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Predicting within-family variability in juvenile height growth of *Salix* based upon similarity among parental AFLP fingerprints

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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 producing willow clones exhibiting desirable extreme phenotypes. Yet, predicting combinations of parents yielding highly variable progeny is not currently possible. Controlled pollinations were completed among 15 *Salix 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. Strong negative 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 that will yield families with large variability.

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Introduction

Growing willows (*Salix* spp.) in intensive culture systems for biomass, which can be used for energy or conversion to high-value products, is gaining increasing worldwide interest. Willows in general are perennial, outcrossing, insect-pollinated species with a long life history and overlapping generations, all contributing to a relativley high degree of heterozygosity and intra- as well as inter-population genetic variation. *Salix eriocephala* is native to North America (Zsuffa 1988) and appears to have large potential for bioenergy production (Aravanopoulos and Zsuffa 1998). Large natural variation is known to exist in *S. eriocephala* (Aravanopoulos et al. 1999). The species is essentially undomesticated, suggesting that large and rapid genetic gains should be attainable through breeding (Kopp et al. 2001).

Genetic improvement strategies for clonally propagated species, such as willows, focus on maximizing the probability of developing exceptional individual clones, rather than on improving progeny means. Strategies for willow genetic improvement should include maximizing recombination and within-family variance to achieve the largest gains per unit effort (Tuskan 1997). If within-family variance of juvenile trees were highly correlated with their variance at maturity, then identifying families with large variance at a juvenile stage would most likely increase the probability of producing exceptional clones. Theoretically, the genetic distance between parents should be predictive of the genetic variance of segregating generations (Martinez et al. 1983; Burkhamer et al. 1998; Bohn et al. 1999).

Amplified fragment length polymorphism (AFLP) is a genetic fingerprinting technique that may be useful in predicting the likelihood that parental combinations will yield highly variable progeny. AFLP is based upon the selective polymerase chain reaction (PCR) amplification

of fragments from restriction enzyme digests of DNA, and the separation and visualization of amplified fragments using denaturing gel electrophoresis. AFLP loci are generally scored simply for the presence or absence of a band of specific size, and thus are characterized as dominant neutral markers (Vos et al. 1995). AFLP has been effectively used to fingerprint willows for genetic distance estimates and clone identity verification (Barker et al. 1999), and to identify willow hybrids in natural stands (Beismann et al. 1997; Hardig et al. 2000).

A study was established in 1998 to obtain basic genetic information on *S. eriocephala* that could be applied to an ongoing willow breeding program. F_1 progeny were produced by controlled pollination of unrelated *S. eriocephala* parents, which were selected for desirable biomass production qualities. Progeny were propagated and tested in a greenhouse trial, and molecular fingerprints were developed for the parents by AFLP. The specific goal was to determine if AFLP fingerprints could assist in identifying parents that will yield families expressing large variability among progeny. The hypotheses were that the amount of similarity between *S. eriocephala* parents estimated from AFLP fingerprints is: (1) inversely related to the amount of variability in height growth within families resulting from crosses among the parents, and (2) strongly correlated to the juvenile height growth of progeny in families produced by crossing the parents.

Materials and methods

Controlled pollinations and experimental design

Eight male and seven female *S. eriocephala* clones were selected from the SUNY-ESF willow clone collection for use as parents in an F_1 progeny test. 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 the goal of maximizing the geographic range from which they were collected. Twelve of the clones were of unknown parentage from native stands in New York, Pennsylvania, and Ontario, Canada. Three clones (S646, S652 and S25) were unrelated F_1 progeny that were produced by researchers at the University of Toronto through controlled breeding of clones collected in Ontario. The mating design was a 7×8 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 °C until use. Female shoots bearing receptive flowers were physically isolated from potential pollen sources to prevent accidental pollination. When seeds were shed, they were immediately collected and sown, then grown in a greenhouse until late summer. Ten seedlings were randomly chosen from every family and the selected seedlings were moved outdoors during fall to promote dormancy. Nine hardwood cuttings approximately 4 cm in length were made from every plant during December 1998 and stored at –20 °C.

The cuttings collected from ten progeny in each family were planted in three different potting mixes in January 1999 and propagated in a greenhouse under intermittent mist. Mix A consisted of silt-loam soil plus Pro-Mix 2:1 (v:v), mix B consisted of clay-loam soil plus Pro-Mix 2:1 (v:v) and mix C was sand plus Pro-Mix 1:1 (v:v). Flats of soil-filled tubes were randomly placed on three greenhouse benches that served as blocks. Each block contained an equal number of flats of each potting mix type. For each clone, cuttings were grouped by size such that those with similar diameters were planted in the same block in order to reduce "C" effects. C effects refer to growth patterns that result from the physical or physiological condition of cuttings when they are planted and are related to the environmental conditions experienced by donor plants, for example cutting size (Libby and Jund 1962). Families were randomly placed in each replication with the restriction that each family was planted in each potting mix once in each replication. Clones were not randomized within families. Temperature in the greenhouse was maintained between 15 and 27 °C, and the photoperiod was maintained at 16 h light and 8 h dark with high-pressure sodium lights. Survival and height of the rooted cuttings were measured in April 1999 after approximately 4 months of growth.

Analyses of variance on height data were completed using the Statistical Analysis System (SAS Institute Inc. 1997) with the following linear model:

$$y_{jvkpq} = \mu + B_j + S_{v(j)} + M_k + F_p + (M^*F)_{kp} + C_q(M^*F)_{(kp)} + e_{jvkpq}, \quad (1)$$

where y_{jvkpq} is the observed value for an individual plant, μ is the overall mean, B_j is the effect of block j, j = 1–3, $S_{v(j)}$ is the effect of soil v in block j, v = 1–3, M_k is the effect of male k, k = 1–8, F_p is the effect of female p, p = 1–7, $(M^*F)_{kp}$ is the interaction effect of male k and female p, $C_q(M^*F)_{(kp)}$ is the effect of clone q from a cross between male k and female p, q = 1–10, e_{jvkpq} is the random pooled error.

The C(M*F), S, and B terms were tested with the experimental error, the M*F term was tested against the mean squares from the C(M*F) interaction, and the M and F terms were tested against the mean squares from the M*F interaction.

Analysis of amplified fragment length polymorphisms

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) as modified by Remington et al. (1999). Cellular DNA was isolated from young foliage produced by cuttings in a greenhouse using a Nucleon Phytopure DNA purification kit (Amersham Pharmacia Biotechnology, Piscataway, NJ). DNA was quantified using a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotechnology Inc., San Francisco, CA). *Eco*RI and *Mse*I restriction endonucleases (New England Biolabs, Beverly, MA) were used to digest 250 ng of DNA for each clone.

Two replications of pre-selective amplification using standard AFLP *Eco*RI (E) and *Mse*I (M) primers with selective nucleotides E + A and M + C were completed for each clone using separate master mixes in each replication. PCR amplifications were as described by Remington et al. (1999) except that annealing was at 56 °C.

For selective amplification, two different selective primer pairs with three selective bases were used: (1) E+ACT/M+CTA and (2) E+AGC/M+CAC. The *Eco*RI primers were fluorescently tagged with IRD-700 (LI-COR, Lincoln, NE). Selective amplification with primer pairs one and two were completed with two and three replications of the pre-selective product, respectively. All amplifications were completed in a Perkin Elmer-9700 thermocycler (PE Applied Biosystems, Foster City, CA), with PCR amplifications as described by Remington et al. (1999). Loading buffer (10 µl) containing 95% (v/v) formamide, 20 mM of EDTA pH 8.0 and 1 mg ml^{-1} of bromophenol blue (Aldrich, Milwaukee, WI) was added to the selective product, which was stored in complete darkness overnight at –20 °C.

Gel electrophoresis was accomplished using a LI-COR Long ReadIR 4200 sequencer and 6.5% (w/v) of KB-plus (LI-COR, Lincoln, NE) polyacrylamide-gel matrix in $1 \times$ TBE (diluted from

5 × stock, Sigma Chemical Company). Samples and molecular-weight standards were heated to 94 °C for 3 min and cooled to 4 °C prior to loading on the gel. Molecular-weight standards (LI-COR) were included in outer lanes and at least two interior lanes on each gel. Electrophoresis conditions were as described by Remington et al. (1999). Molecular weights of all bands were estimated using the piecewise linear method in the RFLPscan Version 3.12 software package (Scanalytics, Fairfax, VA). Gel images were manually scored for presence or absence of bands. Bands were scored as present and included in similarity index calculations if they were present in at least one replication. Bands were considered polymorphic if they were present in less than 95% of the individuals (Hartl and Clark 1989). Data from primer pairs one and two were combined and similarity indexes were computed based on the formula (Weising et al. 1995):

$$(N_{AB}/2)^{*}[(1/N_A)+(1/N_B)] \quad (2)$$

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

The hypotheses that the similarity index differed by male clones, and by female clones, were tested by analysis of variance ($\alpha = 0.05$) using PROC ANOVA in SAS (SAS Institute Inc. 1997) with the following linear models:

$$\text{for males} \quad y_k = \mu + M_k + e_k \quad (3)$$

$$\text{for females} \quad y_p = \mu + F_p + e_p \quad (4)$$

where, y_k (and y_p) is the observed similarity index for a male-female parental combination, μ is the overall mean, M_k is the effect of male k, k = 1–8; F_p is the effect of female p, p = 1–7, e_k (and e_p) is the random error, respectively.

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 Inc. 1997).

Results

The average height of rooted cuttings of *S. eriocephala* progeny grown for 4 months was 17.0 cm, with full-sib family means that ranged from 13.5 cm (family 9843) to 20.3 cm (family 9812) (Table 1). Family standard deviations varied from 2.5 (family 985) to 5.1 (family 9812) (Table 1). Analyses of variance indicated that height growth was significantly ($P < 0.01$) affected by both male and female parents, and soil type. Significant ($P < 0.01$) male by female (family), and clone within male by female interactions were detected for height. The survival across all ramets was 94.5%, while family survival ranged from 77% (family 98016) to 100% (six families).

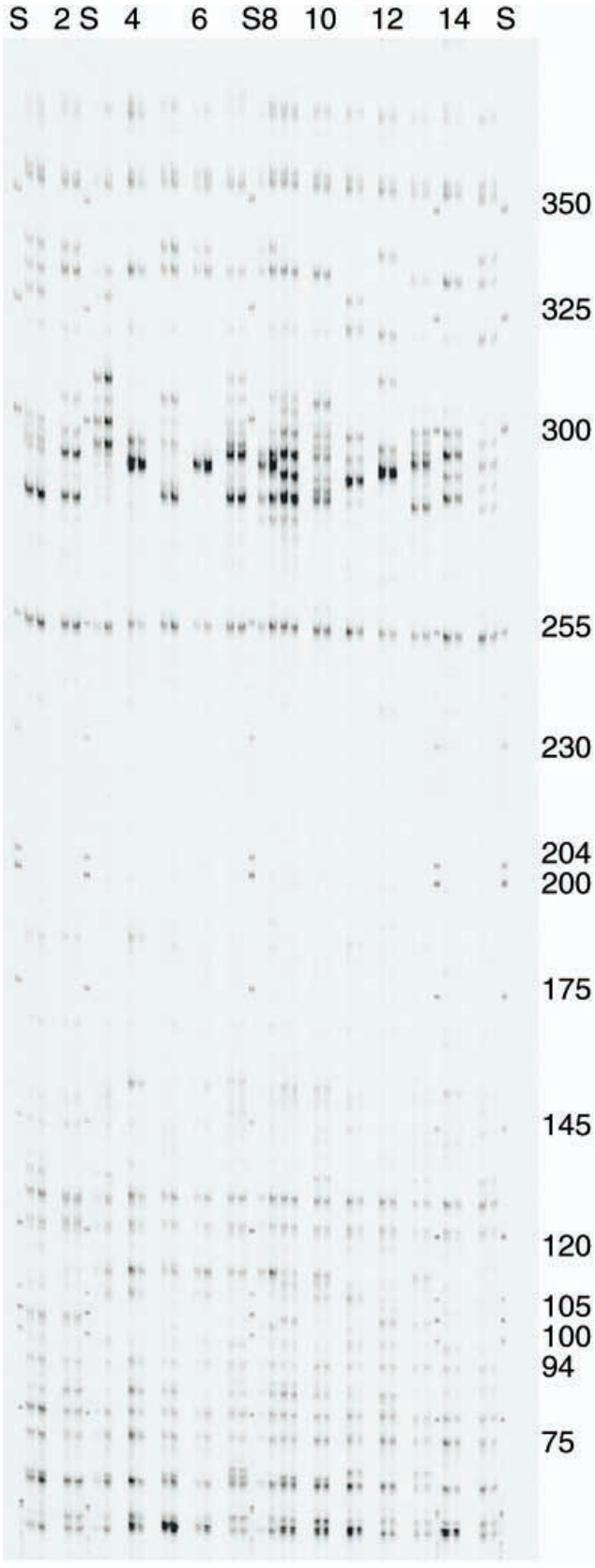

Fig. 1 Image of a representative gel with AFLP fragments amplified from 15 *S. eriocephala* parents with selective primer pair E+ACT/M+CTA. All samples were replicated and the two replicates of each clone were loaded in adjacent lanes. An empty lane or a molecular weight standard separates samples from each clone with the exception of clones 8 and 9. Clones are: *1* = 95019, *2* = S287, *3* = 95331, *4* = S646, *5* = 95061, *6* = S25, *7* = 95024, *8* = 95054, *9* = 96305, *10* = 95311, *11* = 95316, *12* = S652, *13* = 95022, *14* = 95306, and *15* = 95064. *S* indicates lanes with a molecular weight standard and the sizes of those bands (bp) are indicated on the right

Table 1 Heights and standard deviations of greenhouse-grown rooted cuttings of full-sib *S. eriocephala* progeny measured 4 months after planting. Full-sib families are designated ‘‘98#,” average heights (cm) are italicized, standard deviations are in parentheses, m is the height of the tallest tree, *n* is the number of clones per full-sib family and r is the total number of ramets per family. Parents of each family are in bold print along with their geographic origin

Female	Male								Female mean
	S646 (ONT)	**95061** (NY)	**95316** (PA)	**95019** (NY)	**95024** (NY)	**95022** (NY)	**95054** (NY)	**S287** (ONT)	
96305 (PA)	981 *19.3* (3.2) m = 26.5 *n* = 10 r = 90		983 *16.4* (4.2) m = 23.9 *n* = 6 r = 51	984 *15.0* (4.1) m = 22.5 *n* = 8 r = 64	985 *15.4* (2.5) m = 22.4 *n* = 10 r = 90				*16.7* (3.8) m = 26.5 *n* = 34 r = 295
S652 (ONT)	98106 *20.2* (4.7) m = 32.2 *n* = 10 r = 87	986 *17.2* (4.7) m = 29.2 *n* = 7 r = 60	987 *18.3* (4.1) m = 27.5 *n* = 9 r = 81	988 *20.0* (3.8) m = 29.1 *n* = 10 r = 90	989 *17.0* (4.0) m = 30.3 *n* = 10 r = 85	9810 *18.6* (4.0) m = 27.1 *n* = 8 r = 71			*18.6* (4.4) m = 32.2 *n* = 54 r = 474
S25 (ONT)			9811 *19.5* (4.2) m = 30.0 *n* = 8 r = 72	9812 *20.3* (5.1) m = 36.0 *n* = 5 r = 42	9813 *16.1* (4.6) m = 27.7 *n* = 10 r = 79	9814 *18.9* (4.7) m = 28.9 *n* = 10 r = 89	9815 *19.6* (3.7) m = 30.7 *n* = 10 r = 89	9842 *16.7* (3.1) m = 23.5 *n* = 10 r = 84	*18.4* (4.6) m = 36.0 *n* = 53 r = 455
95331 (PA)				9816 *16.8* (3.7) m = 28.5 *n* = 9 r = 62	9817 *14.2* (3.6) m = 25.8 *n* = 10 r = 73	9818 *16.3* (3.7) m = 24.3 *n* = 10 r = 70	9819 *15.7* (4.1) m = 25.0 *n* = 10 r = 75	9843 *13.7* (3.4) m = 21.0 *n* = 9 r = 74	*15.3* (3.9) m = 28.5 *n* = 48 r = 354
95064 (NY)	9826 *18.2* (4.2) m = 25.2 *n* = 10 r = 84	9827 *15.3* (4.7) m = 26.0 *n* = 8 r = 71				9828 *15.1* (4.1) m = 25.0 *n* = 9 r = 76	9829 *15.3* (4.1) m = 26.2 *n* = 9 r = 69	9844 *14.4* (4.3) m = 30.3 *n* = 9 r = 76	*15.7* (4.5) m = 30.3 *n* = 45 r = 376
95306 (PA)	9831 *19.3* (4.5) m = 30.1 *n* = 9 r = 78	9832 *15.8* (3.3) m = 22.8 *n* = 9 r = 78					9834 *17.9* (3.9) m= 31.3 *n* = 9 r = 79	9845 *14.7* (3.4) m = 22.5 *n* = 10 r = 87	*16.9* (4.2) m = 31.3 *n*=37 r = 322
95311 (PA)		9837 *18.2* (3.2) m = 24.2 *n* = 8 r = 72	9838 *18.5* (3.5) m = 26.3 *n* = 10 r = 81	9839 *15.5* (4.0) m = 24.1 *n* = 8 r = 72				9846 17.8 (3.6) m = 29.8 *n* = 10 r = 89	17.6 (3.7) m = 29.8 *n* = 36 r = 314
Male mean	*19.3* (4.2) m = 32.2 *n* = 39 r = 339	*16.6* (4.1) m = 29.2 *n* = 32 r = 281	*18.3* (4.1) m = 30.0 *n* = 33 r = 285	*17.5* (4.6) m = 36.0 *n* = 39 r = 330	*15.7* (3.9) m = 30.3 *n* = 40 r = 327	*17.3* (4.4) m = 28.9 *n* = 37 r = 306	*17.3* (4.3) m = 31.3 *n* = 38 r = 312	*15.5* (3.9) m = 30.3 *n* = 48 r = 410	

Preliminary tests with 15 different selective primer pairs indicated that, of the pairs tested, primer pair E+ACT/M+CTA yielded the largest number of polymorphic AFLP bands that had consistently high intensity. Selective amplification with primer pair E+ACT/M+CTA resulted in unique AFLP fingerprints for all 15 *S. eriocephala* parent clones (Fig. 1). For this primer set, 53 different fragment types were observed across all clones. The number of bands detected per individual clone ranged from 11 (clone S25) to 27 (clone 95311) and averaged 20. Of the 53 fragment types, 49 were polymorphic, and ten of those were amplified from only one clone. Selective amplification with primer pair E+AGC/M+CAC also resulted in unique AFLP fingerprints for all 15 clones, but fewer polymorphic bands were detected than with primer pair E+ACT/M+CTA. Thirty-eight fragment types were amplified across all parents. The largest number of bands per parent was 22,

Table 2 Similarity index of pairs of *S. eriocephala* parents based on 77 polymorphic fragment types amplified using two pairs of selective AFLP primers. Values within the table represent similarity indices for pairwise combinations of parental genotypes; values within the table margins represent similarity values averaged for a given male or female parent

Female	Male								Female mean
	S646 (ONT)	95061 (NY)	95316 (PA)	95019 (NY)	95024 (NY)	95022 (NY)	95054 (NY)	S287 (ONT)	
96305 (PA)	0.45		0.57	0.59	0.55				0.54
S652 (ONT)	0.54	0.37	0.40	0.50	0.36	0.25			0.40
S25 (ONT)			0.40	0.38	0.35	0.25	0.43	0.44	0.38
95331 (PA)				0.58	0.57	0.55	0.41	0.49	0.52
95064 (NY)	0.38	0.42				0.50	0.34	0.52	0.43
95306 (PA)	0.38	0.32					0.38	0.40	0.37
95311 (PA)		0.43	0.69	0.51				0.69	0.58
Male mean	0.44	0.39	0.52	0.51	0.46	0.39	0.39	0.51	

Table 3 Pearson correlation coefficients (probability values in parentheses) between similarity indexes calculated from AFLP data on 15 *S. eriocephala* parents and height growth of progeny

Variables	Similarity index		
	Parental pairs	Male average	Female average
Height	0.17 (P = 0.33)	<0.01 (P = 0.99)	–0.39 (P = 0.38)
Standard deviation	–0.27 (P = 0.11)	–0.22 (P = 0.61)	–0.88 (P = 0.009)
Coefficient of variation	–0.13 (P = 0.46)	–0.21 (P = 0.60)	–0.41 (P = 0.35)

for clone 95019; the least was 16, for clone S646. Twenty-eight of the 38 fragment types detected were polymorphic, while five of the fragment types were amplified from only one parental clone.

Similarity indexes were calculated from 77 polymorphic fragment types amplified using the two AFLP primer combinations. The average similarity index for parents was 0.45, with a low of 0.25 between S25 and 95022 and between S652 and 95022 and a high of 0.69 between 95311 and 95316 and between 95311 and S287 (Table 2). Based on ANOVA results, mean similarity indices among female parents, averaged across male parents, differed significantly ($P < 0.01$) and ranged from 0.37 for female 95306 to 0.58 for female 95311 (Table 2). Mean similarity indexes were not significantly different among male parents averaged across female parents, ranging from 0.39 for male 95061 and 95054 to 0.52 for male 95316 (Table 2).

Pearson's correlation coefficients were calculated between the similarity indices and height growth metrics described above (Table 3). Significant correlation coefficients were detected between the standard deviation for height growth by half-sib progeny of each female parent and female mean similarity indices (r = –0.88, P = 0.009). All other correlations, including those between full-sib and male mean similarity indices and tree height or the coefficient of variation for tree height, were low and non-significant (Table 3).

Discussion

Results of AFLP fingerprinting in this study suggest that there was, in general, large variability among *S. eriocephala* parents. Seventy-seven polymorphic loci were detected in the 15 *S. eriocephala* parents using only two AFLP primer pairs. More than 84% of the fragment types were polymorphic. Every parent could be distinguished with either primer pair alone. These results were similar to those reported for clones of *Salix viminalis* and its hybrids grown for bioenergy in Europe, where AFLP analyses of 29 willow clones with five AFLP primer pairs yielded 919 different fragment types, of which 752 (81.8%) were polymorphic (Barker et al. 1999). The high percentage of polymorphic bands detected in willows in AFLP experiments reflects the low level of domestication in this genus. Large molecular genetic variation was expected in the current study because *S. eriocephala* is an outcrossing species, and maintains large genetic variation within populations and high individual-tree heterozygosity (Aravanopoulos and Zsuffa 1998). Furthermore, *S. eriocephala* has a widespread geographic distribution, widely dispersed seeds, and both sexual and asexual reproduction, which are characteristics that tend to be associated with large genetic diversity (Hamrick et al. 1992).

Although the number of polymorphic bands used to estimate similarity among parental clones was relatively small in this study, there appears to be sufficient polymorphism to provide reliable estimates of genetic diversity and possibly genetic complementation among the tested parental combinations. The number of polymorphic bands necessary to accurately discriminate among individuals is determined by the degree of relatedness among individuals being compared, with distantly related individuals requiring relatively few bands (Tivang et al. 1994). Genetic similarity between inbred maize lines was most accurately estimated with 150 polymorphic AFLP bands, but good estimates could be obtained until less than 50 bands were used (Pejic et al. 1998). Between

82 and 140 polymorphic AFLP markers were sufficient to accurately estimate similarities among rice accessions (Zhu et al. 1998). The number of polymorphic bands necessary to accurately estimate similarity among willows in this experiment should be relatively low because of the broad geographic range from which parents were selected and the high degree of genetic variation that exists in this species.

Considering the large number of polymorphic bands detected per AFLP experiment for willow, and that one of the criteria for selecting parents in this study was that they were from geographically distant areas, the high similarity values for some parent combinations were surprising. A wide range in similarity indices (0.25–0.69) was calculated among pairs of parents in this study. Two of the parents, clone 95311 and clone 95316, yielded a high similarity index (similarity index = 0.69) and were collected from wild stands separated by only 60 km. Parents with an identical similarity index, clone 95311 and clone S287, were from wild stands separated by several hundred kilometers (Table 2). Speculatively, these observations may be associated with the relatively low number or the particular set of alleles sampled, or the number of parents that were tested.

The amount of variability in juvenile height growth was not uniform across families in this study, and the observed variability appears to be due, at least in part, to genetic causes. Limitations in the experimental design, especially the small number of progeny per family, and differing numbers of progeny per family, may have influenced the observed amounts of variability. The standard deviation for height growth of family 9812 (SD = 5.1) was the largest in the experiment and twice that of the family with the smallest standard deviation (family 985, SD = 2.5). The large standard deviation observed for family 9812 may be related to the small number of progeny sampled compared with other families. However, most of the 34 families studied in this experiment had similar numbers of progeny, permitting unbiased comparisons of family standard deviation. Several families with numbers of trees comparable to family 985 had standard deviations exceeding that of family 985 by more than 80%, indicating that differences in family standard deviations were likely to be due to genetic causes.

Correlations between similarity index and height growth were generally low and not significant, suggesting that progeny from parents that differed in AFLP fingerprints did not on the average, grow better than progeny of parents with similar AFLP fingerprints. Certain parents consistently produced full-sib progeny that expressed above-average mean height (e.g., S646, Table 1). These parents were considered good general combiners and their AFLP-based similarity indices were not good predictors of their mean progeny performance. However, AFLP-based estimates of similarity were correlated with the half-sib progeny standard deviation for height. This 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. Clone 95306 represents such a parent, (Table 2) since progeny of this parent are in the top 5% of all progeny, although their mean family height is below average. The relationship between AFLP-based similarity and half-sib standard deviation can also be applied to those female half-sib families that have above-average performance (e.g., S25), as means and variances are independent measures of populations.

The large negative correlation between mean female parent similarity indices, and the standard deviation of height among half-sib progeny from those females, contrasts with the low correlation observed with male parents and their half-sib progeny. An explanation for this difference was not obvious. Both male and female parents significantly affected the height growth of their progeny, so it is unlikely that the significant correlation described above was spurious. The average similarity index for male parents averaged across all female parents was 0.48, while the average similarity index for female parents averaged across all male parents was 0.45, indicating that the amount of variation among males and females was similar. There was no less variation among male parents in terms of their performance or geographic origins than there was among female parents. Thus, it appears that this lack of difference in mean similarity value among male parents was due to the sampling error that occurred between males and the set of female parents they were crossed with, as well as the sampling error that may have occurred during the selection of the male clones. Increasing both the number of crosses per parent and the total number of parents used should reduce this error.

The low correlation between the similarity indices of specific parent combinations and standard deviations for the height of their full-sib progeny may be due to non-additive genetic effects, as suggested by the observed significant male-by-female interaction for juvenile height growth. Non-additive genetic effects contributed significantly to variation in the number of shoots and stem weight in *S. viminalis* full-sib families (Rönnberg-Wästljung et al. 1994).

Because greenhouse juvenile height growth may be a poor predictor of field performance, 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. Promnitz and Wray (1976) report that the field performance of hybrid poplars could not be accurately predicted based on the measurement of morphological variables of greenhouse-grown trees. Furthermore, tree height alone has been shown to be a poor predictor of willow biomass production (Ballard et al. 1999). In *S. eriocephala*, the correlation between variability in height growth and variability in biomass production within families is uncertain.

It is possible that some of the parents used in this study were not pure *S. eriocephala*. Natural hybrids with *Salix 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 and the 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. Comparisons of the number of unique AFLP fragments present in each of the 15 parental clones did not reveal any clones that appeared grossly different from the others, with the number of unique fragments ranging from zero to three. Molecular genetic analyses may be necessary to confirm that parental clones, putatively identified as *S. eriocephala*, are in fact pure *S. eriocephala*.

The large significant negative correlation between the female mean similarity index and the standard deviation of height growth (Table 3), suggests that using AFLP fingerprints to estimate the similarity index may be useful for predicting progeny variance for willow crosses. Confidence in these predictions must be tempered because of the low correlation between the similarity index and the coefficient of variation calculated on both the full-sib and half-sib basis, and the relatively small number of parental genotypes used to estimate the correlation. Tests with a larger number of parents, progeny per family, and AFLP primer pairs will be necessary to confirm the reliability of predicting the standard deviation with similarity indexes calculated from AFLP fingerprints.

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