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Estimation of Combining Ability, Genetic Components and Heterosis in morphological characters of Line × Tester in *Populus deltoides* Bartr.

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ABSTRACT

The present experiments were carried out in the Department of Tree improvement and Genetic Resources, Dr. Y.S.P.U.H.F, Nauni, Solan. The control crossing was carried out in the poplar clones and progenies were evaluated for the morphological characters. The overall performance of G-48 x L-17/92, L-62/84 x S_7C_1, L-62/84 x L-17/92, G-48 xL-124/86 and S_1x S_7C_{11} hybrids were found outstanding for most of the morphological traits. The high significant variances were recorded for most characters in the hybrids. The estimates of SCA were found to be more than the GCA variance for all the characters studied and the ratio of GCA to SCA was found less than unity. In gene action study, dominance variance was observed to be more than that of additive variance as such the ratio of additive genetic variance/ dominance genetic variance was less than unity for all parameters studied whereas lines contribution found to be more than individual contribution of testers or line × tester interaction except for plant height, collar diameter, internodal length, leaf area, maximum width of leaf, shoot bark thickness, root bark thickness, fresh shoot weight, dry shoot weight, dry root weight, root length, total fresh weight and total dry weight.

Key Words:

Combining ability, Crossing, Gene action, Hybrids, Morphological

INTRODUCTION

Populus deltoides (Poplar) is widely used agroforestry tree and have strategic interest in many Northern states of India. It is one of the most popular tree species in the agroforestry system in irrigated plains of Uttarakhand, Western Uttar Pradesh,

Punjab and Haryana. The wood of Poplar is in great demand for paper and pulp, matchwood, plywood, packing cases and light constructional timber all over the world. It plays significant role in national economics, which was the major reason for their study by agronomist, geneticists, breeders and tree scientist (Sharma et al. 2019).

Clones of *P. deltoides* were introduced in India in 1952 to increase the availability of raw material for plywood industries in country. Improved clones *i.e.* G-48 and S_7C_8 currently form the backbone of the Poplar which has been spread in large area of North India. But the major risk associated with these clones is susceptibility to some adverse climatic conditions and disease or pest which may fail after few years (Kadam 2002). Therefore, a large number of clones are urgently required in assembly which will serve as replacement for unpromising clones in our country. So, for long term improvement program, continuous efforts are to be made for selecting new clones and their field testing for desirable output (Sharma et al. 2019)

Keeping in view, the ecological and economical importance of *P. deltoides,* lot of work has been made on cultivation aspects (Bhardwaj et al. 2001, Panwar et al. 2017, Bishnoi and Chauhan 2020)) but meager efforts has been made on the genetic improvement specially through control breeding in India. In control crosses, sufficient amount of variability could be expected which helps in the selection of Poplar clones on the basis of qualitative and quantitative characters and also their mass multiplication can increase species productivity (Dobhal et al. 2017; 2018). It has been reported that the crossing among different sources of trees can result in the superior inter racial F_1 families (Sharma et al. 2018).

Number of genetic markers are used nowadays to monitor the efficiency of various tree improvement activities such as genetic diversity, genetic fidelity analysis within and among wild or generated populations, identification of individuals at a young age that will express a trait at maturity and are also necessary for the construction of genetic linkage maps for aiding breeding. Basic genetic studies such as right from growth, cross ability pattern, estimate of genetic parameters of traits of interest, productivity and adaptability to produce are very much needed. The combination of traditional breeding with modern molecular research could advance genetic improvement of the genus (White et al. 2007).

MATERIALS AND METHODS

Plant material

The experimental was carried out at Dr. YSPUHF, Nauni, Solan, H.P during 2014-15. Experiment site is located between 30˚ 51' N latitude and 76˚ 11' E longitude at the elevation of 1200m above mean sea level. The area experiences wide range of temperature range i.e. minimum of 2˚C in winters to a maximum of 32.6˚C in the summers. The experiment was conducted in the nursery consisting of well drained and sandy loam type soil with pH of 7.2. The flowering branches of male ($S_7C_{11,}$ L-124/86, L-17/92 and S_7C_1) and female (G-48, $S_{1,}$ S_7C_8 and L-62/84) clones were obtained from the Uttarakhand, State Forest Department, Haldwani and Haridwar. The flowering branch of male clones were kept in water buckets to get sufficient amount of pollen for hybridization; whereas flowering branches of female clones were grafted individually on root stock of *Populus deltoides* under moist conditions.

To accomplish artificial pollination, pollen grains were removed from male catkins during anthesis. The pollen grains from each male clone were *in-vitro* tested for viability and used on female clones at stigma receptivity stage. After controlled pollination (single pollen and no pollen mixture), the flowers were bagged and tagged, from which mature seeds were harvested and sown immediately. The F_1 populations of successful crosses were uniformly grown under the environmental conditions, from which 5 best performing

individuals were selected and the progeny was cloned. The cutting of all selected individuals was grown in the RBD in 3 replications at the experimental field. The sixteen F_1 hybrids were needed for Line × Tester (4 ×4 factorial) mating for experimental design using 4 males and 4 females but only twelve F_1 hybrids survived for evaluation in the nursery trial. These twelve survived F_1 hybrids were evaluated for various morphological characters. The experiment was conducted in the nursery, whereas, assessment of paternity verification using molecular marker (Simple Sequence Repeats) techniques was carried out in the procedure described by Sharma et al. (2019).

Verification of F_1 hybrids as well as parents using molecular marker

The cetyltrimethyl ammonium bromide (CTAB) method was used for genomic DNA isolation from young and healthy leaves of 12 hybrids and their parents. A set of 18 SSR markers (Table 1) were used for paternity verification (Samriti et al. 2019).

Statistical Analysis

Variability and genetic parameters

The genotypic, phenotypic and environmental coefficient of variation were calculated as suggested by Burton and De-vane (1953) and Pillai and Sinha (1968).

$$GCV\ (\%) = \frac{\sqrt{Vg}}{X} \times 100$$

$$PCV\ (\%) = \frac{\sqrt{Vp}}{X} \times 100$$

$$ECV\ (\%) = \frac{\sqrt{Ve}}{X} \times 100$$

Where,
GCV =Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, ECV= Environmental coefficient of variation, X=Population mean of character.

Heritability (Broad Sense)

Heritability in broad sense was calculated as suggested by Burton and De-Vane (1953) and Johnson *et al.* (1955).

$$H^2_{b.s} = \frac{Vg}{Vp} \times 100$$

where,
$H^2_{b.s}$ = Heritability (Broad sense)

Genetic Advance

The expected genetic advance at 5 per cent selection intensity was calculated using formula suggested by Lush (1940) and further modified by Burton and De-Vane (1953) and Johnson et al. (1955).

$$\text{Genetic Advance} = \left[\frac{Vg}{Vp}\right] \times \left(\sqrt{Vp}\right) \times K$$

where,
K = 2.06 (Selection differential at 5 per cent selection intensity) (Allard 1960)

Genetic Gain

Genetic gain was worked out using methodology suggested by Johnson et al.(1955) as per following formulae:

$$\text{Genetic Gain (\%)} = \frac{\text{Genetic Advance}}{\overline{X}} \times 100$$

Line x tester analysis

The replication wise mean values of F_1generation of 12 crosses for each trait were subjected to statistical analysis using the following model suggested by Kempthrone (1957) and Singh and Chaudhary (1985 and 2001).

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$

where,
Y_{ijk}=Observation of the crosses involving i^{th} line and j^{th} tester in k^{th} replication; μ = General mean (an effect to all the hybrids in all replications); g_i=General combining ability (GCA) effect of i^{th} line; g_j=General combining ability effect of j^{th} tester; s_{ij}=Specific combining ability (SCA) effect of the cross involving i^{th}line and j^{th} tester; e_{ijk}=Errors associated with ijk^{th} observation i= (1, 2, 3, 4), j= (5, 6, 7, 8), k= (1, 2, 3).

Estimation of general and specific combining ability effects

The GCA and SCA effects were obtained from the two way table of female v/s male parents in which each figure was total over replication. The individual effects were estimated as follows:

Table 1. Details of primers used in present study

S. No.	Primer Names	Sequences
1.	WPMS-03	FP-TTTACATAGCATTTAGCCTTTAGA RP-TTATGATTTTGGGGGTGTTATGGA
2.	WPMS-05	FP-TTCTTTTTCAACTGCCTAACTT RP-TGATCCAATAACAGACAGAACA
3.	ORPM-015	FP- CGTGAGTTTTGAGGCCATTT RP-CATGGAAAGGATCACCCACT
4.	PMGC-451	FP-AATTACAACCACTTTAGCATATTC RP-TGCCGACACATCACACATACC
5.	PMGC-325	FP- CGATTTATGACAGACAGCTTG RP-GTACCGTTGAGGTGGCTAG
6.	PMGC- 333	FP-CTTAGTGGTGAAGTATTC RP-GAG TGGGTGCTGATTCATCC
7.	PMGC- 409	FP-ACGTATATGAAGTTCTTGATTGC RP- GACAGATCATTATGATTACTACAG
8.	PMGC- 420	FP-ATGGATGAGAAATGCTTGTG RP-ACTGGCACACGCTTTAACTGG
9.	PMGC- 422	FP-AACCTCGAATTAAGAATAACCC RP- GTCTCGGTTAAGGTATTGTCGC
10.	PMGC- 433	FP-GCAGCATTGTAGAATAATAAAAG RP- AAGGGGTCTATTATCCACG
11.	ORPM-026	FP- GCTGCAGTCAAATTCCAAAA RP- CGAGCGTCTTCTTCATGGAT
12.	PMGC- 562	FP-TTTTGGGAGGGGAGTCGAG RP-ACAACTCTCAACTTCCTAAC
13.	PMGC- 571	FP-CTGGTACCGATGGAGAAGAC RP-CAAACCAACAACTCACCGTAC
14.	PMGC- 2020	FP- TAAGGCTCTGTTTGTTAGTCAG RP-GAGATCTAATAAAGAAGGTCTTC
15.	PMGC- 2060	FP- CTCTCAAATGCTGATTTACCG RP-TCTTCAGTTGCAGTATTCAAAG
16.	PMGC- 2140	FP- GCTGTCAGAATCAAACACTTC RP- AAGCAGATAACTAAGACATGCC
17.	PMGC- 2143	FP- TCATCATCCATTACTCAACTTG RP- TCATCATCCATTACTCAACTTG
18.	PMGC- 2163	FP- CAATCGAAGGTAAGGTTAGTG RP- CGTTGGACATAGATCACACG

GCA effect of i^{th} lines (g_i) = $\frac{y_{i..}}{rt} - \frac{y...}{Rlt}$

GCA effect of j^{th} testers (g_j) = $\frac{y_{.j.}}{rl} - \frac{y...}{Rlt}$

SCA effect of testers(s_{ij}) = $\frac{y_{ij}}{R} - \frac{y_{i..}}{rt} - \frac{y_{.j.}}{rl} + \frac{y...}{Rlt}$

Significance of different effects was tested by 't' test

SE for GCA effects of lines = $\sqrt{\frac{M_e}{rt}}$

SE for GCA effects of testers = $\sqrt{\frac{M_e}{rl}}$

SE for GCA effects of line X tester = $\sqrt{\frac{M_e}{r}}$

Test of significance

't' calculated values were worked out as follows:

$$\text{'t' value} = \frac{\text{GCA}}{\text{SE}_{\text{GCA}}}$$

$$\text{'t' value} = \frac{\text{SCA}}{\text{SE}_{\text{SCA}}}$$

The 't' calculated values for GCA and SCA were compared with 't' table values at error degree of freedom and P = 0.05. The 't' calculated values > 't' table values were marked as significant and asterix was put on those values only.

Estimation of variance components

The covariances of full sibs (FS) and half sibs (HS) were calculated as methodology suggested by Singh and Chaudhary (1985).

Individual environment

Cov (H.S.) = $\sigma^2 l$(lines) = $\frac{(M(l) - M(lt))}{M(t)}$

Cov (H.S.) = $\sigma^2 t$ (testers) = $\frac{(M(t) - M(lt))}{M(l)}$

$\sigma^2 lt$(line x tester) = $\frac{Mlt - Me}{r}$ = σ^2SCA

Estimation of Cov HS (average) and Cov (FS)

Cov HS (average) = $\frac{(t\sigma_l^2 + l\sigma_t^2)}{(l+t)}$

CovFS (average) = σ_{lt}^2 + 2 Cov (HS)

These can also be calculated from the expected mean squares as:

Cov HS (average) = $\frac{(M\,l + M\,t - 2\,M\,lt)}{r\,(l+t)}$

CovFS = $\frac{M\,l + M\,t + M\,lt - 3Me}{3r}$ + $\frac{6r\,Cov\,(HS) - r\,(l+t)Cov\,(HS)}{3r}$

Estimation of GCA and SCA variances

From the estimation of Cov (HS) and Cov (FS), variance due to GCA and SCA were calculated as:

Variance of GCA = Cov. HS (Covariance of half sibs) = $\frac{(M\,l + M\,t - 2\,M\,lt)}{r\,(l+t)}$

Variance of SCA = Cov. FS – 2 Cov. HS = $\frac{(Mlt - Me)}{r}$

Estimation of additive (σ^2A) and dominance (σ^2D) component of variances

Populus deltoides is a cross pollinating dioecious plant which does not suffer inbreeding depression so inbreeding coefficient F=0 is used in the further analysis.

Cov. HS = $\frac{1}{4}\ \sigma^2_D + \frac{1}{16}\sigma^2_{DD}$ + other forms of epistasis

Cov. FS = $\frac{1}{2}\ \sigma^2_D + \frac{1}{4}\sigma^2_H + \frac{1}{4}\ \sigma^2_{DD} + \frac{1}{8}\sigma^2_{DH} + \frac{1}{16}\ \sigma^2_{HH}$ + other forms of epistasis

Assuming there is no epistasis

σ^2_D (Additive genetic variance) = 4 Cov. HS or 4 σ^2 GCA

σ^2_H (Dominance genetic varience) = 4 [Cov. FS – 2 Cov. HS] or 4 σ^2 SCA

Percent contribution of lines, testers and their interactions

These were computed as per the formulae given by Singh and Chaudhary, 1985.

% contribution of lines = $\frac{SS\ (lines)}{SS\ (crosses)}$ x 100

% contribution of testers = $\frac{SS\ (testers)}{SS\ (crosses)}$ x 100

% contribution of lines x testers = $\frac{SS\ (lines\ x\ testers)}{SS\ (crosses)}$ x 100

Estimation of Heterosis

Heterosis was calculated in terms of percentage increase or decrease of a hybrid against its better control value with respect to individual character, hereafter called standard heterosis (Nadarajan and Gunasekaran 2008).

$$\text{Standard Heterosis} = \frac{F_1 - \text{better control}}{\text{better control}}\ X\ 100$$

Standard error for testing heterosis over better control = $\sqrt{\frac{M_e}{r}}$

Test of significance't' calculated values were worked out as follows:

't' value = $\frac{F_1 - \text{better control}}{SE}$

RESULTS AND DISCUSSIONS

Estimation of GCA and SCA effects

The results of present investigations reveled that tester L-17/92 and line S_1 was a good general combiner with significant positive GCA value for only three characters i.e. leaf area, fresh root weight and total fresh weight. The analysis showed that both lines and testers recorded non-significant

GCA effects for the characters *i.e.* collar diameter and number of leaves/plant (Table 2). The different characters *i.e.* plant height, collar diameter, internodal length, number of leaves/plant, petiole length, fresh shoot weight, dry shoot weight and total dry weight showed non-significant SCA effects. For fresh root weight, L-62/84 x S_7C_1having significant positive SCA effect which was best cross combination (Table 3). However, it is not necessary that parents having higher estimating of general combining ability effects would always give higher estimation of specific combining ability effects. Usually the highest estimated of specific combining ability effects were obtained from crosses involving the diverse parents. Sometimes specific interaction effects, most likely complementary of poor x poor cross indicated that a high magnitude of non-additive component was responsible for confirming the highest rank to the appropriate cross combination. Biabani et al. (2012) for analysis of specific combining ability in his study revealed that some hybrids of *Jatropha curcas*L. presented significant SCA effects for each trait. Moreover, the present results are strongly supported by the findings of Bisoffi (1993), Li and Wu (1996), Kadam (2002), Choudhary (2011) and Saresh (2013).

Estimation of genetic components of variance

In present results, the variability was estimated in terms of mean, range, genotypic and phenotypic coefficient of variation. Genetic parameters were worked out with regards to estimate genetic advance, genetic gain and heritability (broad sense) as per cent of mean. Among all the morphological characters, total fresh weight showed widest range of values (239.84 - 512.85 g and mean 388.57g), followed by fresh shoot weight (154.16 - 338.12 g and mean 268.34 g) indicating the extent of variation existing in the plants. Phenotypic coefficient of variation (PCV) was found to be maximum for shoot bark thickness (48.86%) followed by root bark thickness (48.46 %). Both high heritability and genetic gain were recorded in shoot bark thickness. Highest genetic gain (67.07%) was recorded for shoot bark thickness followed by leaf area (36.88%) and fresh root weight(24.84%) among all the characters suggesting that additive genetic effects would be effective for in the selection these traits (Table 4).Our findings are in conformity with the findings of Johnson *et al.* (1955) whom reported that heritability estimated along with expected gain is more useful and realistic than the heritability alone predicting the resultant effect for selecting the best genotype. Similar findings were reported by Singh (2002) in full-sib progenies of selected clones of *P. deltoides*.

Estimation of proportional contribution of lines, testers and their interaction

In quantitative genetics, genotypic value of an individual is determined by various types of gene actions such as additive, dominance and their interactions (Falconer 1989). Additive and dominance genetic variances are important to breeders in that, they are attributable as how far a particular trait is amenable to selection in segregating generations or is useful for hybrid development. The performance of an individual parent or the performance of specific parents to generate improved progeny can be predicted after characters with large amounts of additive variance have been identified. The proportional contribution of lines ranged from 50.85 % (fresh root weight) to 6.63 % (dry shoot weight), whereas for testers it ranged from 56.71 % (total dry weight)to 12.12 % (petiole length). However, the proportional contribution of line × tester interaction ranged from 56.15 % (plant height) to 26.76 % (fresh shoot weight) indicating the importance of combination of specific parents. The proportional contribution of lines interaction was higher than individual contribution of testers or line x tester interaction except for plant height, collar diameter, internodal length, leaf area, maximum width of leaf, shoot bark thickness, root bark thickness, fresh shoot weight, dry shoot weight, dry root weight, root length, total fresh weight and total dry weight where the interactions contribution was less (Table 5). Likewise, Cameron et al.

Table 2. Effect of different parents on general combining ability of morphological characters in *Populus deltoides*

Parents	General combining ability effects															
	Plant height (cm)	Collar diameter(mm)	Internodal length (cm)	No. of leaves/plant	Petiole length (cm)	Leaf area (cm^2)	Maximum width of leaf (cm)	Shoot bark thickness (mm)	Root bark thickness (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Root length (cm)	Total fresh weight (g)	Total dry weight (g)
Females																
G-48	10.57	0.99	0.006	3.18	-0.61 *	-20.82*	-0.25	-0.034	-0.08	-29.39	-2.59	-19.06*	-8.20*	-0.47	-49.69*	-17.53
S_1	-3.82	-0.41	-0.05	2.68	0.48 *	18.73*	0.11	-0.063	-0.03	20.51	1.43	18.43*	7.49*	2.36*	42.32*	14.44
S_7C_8	-23.52*	-1.24*	-0.31*	-1.34	0.53*	-18.83*	-0.56	0.243*	0.10	19.74	10.55	-4.94	-0.38	-0.57	7.36	16.41
L-62/84	5.40	-0.08	0.26*	-6.03*	-0.02	21.59*	0.59	-0.068	0.04	-7.54	-6.16	1.80	0.05	-1.55*	-3.06	-9.79
Males																
S_7C_{11}	-16.30	-1.16	-0.31*	2.44	-0.08	-38.79*	-0.63*	-0.080*	-0.14	-4.19	2.96	-8.56*	-4.37	2.82*	-11.99	-3.32
L-124/86	-16.35	-1.12	0.08	-3.74	-0.33	1.22	0.87*	0.027	0.20*	-67.20*	-38.91*	-9.70*	10.81*	-0.20	-77.61*	-59.13*
L-17/92	14.33	1.21	0.17	-0.72	0.29	23.38*	0.01	0.121*	0.15	47.95*	19.82*	11.07*	-12.03*	-1.71*	55.99*	36.12*
S_7C_1	20.32*	1.00	-0.001	3.40	0.03	9.59	-0.38	-0.163*	-0.38*	11.17	14.28	5.20	2.97	-0.49	22.42	21.43
SE	9.43	0.60	0.09	1.85	0.18	7.60	0.30	0.03	0.08	14.61	8.97	4.05	2.51	0.62	17.47	11.40
CD	26.87	1.71	0.25	5.27	0.51	21.66	0.85	0.08	0.22	41.63	25.56	11.54	0.05	1.76	49.78	32.49

* Significant at 5 per cent level of significance.

Table 3. Effect of different parents on specific combining ability of morphological characters in *Populus deltoides*

	Specific combining ability effects															
Crosses	Plant height (cm)	Collar diameter (mm)	Internodal length (cm)	Number of leaves/ plant	Petiole length (cm)	Leaf area (cm^2)	Maximum width of leaf (cm)	Shoot bark thickness (mm)	Root bark thickness (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Root length (cm)	Total fresh weight (g)	Total dry weigh (g)
G-48 X S_7C_{11}	-39.50*	-2.21	-0.31	-5.46	-1.04*	7.86	-1.34*	0.10	0.39*	-53.71	-41.38*	-1.71	-1.28	-0.08	-62.83	-46.45
G-48 X L-124/86	22.60	1.78	-0.01	6.05	0.20	-18.93	0.21	0.14*	0.07	42.20	22.89	15.50	10.89*	2.34	50.59	27.67
G-48 X L-17/92	35.50	2.07	0.36	-2.51	0.42	36.37*	1.27*	-0.004	-0.04	39.85	30.42	0.17	2.24	0.58	49.37	33.34
G-48 X S_7C_1	-20.60	-1.57	0.02	0.55	0.50	-20.70	-0.01	-0.10	-0.16	-10.72	-6.72	-8.00	-6.16	-2.17	-17.29	-6.44
S_1 X S_7C_{11}	30.57	2.09	0.38	3.77	0.71	-21.40	0.70	0.01	-0.29	28.97	31.76	-6.09	-0.95	-1.80	22.12	23.73
S_1XL-124/86	-7.25	-0.25	-0.27	-3.82	0.07	7.07	-0.09	0.05	0.03	3.40	-1.90	2.59	-0.48	1.05	6.70	17.10
S_1X L-17/92	-4.98	-0.76	-0.04	2.07	-0.66	28.52	-0.86	-0.14*	0.05	-8.94	-13.73	10.69	7.03	-0.15	4.78	-14.50
S_7C_8 X S_7C_{11}	25.70	0.77	0.29	-2.82	-0.08	34.47*	1.34*	-0.35*	-0.11	-6.24	-5.76	6.63	-0.22	-0.32	15.86	-6.01
S_7C_8 X L-17/92	-23.72	-0.82	-0.15	1.11	-0.12	-19.06	-0.72	0.31*	0.11	-37.52	-17.01	-9.14	-6.22	-0.77	-59.86	-26.78
L-62/84 X L-124/86	-27.50	-2.02	0.08	-2.06	-0.12	-7.63	-0.58	0.02	-0.03	-35.66	-11.31	-29.39*	-13.53*	-4.14*	-66.50	-34.21
L-62/84 X L-17/92	4.57	0.26	-0.04	0.83	-0.01	-46.49*	0.41	-0.30*	-0.20	-13.05	-5.80	-5.59	-5.59	0.48	-14.53	-6.11
L-62/84 X S_7C_1	4.62	0.66	-0.29	2.29	0.14	19.93	-0.33	0.28*	0.26	56.79	21.93	28.36*	17.36*	6.07*	80.24*	41.89
SE	18.86	1.20	0.19	3.70	0.37	15.21	0.61	0.06	0.16	29.22	17.94	8.11	5.03	1.25	34.94	22.80
CD	38.77	2.45	0.40	7.61	0.76	31.26	1.25	0.14	0.34	60.88	36.89	16.67	10.34	2.57	71.83	46.87

* Significant at 5 per cent level of significance

Table 4. Variation in mean, range, GCV, PCV, heritability, genetic advance and genetic gain of morphological characteristics of *Populus deltoides* hybrids

Characters	Mean	Range	Coefficient of variance (%)		Heritability (%)	Genetic advance (K=2.06)	Genetic gain (%)
			Genotypic	Phenotypic			
Height (cm)	255.69	210.45 - 316.11	9.59	16.88	32.31	28.74	11.24
Collar diameter (mm)	16.93	13.68 - 21.22	9.72	16.59	34.33	1.98	11.73
Internodal length (cm)	4.05	3.42 - 4.58	6.63	11.25	34.74	0.32	8.05
Number of leaves/plant	41.51	29.66 - 50.41	10.30	19.72	27.31	4.60	11.09
Petiole length (cm)	8.30	6.55 - 9.42	7.36	11.21	43.12	0.82	9.96
Leaf area (cm^2)	160.87	109.11 - 231.50	22.74	28.88	61.99	59.34	36.88
Maximum width of leaf (cm)	14.68	12.44 - 15.71	5.51	9.59	33.05	0.95	6.53
Shoot bark thickness (mm)	0.65	0.30 - 1.45	39.88	48.86	66.63	0.43	67.07
Root bark thickness (mm)	0.94	0.21 - 1.36	3.46	48.46	0.51	0.004	0.51
Fresh shoot weight (g)	268.34	154.16 - 338.12	11.05	31.03	12.67	21.74	8.10
Fresh root weight (g)	125.31	88.92 - 174.73	17.11	24.29	49.64	31.13	24.84
Dry root weight (g)	73.01	47.52 - 102.10	16.22	24.50	43.86	16.16	22.14
Dry root shoot ratio	0.42	0.28 - 0.66	15.55	34.75	20.03	0.06	14.34
Root length (cm)	37.82	31.13 - 42.39	7.66	11.67	43.08	3.92	10.36
Total fresh weight (g)	388.57	239.84 -512.85	14.30	27.89	26.28	58.69	15.10
Total dry weight (g)	256.93	148.89 - 308.86	11.19	26.15	18.32	25.36	9.87

Table 5. Effect of variance components on morphological characters in *Populus deltoides*

Variance components	Plant height (cm)	Collar diameter (mm)	Internodal length (cm)	No. of leaves /plant	Petiole length (cm)	Leaf area (cm^2)	Maximum width of leaf (cm)	Shoot bark thickness (mm)	Root bark thickness (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Root length (cm)	Total fresh weight (g)	Total dry weight (g)
Variances of GCA (δ^2g)	-18.38	-0.04	0.002	0.80	0.004	32.30	-0.01	-0.0005	0.0009	122.75	12.02	11.73	3.74	0.14	150.01	78.46
Variances of SCA (δ^2s)	662.60	2.96	0.07	21.00	0.40	1388.41	0.72	0.0767	0.0603	2656.51	613.31	597.42	193.17	11.64	5623.41	1910.0
Additive variance (D)	-73.55	-0.19	0.009	3.23	0.01	129.21	-0.04	-0.0023	0.0037	491.02	48.08	46.93	14.97	0.56	600.04	313.84
Dominance variance (H)	2650.42	11.86	0.31	84.00	1.60	5553.64	2.88	0.3068	0.2412	10626.04	2453.2	2389.68	772.70	46.57	22493.6	7640.0
Contribution of lines	14.98	15.63	32.09	46.05	47.61	27.61	16.65	35.8867	9.2483	19.62	6.63	50.85	25.61	36.94	27.29	15.30
Contribution of testers	28.86	32.38	33.95	22.44	12.12	38.85	31.48	14.4800	50.6733	53.61	54.45	13.75	38.59	23.63	39.27	56.71
Interaction (Line x Tester)	56.15	51.97	33.94	31.50	40.25	33.53	51.86	49.6332	40.0782	26.76	38.91	35.38	35.78	39.41	33.42	27.97

(2008) while studying the traits affecting the biomass production of *Salix eriocephala* using an incomplete factorial design reported that a large percentage of total variance was additive for all the traits studied and heritability estimated were low to moderate, suggesting that phenotypic expression of the traits are predictable and can be improved through breeding approaches. Luna and Singh (2009) on the basis of their study on *Eucalyptus* hybrids suggested that growth characters are governed by the genetic makeup of the trait and attribute significantly to the phenotypic performance at early stage giving ample opportunity for selection of the outstanding genotypes. Almost similar findings were reported by Dobhal et al. (2019a, b) for the *P. deltiodes.*

Estimation of heterosis

The selection potential of any cross combination on the basis of heterosis estimated may be effectively used for improvement in a particular trait. The genetic basis of heterosis has been proposed as (a) simple gene action, (b) heterozygosity*per se*, (c) dominant and partially dominant growth factors, (d) physiological aspects, (e) multiple alleles, (f) over or super dominance, (g) cytoplasm and (h) additive effects. A large number of experiments have shown that heterosisis based on directional dominance and epistasis, but there is little evidence of real over dominance (Jinks 1956). Manifestation of heterosis usually depends on genetic diversity of parental lines. The lines are considered diverse if they manifest relatively higher heterosis than those that express little (Hallauer and Miranda 1988).

In present investigation, the presence of positive significant heterosis over better control indicated significant increase of F_1 hybrids as compared to the better control. For number of leaves/plant, out of 12 crosses only nine cross viz; S_1x S_7C_{11}, G-48 x S_7C_1, G-48 x L-124/86, S_1 x L-17/92, G-48 x S_7C_{11}, G-48 x L-17/92, L-62/84 x S_7C_1,S_7C_8x L-17/92 andS_7C_8x S_7C_{11}having showed maximum positive significant heterotic effect over better control. Only two crosses, does not showed any positive significant heterosis for intermodal length and root bark thickness characters (Table 6 and 7). Superiority of intra-specific hybrids has been already demonstrated by earlier workers (Smart et al. 2005; Cameron et al. 2008; Choudhary 2011, Singh and Singh 2004, Ozel *et al.* 2010) in various tree species. Earlier Stott (1984) reported better productivity and higher adaptability of *S. alba* x *S. alba* hybrids as compared to hybrids between species (*S. alba* x *S. fragilis*). In Jatropha (*Jatropha curcas* L.) high mid parent heterosis (254.13 %) and better parent heterosis (202.36 %) were found for seed yield per plant in cross P_2 x P_5 and P_1 x P_3 respectively (Islam et al. 2011).

Paternity verification using SSR marker

The DNA extracted from Doyle and Doyle (1987) with slight modification protocol registered an absorbance ranging in females (1.53 to 1.71), males (1.88 to 1.96) and hybrids (1.24 to 1.71) & concentration ranging in females (534.6 to 2051.9 ng/µl), males (222.7 to 777.4 ng/µl) and hybrids (488.5 to 1827.4 ng/µl). The resulted DNA extracted from young leaves of male parents, registered the best absorbance (1.88 to 1.96) than female parent and their hybrids. The DNA extracted from both female parents and their hybrids contained impurities and with protein contamination (Table 8). Among 18 SSR markers, fifteen markers (Table 1) showed monomorphic allelic pattern, the remaining three markers (PMGC-2060, PMGC-2020 and PMGC-451) showed polymorphic pattern and were used to confirm the hybrids on the basis of banding pattern. A close appraisal of the SSR banding pattern obtained after the amplification of genomic DNA of both the parents and their hybrids revealed that, all the hybrids were true to type. The F_1 hybrids exhibited the alleles of both parents confirming the heterozygosity of the hybrid by having two bands (one allele per parent) in PMGC-2060, PMGC-2020 and PMGC-451. The identified SSR in F_1 hybrids showed complementary banding pattern of

Table 6. Effect of different morphological characters on magnitude of heterosis (% deviation) over better control

	Plant height		Collar diameter		Internodal length		Number of leaves/plant		Petiole length		leaf area		Maximum width of leaf	
Crosses	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrids	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrids	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrids	% increase (+) or decrease (-) over better control	F_1hybrids	% increase (+) or decrease (-) over better control
G-48 X S_7C_{11}	210.45	7.83	14.55	3.89	3.42	-18.43*	41.66	49.70*	6.55	-11.36	109.11	10.61	12.44	0.54
G-48 X L-124/86	272.52	39.63*	18.58	32.65*	4.12	-1.83	47.00	68.86*	7.55	2.16	122.33	24.01	15.51	25.31*
G-48 X L-17/92	316.11	61.96*	21.22	51.45*	4.58	9.24	41.44	48.90*	8.41	13.80	199.80	102.54*	15.71	26.93*
G-48 X S_7C_1	265.98	36.28*	17.36	23.91	4.07	-2.90	48.65	74.79*	8.22	11.27	128.93	30.70	14.02	13.30
S_1 X S_7C_{11}	266.12	36.35*	17.45	24.56	4.05	-3.37	50.41	81.14*	9.42	27.41*	119.40	21.04	14.86	20.03*
S_1 X L-124/86	228.25	16.95	15.14	8.07	3.79	-9.69	36.63	31.62	8.53	15.42	187.89	90.47*	15.57	25.79*
S_1 X L-17/92	261.21	33.83*	16.97	21.11	4.11	-2.09	45.55	63.65*	8.42	13.89	231.50	134.68*	13.94	12.60
S_7C_8 X S_7C_{11}	241.56	23.77	15.29	9.15	3.71	-11.54	39.78	42.93*	8.67	17.31*	137.71	39.60	14.82	19.71*
S_7C_8 X L-17/92	222.77	14.14	16.07	14.73	3.75	-10.73	40.55	45.69*	9.01	21.91*	146.35	48.36	13.39	8.19
L-62/84 X L-124/86	217.23	11.30	13.68	-2.31	4.47	6.58	29.66	6.59	7.82	5.77	176.04	78.46*	15.56	25.74*
L-62/84 X L-17/92	280.00	43.46*	18.32	30.75*	4.43	5.59	35.58	27.84	8.56	15.78	159.35	61.54*	15.70	26.82*
L-62/84 X S_7C_1	286.03	46.55*	18.52	32.19*	4.01	-4.43	41.17	47.94*	8.46	14.43	211.99	114.90*	14.55	17.56*

* Significant at 5 per cent level of significance

Table 7. Effect of different morphological characters on magnitude of heterosis (% deviation) over better control

	Shoot bark thickness		Root bark thickness		Fresh shoot weight		Dry shoot weight		Fresh root weight		Dry root weight		Root length		Total fresh weight		Total dry weight	
Crosses	F_1hybrids	% increase (+) or decrease (-) over better control	F_1hybrids	% increase (+) or decrease (-) over better control	F_1hybrids	% increase (+) or decrease (-) over better control	F_1hybrids	% increase (+) or decrease (-) over better control	F_1hybrids	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control	F_1 hybrid	% increase (+) or decrease (-) over better control
G-48 X S_7C_{11}	0.63	30.63	1.09	51.72	181.03	47.63	148.85	38.47	95.96	27.67	59.15	-4.21	40.0	26.10*	264.05	54.44	189.6	13.20
G-48 X L-124/86	0.78	61.57	1.13	57.41	213.94	74.47	171.25	59.30	112.0	49.06	63.67	3.12	39.4	24.23*	311.85	82.40	207.9	24.14
G-48 X L-17/92	0.73	49.71	0.95	32.48	326.75	166.46*	237.51	120.95*	117.5	56.32	77.87	26.11	36.2	13.91	444.25	159.83*	308.8	84.40*
G-48 X S_7C_1	0.30	-38.41	0.21	-69.57	234.03	90.85	191.45	78.10	99.49	32.37	58.54	-5.18	33.5	5.69	335.35	96.14	251.1	49.95
S_1 X S_7C_{11}	0.49	0.82	0.43	-39.55	323.89	164.13*	226.75	110.93*	138.3	84.00*	78.92	27.81	42.3	33.37*	381.15	122.93*	299.0	78.51
S_1 X L-124/86	0.64	31.43	1.11	54.12	235.31	91.89	151.2	40.65	145.8	94.03*	71.74	16.18	42.2	32.86*	512.85	199.95	236.5	41.23
S_1 X L-17/92	0.53	8.44	1.08	50.05	338.12	175.74*	198.10	84.29	174.7	132.4	102.1	65.36*	39.5	24.26*	462.19	170.32	300.2	79.23
S_7C_8 X S_7C_{11}	0.57	17.92	0.82	15.04	287.51	134.47	202.88	88.73	115.9	54.29	67.83	9.86	39.4	24.10*	403.49	135.99	272.2	62.52
S_7C_8 X L-17/92	1.45	197.71*	1.36	89.70	308.38	151.48*	208.50	93.95	119.8	59.42	77.03	24.75	34.4	8.40	395.75	131.46*	290.9	73.67
L-62/84 X L-124/86	0.59	22.72	1.17	62.95	154.16	25.72	130.39	21.30	88.92	18.31	47.52	-23.04	31.1	-2.06	239.84	40.28	148.8	-11.11
L-62/84 X L-17/92	0.36	-24.84	0.94	30.59	291.92	138.06	194.64	81.06	133.5	77.61*	78.31	26.83	34.2	7.75	425.43	148.82*	272.2	62.54
L-62/84 X S_7C_1	0.67	37.57	0.88	22.96	324.99	165.03*	216.84	101.71*	161.6	115.0	93.43	51.32*	41.0	29.19*	486.64	184.62*	305.5	82.43*

* Significant at 5 per cent level of significance

Table 8. The quantitative details of the samples of *Populus deltoides* clones and their hybrids

Sr. No.	Samples	Concentration (ng/µl)	Ratio
	Females		
1	G-48	802.7	1.64
2	S_1	2051.9	1.71
3	S_7C_8	902.9	1.66
4	L-62/84	534.6	1.53
	Males		
1	S_7C_{11}	578.0	1.96
2	L-124/86	777.4	1.89
3	L-17/92	222.7	1.88
4	S_7C_1	500.2	1.92
	Crosses		
1	G-48 X S_7C_{11}	746.4	1.61
2	G-48 X L-124/86	1461.5	1.67
3	G-48 X L-17/92	623.1	1.56
4	G-48 X S_7C_1	1265.2	1.67
5	S_1 X S_7C_{11}	747.2	1.57
6	S_1X L-124/86	679.6	1.56
7	S_1X L-17/92	938.0	1.61
8	S_7C_8X S_7C_{11}	493.9	1.51
9	S_7C_8 X L-17/92	1573.9	1.67
10	L-62/84 X L-124/86	488.5	1.24
11	L-62/84 X L-17/92	1827.4	1.71
12	L-62/84 X S_7C_1	507.7	1.48

both the parents and found vital to distinguish the F_1 from their male and female parents. The result of identification showed that there were banding pattern similar to the male parent, it seemed that mixing occurs during harvesting seed or processing activities, while the presence of the same banding pattern with female parent indicated that selfing occurred in the production process due to inaccuracies in detasseling. There were two bands in male parent, it shows that there is more contribution of male than female parent in formation of hybrids. The results of the present investigation suggested that, SSR markers are very useful for confirming paternity of hybrids. Molecular markers are especially useful when hybridity is questioned by morphological reasons or for early screening of large putative hybrid populations (Rajendra 2009). SSR markers have been successfully used for genetic fingerprinting including verification of controlled crosses (hybrids) in tree species (Singh et al. 2013). SSR markers based on the presence or absence of polymorphism among group of individuals were employed for hybrid verification along with parents. Our results are in confirmatory with the findings of Khasa et al. (2003) who optimized seventeen microsatellite or simple sequence repeat (SSR) markers in seven species of genus *Populus (P. balsamfera, P trernuloides, P. deltoides, P. davidiana, P alba, P. tremula* and *P. nigra*) in which they found that fourteen out of 17 primer pairs amplified SSR loci exhibiting variable amounts of polymorphism across the species studied. Similar results reported by Rahman et al. (2000) in *P. tremuloides.* Smulders et al. (2001) also reported polymorphism in *P. nigra*, *P. deltoides, P. tricocarpa, P. tremula, P. tremuloides, P. candicans, P. lasiocarpa*). Our results also find support from findings Fossati et al. (2005) who reported 96 per cent polymorphism in *Populus* × *canadensis*. Our findings are in the line with the

findings of Gao et al. (2006) who reported 84% polymorphism among the *Populus L.* cultivars using the Inter-Simple Sequence Repeat (ISSR) markers. Almost similar findings were reported by Grewal et al.(2013) who studied 32 simple sequence repeat (SSR) markers in *Populus deltoides* in which only 22 markers showed polymorphic pattern and amplified a total of 102 alleles.

CONCLUSIONS

Line x tester analysis for combining ability revealed that line S_1 and tester L-17/92 were found to be good general combiners and thus appeared to be worthy of exploiting in *Populus deltoides* improvement through breeding and recurrent selection followed by cloning for developing commercial superior clones. On the basis of heterosis over better parent, mean performance and significant desirable SCA effects for all morphological characters, the combinations L-62/84 X S_7C_1 and L-62/84 X L-17/92 were found to be the most promising families. Among 18 SSR markers, fifteen markers showed monomorphic allelic pattern, the remaining three markers (PMGC-2060, PMGC-2020 and PMGC-451) showed polymorphic pattern and were used to confirm the hybrids on the basis of banding pattern. There were two bands in male parent, it shows that there is more contribution of male parent than female parent in formation of hybrids.

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