Predicting Within-Family Variability In Juvenile Height Growth Of *Salix* Based Upon Similarity Among Parental AFLP Fingerprints

Richard F. Kopp[1], Lawrence B. Smart[1], Charles A. Maynard[2], Gerald A. Tuskan[3] and Lawrence P. Abrahamson[1,2]

[1]*State University of New York College of Environmental Science and Forestry, Faculty of Environmental and Forest Biology, 1 Forestry Drive, Syracuse, NY 13210*
[2]*State University of New York College of Environmental Science and Forestry, Faculty of Forestry , 1 Forestry Drive, Syracuse, NY 13210*
[3]*Oak Ridge National Laboratory, Environmental Sciences Division, P.O. Box 2008 Mail Stop 6422, Oak Ridge, TN 37831*

rfkopp@syr.edu

ABSTRACT

Willow is being developed as a crop for biomass plantations in the Northeast and North-central United States, but has only recently been the subject of controlled breeding to generate improved genotypes. Maximizing variability among progeny within full-sib families produced by controlled pollination may increase the probability of generating willow clones exhibiting desirable phenotypes. These superior individuals can then be vegetatively propagated. Yet, it is currently not possible to predict combinations of parents that will yield highly variable progeny. Controlled pollinations were completed among 15 unrelated *S. eriocephala* clones, and the resulting progeny were vegetatively propagated and planted in a greenhouse progeny test. Heights of rooted cuttings were measured after 4 months of growth. Genetic similarity among parents was estimated based on 77 polymorphic AFLP bands produced by amplification with two selective primer pairs. Strong correlation (r=-0.88) was detected between mean female parent similarity indices and the standard deviation of height among half-sib progeny from those females. Parent combinations that had relatively low similarity indices tended to produce progeny that had greater variability in height. This negative relationship suggests that AFLP fingerprints of *S. eriocephala* parents may be useful for predicting parent combinations, and specific female parents in particular, that will yield families with large variability.

INTRODUCTION

Genetically improved *Salix* clones with higher productivity, upright coppice form, and insect and disease resistance are necessary to improve the economic potential of growing willows as a feedstock for bioenergy or bio-based products. At the State University of New York College of Environmental Science and Forestry, genetic improvement of willows through selection and controlled breeding has focused on *Salix* species native to or naturalized in North America. The genetic improvement strategy includes maximizing recombination and within-family variance, and then selecting and vegetatively propagating desirable individuals from promising families (Kopp et al., 2001). However, predicting combinations of parents that yield highly variable progeny is not currently possible. Theoretically, genetic diversity in parents should be predictive of the genetic variance of segregating generations (Burkhamer et al., 1998; Bohn et al., 1999).

Salix eriocephala is native to North America (Zsuffa, 1988) and appears to have large potential for bioenergy production (Aravanopoulos, 1998). Large natural variation is known to exist in *S. eriocephala* (Aravanopoulos et al., 1999), and the species is essentially undomesticated, suggesting that large and rapid genetic gains should be attainable through breeding (Kopp et al., 2001).

An experiment was completed to determine if amplified fragment length polymorphism (AFLP) fingerprints may be used to predict which *S. eriocephala* clones are desirable parents. The hypotheses were that the amount of similarity between *S. eriocephala* parents estimated from AFLP fingerprints would be inversely related to 1) the level of variability in height growth within families resulting from crosses among the parents and 2) mean juvenile height growth of progeny in families produced by crossing the parents.

MATERIALS AND METHODS

Controlled pollinations were completed among seven female and eight male parents. All clones were selected for one or more traits related to biomass production, including: tree height, stem number, stem diameter, and coppice form based on field trials. Clones were also chosen with a goal of maximizing the geographic range from which they were collected. The mating design was a 7 x 8 incomplete factorial, in which every clone was mated with at least four clones of the opposite sex. Controlled pollinations were completed in a greenhouse during the winter of 1997-1998 using flower-bearing shoots placed in water, and pollen that was extracted with carbon tetrachloride and stored at 20^{o}C until use. Female shoots bearing receptive flowers were physically isolated from potential pollen sources to prevent accidental pollination. Thirty-four families were produced from these crosses. Ten seedlings were randomly selected from each family, grown through summer 1998 in a greenhouse, and moved outdoors during fall 1998 so they would become dormant. Dormant seedlings were vegetatively propagated by producing nine cuttings that were each 4 cm in length, and cuttings were planted in each of three potting mixes in a replicated greenhouse study. Heights of the rooted cuttings were measured after four months of growth.

Amplified fragment length polymorphism (AFLP) fingerprints were generated for the 15 *S. eriocephala* clones used as parents according to the procedure of Vos et al. (1995) modified by Remington et al. (1999). DNA was isolated from young foliage, and AFLP fingerprints were produced for each of the parents using two selective primer pairs. Presence or absence of bands was scored manually, and bands present or absent in at least one of the 15 parents were classified as polymorphic. Data from the two primer pairs were combined and similarity indexes were computed based on the formula (Weising et al., 1995):

$$(N_{AB}/2) * ((1/ N_A) + (1/N_B)) \qquad (1)$$

where,

N_{AB} is the number of polymorphic bands that individual A and B have in common,
N_A is the total number of polymorphic bands observed for individual A, and
N_B is the total number of polymorphic bands observed for individual B.

ANOVA tests were completed to determine if average similarity indices for female clones differed, and likewise for male clones. Pearson's correlation coefficients were calculated between similarity indices and height measurements, standard deviations, and coefficients of variation of height measurements. All correlations were calculated using PROC CORR in SAS (SAS Institute, 1997).

RESULTS AND DISCUSSION

The average height of rooted cuttings of *S. eriocephala* progeny grown for four months was 17.0 cm, with full-sib family means that ranged from 13.5 cm to 20.3 cm. Family standard deviations varied from 2.51 cm to 5.06 cm (Table 1). The survival across all ramets was 94.5%, while family survival ranged from 77% to 100%.

A large number of polymorphic loci (77) were detected in the 15 *S. eriocephala* parents using only two AFLP primer pairs. More than 84% of all fragments detected were polymorphic. Every parent could be distinguished with either primer pair alone. The average similarity index for parents of crosses that were completed was 0.45, with a low of 0.25 and a high of 0.69 (Table 1). Based on ANOVA results, similarity indices significantly differed ($P < 0.01$) among female parents and ranged from 0.37 to 0.58. Similarity indexes were not significantly different among male parents, ranging from 0.39 to 0.52. The mean similarity index for all possible parental combinations, including male-by-male, female-by-female, and male-by-female combinations for which no seedlings were produced, was 0.46, with a low of 0.25 and a high of 0.70 (Table 1).

Strong correlation ($r=-0.88$, $P=0.009$) was detected between mean female parent similarity indices and the standard deviation of height among half-sib progeny from those females. Some female clones which had low average similarity indices and whose progeny had below-average height growth yielded progeny that were among the tallest 5% of all progeny in the experiment (Fig 1). Parent combinations that had relatively low similarity indices tended to produce progeny that had greater variability in height. All other correlations, including those between full-sib and male mean similarity indices and tree height or coefficient of variation for tree height, were low and non-significant. Progeny of parents with very different AFLP fingerprints did not grow, on the average, better than progeny of parents with similar AFLP fingerprints. Certain parents consistently produced full-sib progeny that expressed above average mean height. These parents are considered to have good general combining ability, but their AFLP-based similarity indices are not good predictors of the mean performance of their progeny.

Results of AFLP fingerprinting in this study suggest that there was large variability among *S. eriocephala* parents. These results were similar to those reported for clones of *S. viminalis* and its hybrids grown for bioenergy in Europe (Barker et al., 1999). AFLP analyses of 29 willow clones with five AFLP primer pairs yielded 919 different fragment types, of which 752 (81.8%) were polymorphic. The high percentage of polymorphic bands detected in willows in AFLP experiments probably reflects the low level of domestication and the outcrossing nature of *Salix* spp., which are dioecious.

It is possible that some of the parents used in this study were not pure *S. eriocephala*. Natural hybrids with *S. sericea* can be difficult to detect based on morphological characteristics (Hardig et al., 2000). Interspecific crosses tend to yield progeny that have large variability in growth rates, a higher than normal percentage of seedlings with abnormal morphology, and reduced fertility (Mosseler, 1990). High survival percentages and observed flowering among most of the progeny after two growing seasons reduces the likelihood that the parents used in this study were hybrids, though this conclusion should be validated. Molecular genetic analyses will be necessary to confirm that parental clones, putatively identified as *S. eriocephala,* are in fact pure *S. eriocephala*.

Table 1. Similarity indices of pairs of *Salix eriocephala* clones based on AFLP fingerprints, and standard deviation of height growth (in italics) by progeny produced by controlled pollination.

		Males							Females						
		S646 ONT	95019 NY	95022 NY	95024 NY	95054 NY	95061 NY	95316 PA	S25 ONT	S652 ONT	95064 NY	95306 PA	95311 PA	95331 PA	96305 PA
Males	S287 ONT	0.44	0.49	0.45	0.47	0.55	0.48	0.60	0.44 ***3.10***	0.39	0.52 ***4.29***	0.40 ***3.36***	0.67 ***3.55***	0.49 ***3.40***	0.54
	S646 ONT		0.48	0.41	0.42	0.51	0.48	0.42	0.60	0.54 ***4.71***	0.38 ***4.21***	0.38 ***4.45***	0.53	0.56	0.45 ***3.17***
	95019 NY			0.59	0.42	0.46	0.57	0.61	0.38 ***5.06***	0.50 ***3.82***	0.50	0.29	0.51 ***3.97***	0.58 ***3.69***	0.58 ***4.05***
	95022 NY				0.47	0.42	0.70	0.38	0.25 ***4.65***	0.25 ***3.99***	0.50 ***4.07***	0.26	0.52	0.55 ***3.66***	0.45
	95024 NY					0.42	0.51	0.45	0.35 ***4.64***	0.36 ***3.99***	0.55	0.39	0.46	0.57 ***3.56***	0.55 ***2.51***
	95054 NY						0.56	0.41	0.43 ***3.74***	0.39	0.34 ***4.09***	0.38 ***3.87***	0.43	0.41 ***4.12***	0.48
	95061 NY							0.42	0.35	0.37 ***4.67***	0.42 ***4.69***	0.32 ***3.34***	0.43 ***3.18***	0.62	0.45
	95316 PA								0.40 ***4.24***	0.40 ***4.06***	0.49	0.35	0.69 ***3.49***	0.63	0.57 ***4.17***
Females	S25 ONT									0.45	0.32	0.47	0.49	0.57	0.50
	S652 ONT										0.26	0.37	0.42	0.45	0.44
	95064 NY											0.28	0.47	0.45	0.39
	95306 PA												0.54	0.42	0.45
	95311 PA													0.55	0.67
	95331 PA														0.54

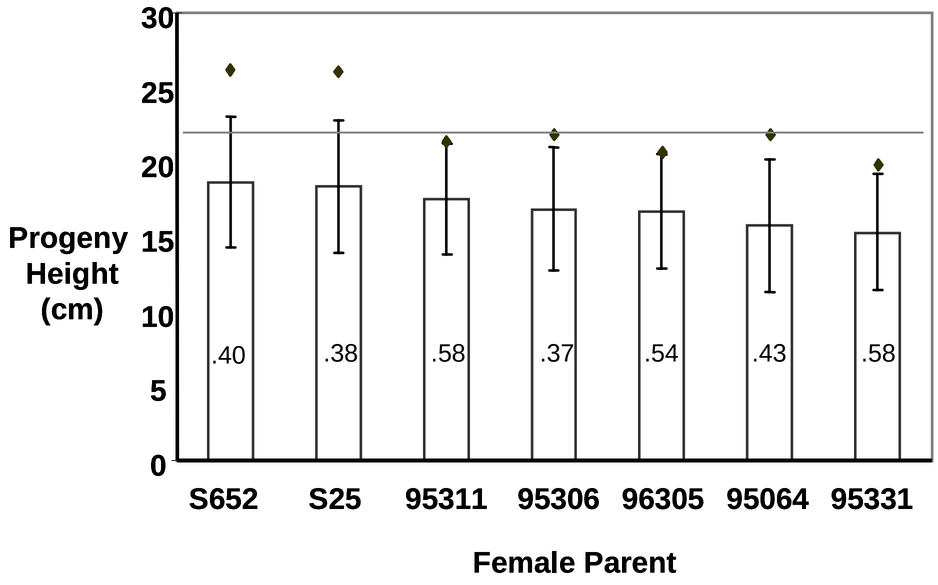

Figure 1. Height of rooted cuttings after four months of growth, averaged by female parent, with standard deviation bars. Symbols indicate the height of the tallest plant produced by a female parent and numbers within height bars indicate the average similarity index calculated by comparing AFLP fingerprints of females with those of males to which they were mated. Points touching or above the horizontal line at 21.5 cm represent clones ranked in the top 5% for height compared with the entire experimental population. The correlation between standard deviation for height growth and similarity index was large and statistically significant (r=-0.88, P=0.009).

An underlying assumption for selecting the parents used in this study was that clones originating from distant geographic regions are genetically more dissimilar from each other than clones from relatively close proximity. AFLP similarity index data did not support this assumption because, a clear relationship between similarity index and geographic origin was not observed. A low correlation (r=-0.28, P=0.11) between similarity index and linear geographic distance was detected. For example, two of the parents in this study, clones 95054 and 95331, were collected from wild stands separated by only 40 km and had a similarity index of 0.41 (Table 1). Parents with a nearly identical similarity index, clones S287 and 95306 (similarity index =0.40), were from wild stands separated by 420 km. The similarity index for clones S287 and 95306 was expected to be much lower than for clones 95054 and 95331. Though this study was not designed to test the relationship between geographic origin and AFLP similarity index, and inferences are based on a small sample size, preliminary indications suggest that large genetic variability in *S. eriocephala* may be present in a relatively small range. This is similar to sugar maple populations sampled from across their native range, where differences among regions represented less than 2% of the total genetic variation, and differences among stands within regions were 10.5% or less based on RAPD markers, with the remainder of the variation being within stands (Gunter et al., 2000). Similarly, variation among eight trembling aspen populations was only 2.6% of the total variation based on RAPD markers, with 97.4% of the variation attributable to within-population differences (Yeh et al., 1995).

In conclusion, this study suggests that AFLP fingerprints could be used to select parents whose progeny will be highly variable, thus offering an opportunity to select within families for superior individuals that far exceed mean family performance. Confidence in these predictions must be tempered, because correlations between similarity index and coefficient of variation calculated on both the family and half-sib bases were low, and there were relatively few parental genotypes used to estimate the correlation. Correlations between similarity index and juvenile height growth were low and not significant. Results from full-rotation field trials will be necessary to confirm the utility of AFLP fingerprints for predicting family combinations that contain a high degree of variability, because greenhouse juvenile height growth may be a poor predictor of field performance. Field performance of hybrid poplars could not be accurately predicted based on measurement of morphological variables of greenhouse-grown trees (Promnitz and Wray, 1976). Furthermore, tree height alone was shown to be a poor predictor of willow biomass production (Ballard et al., 1999). The correlation between variability in height growth and variability in biomass production within families is uncertain. Tests with a larger number of willow species, parents, progeny per family, and AFLP primer pairs are necessary to determine if results of this study can be generalized to other willow breeding populations.

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