-1 and 71 of E.Cam-3 x E.Gra-2. Height of all trees was measured to the nearest 5cm using height poles at age 5.5 and 12 months. Full details of trial designs, site descriptions and irrigation treatments are detailed in Sasse et al. (2000).

Genetic mapping and QTL analysis

Genetic maps based on RAPD markers were constructed for each parent of family E.Cam-3 x E.Gra-1 using a subset of 96 genotypes from the 192 screened for foliar chloride. The remaining 96 genotypes were subsequently genotyped with scaffold markers. QTL analysis for foliar chloride concentration, as determined from glasshouse pot trials, was carried out using the Multiple Interval Mapping approach (Kao et al. 1999).

RESULTS

Genetic mapping

QTL analysis identified six putative loci influencing foliar chloride levels under glasshouse pot trial conditions in family E.Cam-3 x E.Gra-1. Three QTL were contributed by the relatively more salt tolerant parent (*E. camaldulensis*), and three by the relatively less salt tolerant parent (*E. grandis*). Individual QTL effects ranged from 3 to 5% relative to the population mean. The co-efficient of variation for foliar chloride was 13%. Despite small individual effects, selection for a combination of, say three QTL, could reduce foliar chloride by 14% or just over one standard deviation. It is of note that QTL for reduced foliar chloride were identified in both the phenotypically tolerant parent (*E. camaldulensis*) and the more salt sensitive parent (*E. grandis*).

Field trials

Height and variance statistics of the two *E. camaldulensis* x *E. grandis* families and parental controls included in the Kyabram trial are summarized in Table 2. Ranking of taxa is hybrids > *E. grandis* > *E. camaldulensis*. Height growth of the E.Cam-3 x E.Gra-1 hybrid is significantly greater ($P = 0.05$) than the *E. camaldulensis* parent, and the E.Cam-3 x E. Gra-2 hybrid, but not the average of open pollinated seedling progeny of E.Gra-1 and E.Gra-2. There is no significant difference between seedling progeny of the pure *E. grandis* parents and the clonal replicates *E. camaldulensis* parent.

Correlations between foliar chloride and early field growth

Sixty five genotypes of family E.Cam-3 x E.Gra-1 assessed for foliar chloride in glasshouse screening were also included in the Kyabram field trial. Height growth at 5 and 12 months displayed no significant correlation with foliar chloride ($r^2 = .00004$ and $.0029$ respectively) (Figure 2). This remained true when foliar chloride was expressed as a percentage of dry weight, fresh weight and a percentage of tissue water.

Table 2: Height growth statistics for the *E. camaldulensis* x *E. grandis* families and parental controls at 12 months in the Kyabram trial.

Entry	Height (m)	No. of genotypes	No of plants	Standard deviation	Co-efficient of variation	Statistical significance
E.Cam-3 x E.Gra-1	2.9	73	286	0.47	16.2%	a
E.Cam-3 x E.Gra-2	2.8	71	247	0.56	20.0%	b
E.Cam-3	2.5	1	10	0.31	12.4%	b
E.Gra-1	2.7	8	8	0.43	15.9%	ab
E.Gra-2	2.7	9	9	0.41	15.2%	ab

The frequency distribution of the mapped hybrid family (E.Cam-3 x E.Gra-1) is illustrated in Figure 1. It can be seen from this figure and Table 2 that the average performance of the hybrids exceeds the mid-point of the parental means.

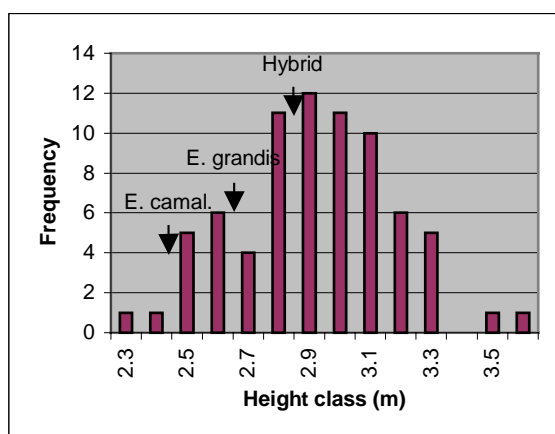


Figure 1: Frequency distribution of height for the E.Cam-3 x E.Gra-1 F₁ hybrid population. Means for open pollinated seedlings of the *E. grandis* parent, clonal replicates of the *E. camaldulensis* parent and the hybrid are indicated by arrows.

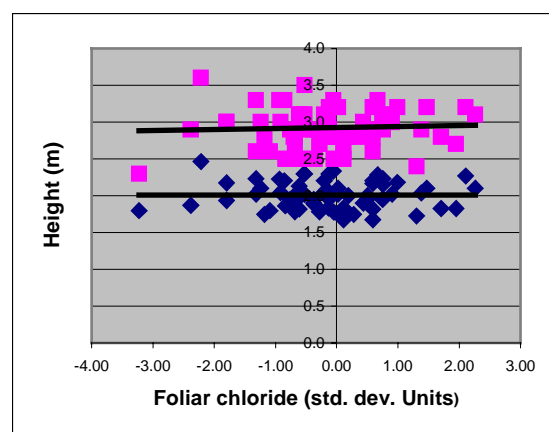


Figure 2: Correlation between standardised foliar chloride value (based on foliar chloride as a percentage of dry weight) and field height at 5 and 12 months.

DISCUSSION

Studies of inter-specific hybridization in both tree and crop species have demonstrated the presence of complementary alleles in each parent (deVincente and Tanksley 1993; Lee et al. 1999). Transgressive segregation, or the occurrence of hybrid progeny with extreme phenotypes relative to the mid-point of the parents is observed where complementary alleles are contributed by both parents (deVincente and Tanksley 1993). Results from this study

support the occurrence of complementary alleles being contributed to the F₁ hybrid of *E. camaldulensis* x *E. grandis* for both foliar chloride concentration and early field growth.

E. grandis is typically classified as a species with slight tolerance to salinity (2-4dS/m), while *E. camaldulensis* is considered tolerant of moderate salinities (4-8dS/m) and waterlogging (Lambert and Turner *in press*). Genetic mapping results from the present study indicate alleles for reduced foliar chloride concentration in glasshouse grown plants irrigated with 34mM NaCl solution are contributed by both the relatively more salt tolerant *E. camaldulensis* and relatively less tolerant *E. grandis*.

The presence of alleles for reduced foliar chloride concentration in *E. grandis* with an effect opposite to that expected by its phenotype may reflect the underlying mechanism of salt tolerance. Elevated soil salinity results in numerous stresses on non-halophytic plants, with the primary physiological effects being desiccation and toxicity (Frommer et al. 1999; Lambert and Turner *in press*). The most important requirement for salt tolerant plants is an ability to keep levels of ions in young leaves and shoot apices low (Niknam and McComb *in press*). It is generally accepted for eucalypts that at low to moderate levels of salt, active ion exclusion from uptake into the roots may be the most important tolerance mechanism, while at higher salt concentrations, salt uptake becomes unavoidable, and elevated concentrations of sodium and chloride in the leaves is avoided through compartmentalization in vacuoles and deposition into bark, ray cells, tracheid walls, lumens and old senescent leaves (Lambert and Turner *in press*; Niknam and McComb *in press*).

In a study by Sun and Dickinson (1993), *E. grandis* was found to be among the most tolerant of 16 Eucalypt species tested at 50 to 150mM NaCl, but all plants died at 200mM. This suggests that *E. grandis* has a strong ability to maintain active exclusion of sodium and chloride ions up to a threshold of around 150mM, but at higher salt concentrations, this mechanism breaks down and the capacity of *E. grandis* to compartmentalise salt is poor, confirming its classification as a slightly salt tolerant species. These results further suggest the possibility that the three QTL from *E. grandis* identified in the present study may represent genes involved in active exclusion of sodium and chloride ions at salt concentrations below 150mM. While this hypothesis requires further study, it illustrates the application of genetic mapping to understanding the genetic architecture of physiologically complex traits.

The early field trial results indicating that the performance of the hybrids exceeds the mid-point of the parental means may be explained by the contribution of complementary alleles from each parent. In constructing the hybrids, it was hypothesised that genes for growth and form would be contributed by *E. grandis*, and genes controlling salinity tolerance mechanisms involving avoidance through compartmentalisation/deposition would be contributed by *E. camaldulensis*. The occurrence of transgressive segregation for early height growth suggests that alleles for growth and form may have been contributed by *E. camaldulensis*, the phenotypically inferior parent for these traits. This hypothesis will be answered through future QTL analysis. In terms of practical application, the present results clearly indicate the potential to select and clonally deploy individual hybrid genotypes whose growth performance significantly exceeds that of either pure species parent (Figure 1).

The lack of any significant correlation between foliar chloride assessed at slight salinity (34mM) in the glasshouse, and early field height growth in moderately saline soil (8dS/m ~ 88mM) is not unexpected given the early age of the field trial. Growth modelling data for pure *E. grandis* on contrasting sites in Victoria indicate little discrimination between low (maximum MAI = 15m³/ha/yr_{age 14}) and high (maximum MAI = 40m³/ha/yr_{age 9}) yielding

sites at age one. It is possible that significant correlations may develop between glasshouse results for foliar chloride and field growth as greater growth variation is expressed with increasing trial age and as saline irrigation is applied. If no correlations develop with time, it may be a reflection of different salt tolerance mechanisms being tested in the field compared to the glasshouse. Foliar chloride levels expressed in the glasshouse trial may represent the effect of genes involved in exclusion of sodium and chloride ions at low salinities, while growth in the field may represent the net effect of independent genes for inherent growth potential and salinity tolerance arising from a combination of exclusion, compartmentalisation and tolerance mechanisms, plus other variable effects including preferential root growth in soil pockets at lower than average salinity, opportunistic utilisation of fresh water inputs, a reduction of inherent soil salinity through flushing by freshwater irrigation in the first five months following planting, dispersive effects of sodium ions in the soil leading to poor soil aeration, and periodic waterlogging stress following flood irrigation. Notwithstanding, the trial analysed here (Kyabram) represents the first known trial to compare glasshouse screening results for foliar chloride with field performance for the same genotypes across a segregating family (Nickham and McComb *in press*). The second trial (Deniliquin) will extend this comparison to field performance under saline and non-saline irrigation. Both trials will provide a valuable source of information on the correlation of glasshouse and field screening methods as they develop.

The complexity of the set of chemical, physical and environmental factors that lead to plant stress as a consequence of salinity, the variety of physiological mechanisms employed to avoid or tolerate salt and associated stresses, and the interaction of tolerance/avoidance mechanisms with salt concentration and other stresses (particularly hypoxia associated with water-logging), indicate the value of understanding the different mechanisms of salinity tolerance in different species. In this regard, genetic mapping provides a potentially powerful tool in dissecting the genetic architecture of salt tolerance. Chromosomal segments controlling tolerance/avoidance mechanisms at different ages, different salinity levels and different conditions can potentially be identified and used in the F₁ generation to select genotypes with a desirable combination of tolerance mechanisms; in the F₂ to combine desirable genes from different species, or to rapidly introgress selected genes from one species into another in the BC₁. The present trials should provide the basis to uncover and manipulate QTL for different mechanisms of salt and stress tolerance, age dependent mechanisms of salt tolerance and for growth under saline conditions.

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