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Leaf Morphological Variation and Heterosis on Hybrid Progenies of *Populus ussuriensis* and *P. simonii* × *P. nigra*

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ABSTRACT: Hybridization remains an important method for breeding new poplar varieties. It results in significant variation in leaf phenotype among parents and offspring, and among offspring themselves. This study aimed to investigate whether leaf shape variations were similar in offspring produced from reciprocal crosses. Specifically, two hybrid combinations were produced: the direct cross with *Populus ussuriensis* as the maternal parent and *P. simonii* × *P. nigra* as the paternal parent (HY53), and the reciprocal cross with *P. simonii* × *P. nigra* as the maternal parent and *P. ussuriensis* as the paternal parent (HY268). Using 3-month-old rooted cuttings from 40 clones (36 F₁ hybrids and their parents) growing in a greenhouse, we measured and analyzed 14 leaf morphological traits to assess genetic variation and heterosis. The results showed HY53 clones generally exhibited greater average height than HY268 clones. Leaf phenotypes differed between the two hybrid combinations, with significant differences observed among parents and offspring for almost all traits, as revealed by analysis of variance (ANOVA). The phenotypic coefficient of variation was higher in HY268 clones. Additionally, leaf traits demonstrated high repeatability. Notably, some hybrid offspring exhibited positive or negative mid-parent heterosis, as well as over-parent heterosis for certain leaf phenotypes. The systematic cluster analysis further indicated distinct separation among HY268 clones. This research provides valuable materials for poplar breeding and offers insights into hybrid vigor in wood plants. The findings highlight the importance of reciprocal crossing in influencing leaf phenotype variation and heterosis, offering practical insights for future breeding strategies.

KEYWORDS: Heterosis; reciprocal crosses; leaf phenotypes; *Populus* breeding

1 Introduction

Poplar (*Populus* spp.) is an economically and ecologically valuable genus predominantly growing in temperate regions worldwide [1], covering over 31.4 million hectares globally, with approximately 8.25 million hectares in China [2]. As conventional crossbreeding remains a primary method for poplar improvement, exploiting heterosis (hybrid vigor) is crucial for breeding new poplar varieties [3]. In natural settings, the *Aigeiros* poplars and *Tacamahaca* poplars could undergo natural hybridization and exhibit significant hybrid vigor [4]. Previous studies had shown that the *P. deltoides* × *P. trichocarpa* hybrids were significantly more productive than the *P. deltoides* × *P. nigra* hybrids, irrespective of site [5]. Now, *Populus simonii* × *P. nigra*



shows strong drought and salt tolerance, and it has been widely planted in the arid and cold regions of western Heilongjiang province [6]. *Populus ussuriensis* exhibited rapid growth and produced high-quality timber but had weak drought and salt-alkali resistance [7]. Therefore, leveraging the desirable traits of *P. ussuriensis* and *P. simonii* \times *P. nigra* through intersectional hybridization and gene recombination is highly valuable. The objective was to create new variations and breed superior varieties. This approach held significant practical significance in the development of poplar varieties that could withstand diverse environmental challenges.

Leaf morphology, a highly heritable trait, has significant phenotypic diversity in different species or different parts of the same species, even in different environmental factors [8]. Leaf shape and size were closely related to overall growth and biomass yield [9]. It served as a key indicator for early growth selection and heterosis prediction [10]. Understanding the inheritance pattern of growth traits was fundamental for breeding programs [11]. Leaf traits were strongly genetically controlled, and interspecific or intraspecific hybridization often resulted in significant variation in offspring, and previous studies had demonstrated that hybrid poplars often exhibited enhanced leaf traits relative to their parents. For instance, hybrids of *Populus trichocarpa* \times *P. deltoides* developed larger leaves than either parent, with the increased biomass attributed to inheritance of larger cell number from *P. deltoides* and larger cell size from *P. trichocarpa* [12]. Similarly, in *P. deltoides* \times *P. nigra* hybrids, greater total leaf area resulted in higher biomass productivity [13]. Additionally, in triploid progenies, leaf size and shape more closely resembled the female parent *P. trichocarpa* than the male *P. deltoides*. Triploid hybrids inherited two alleles from the female parent and one from the male parent at each locus [14]. However, the extent to which direct and reciprocal crosses influence leaf phenotypic inheritance in poplar remains insufficiently explored.

In this study, 19 HY53 clones (the direct cross with *P. ussuriensis* as the female and *P. simonii* \times *P. nigra* as the male) and 17 HY268 clones (the reciprocal cross with *P. simonii* \times *P. nigra* as the female and *P. ussuriensis* as the male) and their parents (4 clones) were used to measure leaf morphological traits. The aim of the study was to determine whether direct and reciprocal hybrids exhibited consistent performance in leaf morphology. This study could provide useful insights into maternal genetic effects and growth variation, and provide excellent materials for breeding new poplar varieties.

2 Materials and Methods

2.1 Experimental Materials

Flower branches of *P. ussuriensis* female and male (clones Pus 5 and Pus 8, respectively) were collected from Weihe Forestry Bureau, Shangzhi City, Heilongjiang Province, and male and female flower branches (clones Psn 3 and Psn 26, respectively) of *P. simonii* \times *P. nigra* were obtained from Northeast Forestry University. The hybrid combination with Pus 5 as the female and Psn 3 as the male was named HY53, while the hybrid combination with Psn 26 as the female and Pus 8 as the male was named HY268. The F₁ progeny seedlings were obtained through hybridization in the spring of 2022. In March 2024, 19 vigorous and disease-free individuals from HY53 and 17 superior clones from HY268 were selected and clonally propagated via stem cutting. The cuttings were planted in pots measuring 14 cm in diameter and 12 cm in height, filled with a growth medium composed of black soil, turf soil, and vermiculite in a 10:1:1 ratio. Each clone was replicated with twelve plants. All seedlings were cultivated in a greenhouse under natural light conditions, with temperatures maintained between 10°C and 20°C, and watered once per day.

2.2 Leaf Morphology Measurements

In June 2024, the tree height (H) for seedlings (at 3 months of age) was measured using a tower ruler. For leaf trait measurements, three healthy, uniformly growing plants without pests and diseases were selected for each clone. For each plant, one leaf among the 8th to 10th positions from the apex was collected. The petiole was retained, and each sample was put into sealed plastic bags, numbered, labeled, and transported to the laboratory for phenotypic analysis. Leaf fresh weight (LFW) and Leaf dry weight (LDW) were measured using an electronic balance with 0.1 mg precision, and leaf water content (LWC) was calculated accordingly. Leaf length (LL), leaf width (LW), and petiole length (PL) were measured using a ruler. Leaf index (LI) was calculated as the ratio of leaf length to width. The leaf area (LA) was estimated using the grid method [15]. Specific leaf area (SLA) was calculated as the ratio of leaf area to dry mass, serving as a major indicator of plants' relative growth rate [16]. Leaf tip angle (LTA) and leaf basal angle (LBA) were measured with a protractor. Meanwhile, the stomatal characteristics, including stomatal length (SL), stomatal width (SW), and stomatal density (SD), were examined under an optical microscope. For these measurements, three trees per clone were randomly selected, and one leaf from the 4th to 6th leaf below the apex was sampled from each tree. For each clone, 30 observations of stomatal lengths and stomatal widths were recorded. Stomatal density was assessed based on three replicate measurements per leaf.

2.3 Statistical Analysis

Statistical analysis was carried out using SPSS 25.0 software. Analyses of variance (ANOVA) for different traits among clones were performed according to the following linear model, and *F* tests were performed to estimate the significance of ANOVA, and a 0.05 significance level was used to test the significance of the differences among clones [17]:

$$Y_{ij} = \mu + C_i + e_{ij}$$

where Y_{ij} was the phenotypic value of the j th individual in clone i , μ was the overall mean, C_i was the effect of clone i th, and e_{ij} was the random error.

The coefficient of phenotypic variation (PCV) was calculated using the formula [18]:

$$PCV = \frac{SD}{X}$$

where X and SD were respectively the phenotypic mean and standard deviation of the trait.

Repeatability (R) was estimated following Yin et al. [17] as follows:

$$R = 1 - \frac{1}{F}$$

where F value was from the analysis of variance.

The percentage of heterosis was estimated with two methods, the mid-parent heterosis (Hm) and the over-parent heterosis (Ho) [19]. Hm was calculated based on the mean value of both parents, while Ho was calculated based on the optimal phenotype value of both parents [20].

$$Hm = \frac{F_1 - 1/2(P_1 + P_2)}{1/2(P_1 + P_2)}$$

$$Ho = \frac{F_1 - P_s}{P_s}$$

where F_1 was the mean performance. P_1 and P_2 were the mean values of the two parents, respectively. P_S was the value of the better parent.

Using raw data of different leaf traits without standardization, hierarchical clustering analysis was used to group different clones based on multiple leaf phenotypes [21].

3 Results

3.1 Traits Variations and Genetic Parameters

P. simonii × *P. nigra* leaves were diamond-shaped, while those of *P. ussuriensis* were oblong. The leaf phenotype of HY53 clones closely resembled that of *P. ussuriensis*, while HY268 clones showed greater morphological divergence from their parents (Fig. 1). Significant variations in leaf morphological traits were observed among offspring of both HY53 and HY268 clones (Table 1). No significant differences were detected in LDW, LA, SLA, LTA, and SD between the parents Pus 5 and Psn 3. Similarly, traits including LFW, LWC, LA, SLA, LTA, SD, and SL did not differ significantly between Psn 26 and Pus 8. The descriptive statistics, together with phenotypic coefficients of variation (PCV) and repeatability (R) were presented in Table 2. Among Pus 5 and Psn 3, the PCV value ranged from 4.78% (SLA) to 77.61% (PL), while for Psn 26 and Pus 8, they varied from 3.26% (LWC) to 86.35% (PL). For the offspring, PCV ranged from 4.34% (LBA) to 36.54% (PL) in HY53, and from 3.24% (LWC) to 45.74% (PL) in HY268. Compared to Pus 5 and Psn 3, Pus 8 and Psn 26 showed higher PCV value for most traits, except for LWC, LL, SD, and SL. Similarly, PCVs were generally higher among HY268 clones than HY53, except for LWC and SLA. Related to parents, offspring exhibited higher PCV in LFW, LDW, LA, SLA, and SD, but lower values in LI, PL, and LBA. The repeatability (R) was consistently high ($R > 0.9$) for all traits across both parental combinations. Among the offspring, all traits showed high repeatability ($R > 0.730$), with HY268 clones generally exhibiting higher repeatability values than HY53.

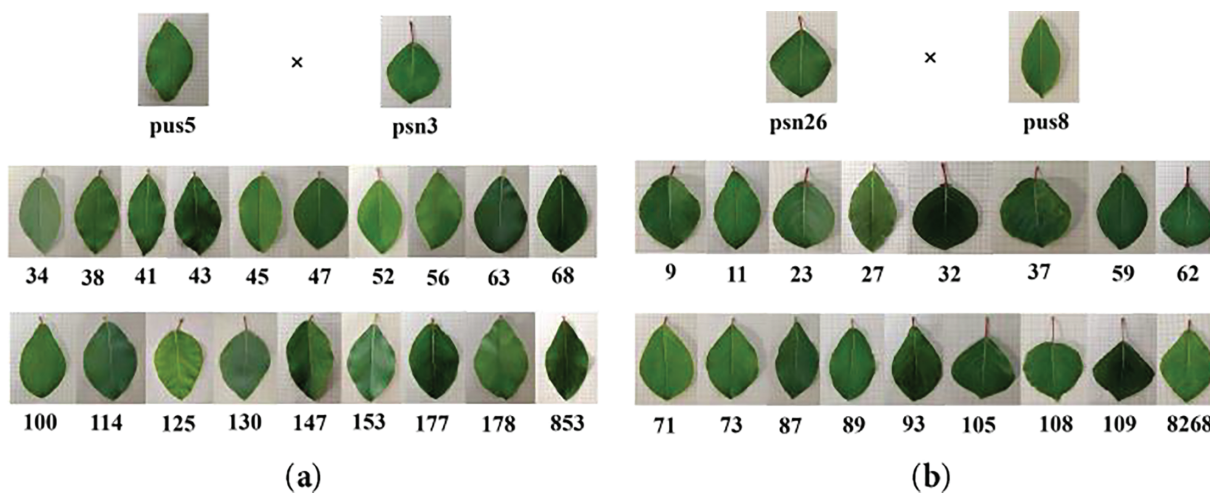


Figure 1: Leaf shape variation among parents and progeny of *P. ussuriensis* and *P. simonii* × *P. nigra*. (a): HY53. (b): HY268. Note: The solid line scale was 10 mm.

Table 1: Analysis of variations of leaf morphological traits for parents and offspring

Trait	Pus 5 and Psn 3 (df = 1)		HY53 (df = 18)		Psn 26 and Pus 8 (df = 1)		HY268 (df = 16)	
	MS	F	MS	F	MS	F	MS	F
H	2.667	0.124	516.436	15.696**	770.667	50.561**	1155.272	44.560**
		ns						
LFW	0.021	8.562*	0.025	3.934**	0.019	5.069	0.086	14.084**
		ns						
LDW	0.000	1.067	0.003	3.864**	0.002	21.600*	0.009	15.832**
		ns						
LWC	55.998	40.002**	28.564	4.552**	0.138	0.024	10.479	5.045**
		ns						
LL	12.907	91.106**	1.255	3.705**	2.535	16.533*	9.790	46.058**
LW	1.307	11.200*	0.717	4.346**	4.860	26.509**	2.030	10.001**
LI	0.939	107.108**	0.098	14.471**	1.335	189.784**	0.274	78.398**
PL	7.042	145.690**	0.281	9.211**	4.167	7.813*	0.821	22.259**
LA	13.500	1.588	429.610	8.006**	8.167	0.280	387.297	9.153**
		ns						
SLA	44.237	0.410	13,946.597	25.592**	1274.292	0.831	6023.369	13.299**
		ns						
LTA	352.667	3.062	307.318	4.387**	1232.667	2.616	1012.324	10.625**
		ns						
LBA	2166.000	58.541**	379.125	9.331**	2730.667	56.497**	1416.020	17.840**
SD	281,666.67	4.225	35,572,631.58	21.294**	135,000.00	2.793	58,371,764.71	69.167**
		ns						
SL	924.887	122.056**	101.188	17.322**	7.245	0.981	220.020	30.112**
		ns						
SW	55.507	16.081**	14.632	4.576**	78.501	19.640**	74.987	23.639**

Notes: H, height; LFW, leaf fresh weight; LDW, leaf dry weight; LWC, leaf water content; LL, leaf length; LW, leaf width; LI, leaf index; PL, petiole length; LA, leaf area; SLA, specific leaf area; LTA, leaf tip angle; LBA, leaf basal angle; SD, stomatal density; SL, stomatal length; SW, stomatal width; MS, mean square; F, F value in analyses of variance; ns, No significance; *, Significance at the 0.05 level (2-tailed); **, Significance at the 0.01 level (2-tailed).

Table 2: The genetic parameters of leaf morphological traits for parents and offspring

Trait	Pus 5 and Psn 3			HY53			Psn 26 and Pus 8			HY268		
	Average	PCV	R	Average	PCV	R	Average	PCV	R	Average	PCV	R
H	42.25	10.78%	–	47.11	17.92%	0.936	33.50	20.70%	0.980	39.46	27.17%	0.978
LFW	0.705	11.19%	0.883	0.650	17.09%	0.746	0.443	18.73%	–	0.507	35.11%	0.929
LDW	0.198	5.95%	–	0.198	17.94%	0.741	0.150	14.29%	0.954	0.165	34.00%	0.937
LWC	0.716	4.91%	0.975	69.42%	5.28%	0.780	0.659	3.26%	–	67.28%	3.24%	0.802
LL	8.433	19.46%	0.989	8.61	9.25%	0.730	6.683	11.87%	0.940	6.57	27.54%	0.978
LW	5.567	10.70%	0.911	5.27	11.11%	0.770	4.433	23.86%	0.962	4.98	17.81%	0.900
LI	1.551	28.46%	0.991	1.65	11.51%	0.931	1.605	32.53%	0.995	1.32	22.71%	0.987
PL	1.550	77.61%	0.993	0.91	36.54%	0.891	1.300	86.35%	0.872	1.17	45.74%	0.955

(Continued)

Table 2 (continued)

Trait	Pus 5 and Psn 3			HY53			Psn 26 and Pus 8			HY268		
	Average	PCV	R	Average	PCV	R	Average	PCV	R	Average	PCV	R
LA	40.500	7.61%	–	50.46	26.18%	0.875	28.833	17.33%	–	37.82	32.67%	0.891
SLA	204.056	4.78%	–	259.805	26.81%	0.961	194.867	19.76%	–	236.180	20.02%	0.925
LTA	92.333	13.81%	–	86.60	13.97%	0.772	86.667	28.81%	–	104.43	18.88%	0.906
LBA	279.00	7.71%	0.983	281.82	4.34%	0.893	282.00	8.58%	0.982	269.02	8.37%	0.944
SD	2983	11.10%	–	3370	24.79%	0.953	3083	8.31%	–	3157	34.75%	0.986
SL	27.279	17.63%	0.992	25.25	11.79%	0.942	25.499	10.66%	–	25.87	14.46%	0.967
SW	14.493	14.36%	0.938	13.63	13.84%	0.781	15.085	15.20%	0.949	13.98	16.67%	0.958

Notes: H, height; LFW, leaf fresh weight; LDW, leaf dry weight; LWC, leaf water content; LL, leaf length; LW, leaf width; LI, leaf index; PL, petiole length; LA, leaf area; SLA, specific leaf area; LTA, leaf tip angle; LBA, leaf basal angle; SD, stomatal density; SL, stomatal length; SW, stomatal width; PCV, phenotypic coefficients of variation; R, repeatability. The units of H, LFW, LDW, LL, LW, PL, LA, SLA, LTA, LBA, SD, SL, and SW were cm, g, g, cm, cm, cm, cm², cm²/g, °, °, /cm², µm, and µm. The same as follows. For indicators where the analysis of variance showed no significant differences, the calculation of repeatability was not conducted.

3.2 Heterosis

The average H for each clone was shown in Table 3. Among the HY53, Clone 153 exhibited the greatest height (60.92 cm), followed by Clone 130 and 125, whereas Clone 8268 showed the lowest H value. The average H of the parental clones Psn 3 and Pus 5 was 41.92 and 42.58 cm, respectively, and the total average H of HY53 clones was 47.11 cm. For the HY268 family, the parental clones Psn 26 and pus 8 had average heights of 51.50 and 34.00 cm, respectively, and the total average H of HY268 clones was 39.46 cm. Mean values for leaf traits across clones were provided in Supplementary Table S1 (HY53) and Table S2 (HY268). Among HY53 offspring, the female parent Pus 5 exhibited higher value in LWC, LL, LI, and LBA, but lower PL compared to its progeny. The male parent Psn 3 showed greater LW, PL, LTA, SL, and SW related to the offspring, though its LL and LI were lower. Mid-parent heterosis for leaf traits ranged from –41.29% (PL) to 27.32% (SLA). Six traits, including SLA, displayed positive mid-parent heterosis, while eight traits, including PL, showed negative values. Most traits exhibited negative over-parent heterosis, except for LA, SLA, and SD. In the HY268 population, the female parent Psn 26 had notably high petiole length, while Pus 8 showed higher LI and LBA, but lower LW, PL, and LTA. Among the offspring, Clone 93 displayed the highest values for LFW, LDW, LWC, LL, LW, LA, and LBA, whereas Clone 108 showed the lowest value across multiple traits, including LFW, LDW, LL, LW, LA, LTA, and LBA. Mid-parent heterosis in HY268 ranged from –17.76% (LI) to 31.16% (LA), with nine traits showing positive and five traits showing negative values. Positive over-parent heterosis was observed in LFW, LWC, LA, SLA, LTA, and SL, while the remaining traits exhibited negative value. Overall, hybrid progenies showed consistent heterotic patterns: LA, SLA, and SD displayed positive mid-parent heterosis, whereas PL and SW showed negative mid-parent heterosis. For over-parent heterosis, LA and SLA were positive over-parent heterosis, while LDW, LL, LW, LI, PL, LBA, and SW were negative over-parent heterosis.

3.3 Cluster Analysis

To investigate the leaf morphological variation among 36 hybrid offspring and their parents, hierarchical cluster analysis was performed. Initially, clustering analysis based on all traits revealed that extreme values—such as those of leaf fresh weight—caused certain clones with divergent leaf shapes to group together. Since the study focuses primarily on leaf shape variation, subsequent clustering incorporated the following morphological traits: leaf length, leaf width, leaf area index, petiole length, leaf tip angle, and leaf base angle.

Using Euclidean distance, the hierarchical clustering result for 40 poplar clones was presented in Fig. 2. At a genetic distance of 15, the clones were grouped into three major categories: Class I comprised 33 clones, Class II contained 6 clones, and Class III consisted of a single clone (108). All 19 HY53 clones were classified into Class I, while all 6 clones in Class II belonged to the HY268 family. HY53 offspring had leaf phenotypes more similar to the maternal parent (Pus 5). At a genetic distance of 10, Class I was further divided into three subgroups. Subclass I-1 included Pus 5 and 24 progeny clones. Subclass I-2 contained Clone 105, 109, 125, 130, along with parents Psn 3 and Psn 26. Subclass I-3 consisted of Clone 41 and Pus 8. The mean values of leaf phenotypic traits for each group and subclass were summarized in Table 4. The average leaf length in Class I was significantly greater than that of Class II and Class III. Clone 108 (Class III) exhibited the smallest leaf base angle (213.33°), which was significantly smaller than the overall mean (276.25°) and the average of both Class II and Class III. Further comparison among subclasses within Class I revealed that Subclass I-2 had smaller leaf length (6.98 cm) and leaf basal angle (259.50°), but greater petiole length (1.98 cm). In contrast, Subclass I-3 displayed smaller leaf width (3.97 cm), and larger leaf index (2.12) and leaf basal angle (301.33°).

Table 3: The average height for different clones

Gene	H	Gene	H
34	52.33 ± 5.28	9	23.75 ± 7.07
38	47.25 ± 7.70	11	42.33 ± 6.72
41	46.58 ± 5.71	23	33.33 ± 5.53
43	47.33 ± 7.63	27	49.83 ± 3.64
45	43.50 ± 7.19	32	53.83 ± 2.92
47	46.00 ± 5.56	37	30.83 ± 6.59
52	48.42 ± 8.70	59	41.75 ± 5.26
56	35.58 ± 3.23	62	42.08 ± 4.10
63	43.33 ± 4.25	71	36.25 ± 3.47
68	47.08 ± 6.96	73	34.42 ± 5.02
100	41.50 ± 4.98	87	45.33 ± 6.14
114	39.17 ± 6.06	89	53.58 ± 5.60
125	55.00 ± 2.95	93	49.75 ± 3.05
130	56.92 ± 3.99	105	37.58 ± 5.42
147	36.17 ± 5.39	108	37.17 ± 4.24
153	60.92 ± 5.05	109	40.92 ± 5.47
177	47.50 ± 5.33	8268	18.00 ± 3.54
178	50.83 ± 4.43	average	39.46 ± 10.72
853	49.67 ± 4.79		
average	47.11 ± 8.44		
psn3(σ^7)	41.92 ± 5.11	psn26(φ)	51.50 ± 1.00
pus5(φ)	42.58 ± 4.12	pus8(σ^7)	34.00 ± 3.16

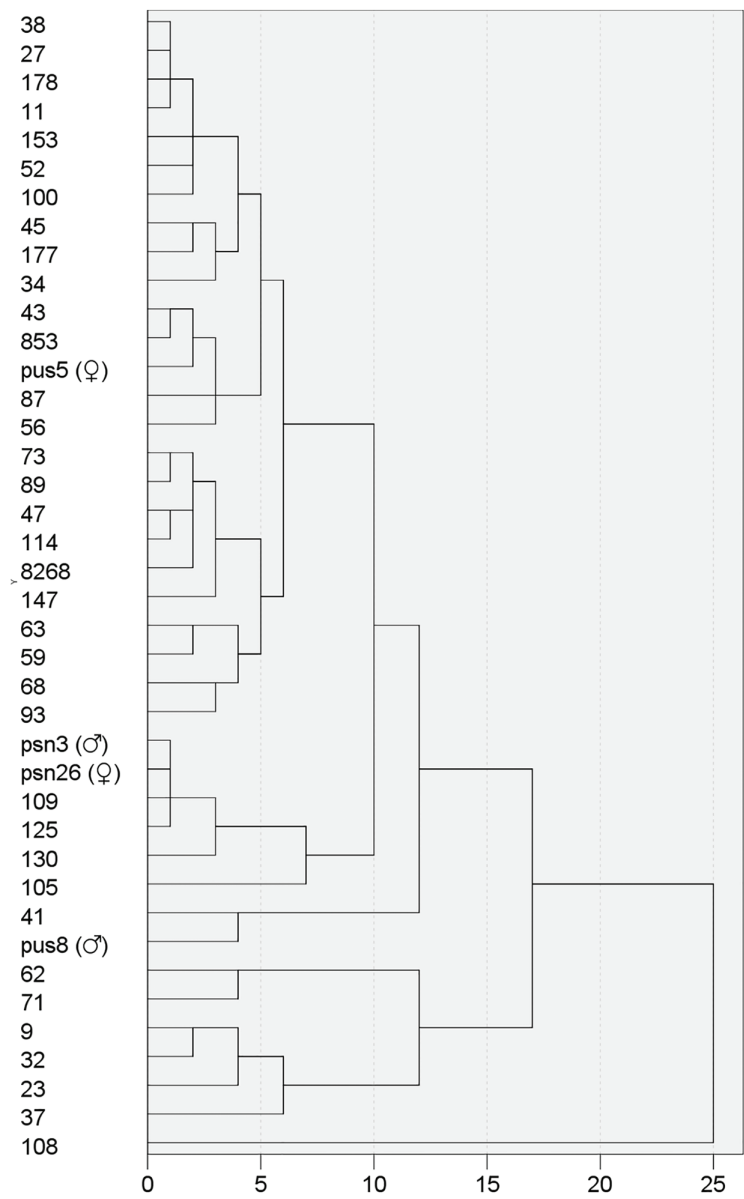


Figure 2: Clustering analysis of F₁ generation and their parents based on leaf phenotypic traits

Table 4: Mean value of leaf phenotypic traits among different groups

Group	LL	LW	LI	PL	LTA	LBA
Class I-1	8.42	5.15	1.64	0.85	89.16	284.64
Class I-2	6.98	5.89	1.19	1.98	97.39	259.50
Class I-3	8.38	3.97	2.12	0.62	67.33	301.33
Class II	5.36	4.83	1.11	1.15	126.34	260.17
Class III	4.07	3.80	1.07	1.80	72.67	213.33

4 Discussions

4.1 Variation in Leaf Morphology for F_1 Progenies and Parents

In China, the poplar varieties widely utilized in forestry production were predominantly developed through artificial cross-breeding. Since its initiation in the 1940s, cross-breeding programs have successfully selected and cultivated improved poplar varieties in major growing regions, yielding substantial economic and social benefits [22]. Cross-breeding not only enriched forest tree germplasm resources but also combined favorable traits from both parents, facilitating the development of novel superior varieties. Thus, it remained a vital strategy for poplar breeding both currently and in the foreseeable future. In this study, F_1 hybrid progenies were generated through reciprocal crosses between *P. ussuriensis* and *P. simonii* \times *P. nigra*, and their leaf traits were systematically measured and analyzed. Analysis of variance (ANOVA) revealed significant differences in all leaf morphological traits among clones, indicating substantial phenotypic variation. The average tree height for HY53 progeny was higher than that of HY268, this result was similar to the findings of Liu et al. [23], who reported that over 17 years, the *Populus deltoides* \times *P. maximowiczii* (D \times M) population exhibited significantly larger diameter at breast height (DBH) and tree height than the reciprocal cross (M \times D). The phenotypic coefficients of variation exceeding 20% were observed for traits including LFW, LDW, LL, LI, PL, LA, SLA, and SD, reflecting broad genetic variability in the F_1 population. Such variations provided a promising basis for subsequent selective breeding of superior genotypes. Notably, HY268 clones exhibited higher PCV values for nearly all traits compared to HY53, except for LWC and SLA, suggesting greater segregation of characteristics within the HY268 group. Additionally, the repeatability for leaf traits was high for both hybrid combinations, with all values exceeding 0.730—a pattern previously reported in hybrid progeny of *Populus deltoides* [24] and consistent with high repeatability [25]. The higher R values observed in HY268 clones further indicated that these traits were strongly genetically controlled and suitable for early selection [26].

4.2 Heterosis in Leaf Morphology for F_1 Progenies

Hybridization can lead to heterosis (hybrid vigor), in which hybrids outperform their parents [27]. In trees, heterosis has been extensively studied in growth traits due to its economic importance [28]. Tree species are highly heterozygous, and F_1 hybrid population typically exhibits significant segregation and variation in phenotypic traits [29]. Furthermore, the range of leaf shape variation in hybrid progeny was largely influenced by phenotypic divergence between the parents [14], highlighting its potential utility in breeding programs aimed at exploiting heterosis. In this study, the leaves of *P. simonii* \times *P. nigra* were broad and short, while those of *P. ussuriensis* were narrow and long. Analysis of leaf morphological traits revealed that most traits exhibited negative over-parent heterosis (H_o), indicating that hybrid performance was lower than the superior parents for traits such as LDW, LL, LW, LI, PL, LBA, and SW. Similarly, negative mid-parent heterosis (H_m) reflected hybrid weakness [30]. For instance, H_m for LL and LW in HY53 clones was 2.07% and -5.30%, respectively, whereas in HY268 clones, it was -1.65% and 12.42%, respectively. These results suggested that leaf dimensions were strongly influenced by the maternal parent, consistent with findings in tea [31], underscoring the role of maternal inheritance or cytoplasmic factors. Notably, LA and SLA displayed significant positive over-parent heterosis, indicating a potential morphological advantage inherited from parental lines. Similar parental leaf shape contrasts—e.g., small rhombic-ovate leaves in *P. simonii* and large triangular leaves in *P. nigra* [32]—had been reported, with hybrid leaf area often showing a paternal bias in frequency distribution [33]. Whether these morphological advantages translated into functional benefits, such as enhanced growth, remains unclear. Future studies should therefore integrate functional-morphological experiments, such as A-Ci curve analyses and resource-use efficiency assessments, to evaluate the physiological and ecological implications of these leaf traits. It should also be noted that heterosis

expression was often context-dependent and influenced by environmental conditions [5]. This study was conducted under controlled greenhouse conditions with seedlings of a specific age, which might not fully represent trait expression under field environments or across development stages. Future validation using larger populations, multiple environments, and mature plants was warranted. Furthermore, future research should employ genomic approaches, such as quantitative trait locus (QTL) mapping or genome-wide association studies (GWAS) on this population, to identify the specific chromosomal regions and candidate genes controlling these leaf traits. Integrating genomic validation, field performance, and physiological assessments will be crucial for translating these findings into applied breeding strategies.

4.3 Cluster Analysis for Leaf Morphology for *F₁* Progenies and Parents

Cluster analysis divided the 36 progeny and their parents into three groups. Class III contained only Clone 108, which was morphologically distinct due to its significantly smaller leaf basal angle and inverted triangular leaf shape. All 19 HY53 clones were clustered within Class I, whereas the 17 HY268 clones were distributed across groups, reflecting greater leaf shape variation within this cross. Previous studies had also reported that complementary inheritance of leaf traits occurred in poplar hybrids. For example, when hybridized with the same maternal parent (*P. deltoides*), progeny sired by *P. trichocarpa* displayed significant larger leaf area than either parent, whereas those sired by *P. nigra* exhibited intermediate leaf area [5]. Ridge et al. [12] similarly observed transgressive leaf area in *P. trichocarpa* × *P. deltoides* hybrids. Strong maternal effects had been reported in crosses between *P. deltoides* and *P. simonii*, where hybrid leaf morphology closely resembled the maternal parent, though leaf size showed paternal influence [34]. In contrast, no pronounced leaf morphology differences were detected between direct and reciprocal crosses of *P. tremula* and *P. alba* [35], suggesting genetic architecture of leaf traits was cross-specific. Although genome-wide association analysis (GWAS) had been applied to leaf shape traits in poplar [36], no significant SNPs were identified for leaf length, width, area, or aspect ratio, indicating these traits may be controlled by polygenic or non-genomic mechanisms.

5 Conclusions

In this study, the leaf phenotypic characters of 36 hybrid offspring and their parents were measured, which revealed significant genetic variation and heterosis. Reciprocal hybrids showed maternal influence and trait segregation, with several traits demonstrating positive or negative over-parent heterosis. These findings enhance our understanding of early-stage leaf morphology variation and heterosis in poplar, and also provide valuable genetic material for poplar breeding programs.

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