



# Morphological, physiological and molecular analysis of Line $\times$ Tester in *Populus deltoides* Bartr

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Received: 1 September 2017 / Accepted: 21 November 2019  
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**Abstract** In the present investigation, male and female clones/strains/species of *Populus deltoides* procured from State Forest Department, Haldwani and Shyampur, Haridwar Forest Division and maintained in the germplasm block of Naganji nursery from which 11 male and 13 female clones were included in the reproductive study and the selected plant material of 4 females (G-48, S 1, S 7 C 8 and L-62/84) and 4 males (S 7 C 11, L-17/92, L-124/86 and S 7 C 1) clones were crossed using Line  $\times$  Tester (4  $\times$  4) mating design. Control crossing was done and seedlings were raised in the nursery at stage-1 and were evaluated for morphological characters. In stage-2 clonal cuttings of selected individuals were raised in RBD and were evaluated for morphological, physiological and wood characters. The gene action study revealed that dominance variance was observed more than the additive variance for all the parameters studied. The proportional contribution of lines

was higher than individual contribution of testers or line  $\times$  tester interaction except for plant height, collar diameter, internodal length, leaf area, maximum width of leaf, angle between the mid rib and 2 nd lower lateral mid rib, shoot bark thickness, root bark thickness, fresh shoot weight, dry shoot weight, dry root weight, dry root shoot ratio, root length, total fresh weight, total dry weight, stomatal conductance and fibre length. Among 18 SSR markers, fifteen markers showed monomorphic allelic pattern, the remaining three markers (PMGC-2060, PMGC-2020 and PMGC-451) showed polymorphic 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 similar to their parents.

**Keywords** *Populus deltoides* · Control crossing · Nursery · Morphological analysis · SSR analysis

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## Introduction

*Populus deltoides* clones were introduced in India in 1952 to increase the availability of raw material for plywood industries in the country. It is one of the most popular tree species in the agroforestry system in irrigated plains of Western Uttar Pradesh, Uttarakhand, Punjab and Haryana. Its wood is in demand for pulp and paper, plywood, matchwood, packing cases and light constructional timber all over the world (Rizvi et al. 2008).

Improved clones of G-48 and S<sub>7</sub>C<sub>8</sub> presently form the backbone of the poplar planting programme, which has spread in large areas in North India. But there is always potential risk that they may fail after a few years, due to their susceptibility to some adverse climatic conditions or

disease or pest in their habitat (Kadam 2002). Clones like D-100 and D-121, which were most popular at one time, but now, have been eliminated out due to their susceptibility to various pests and diseases. Therefore, a large number of clones in assembly line are urgently required to serve as replacements for un-promising clones in our country. So for long term improvement plan, the efforts for selecting new clones and their field testing must continue to get desirable output (Stanton et al. 2019).

Keeping in view its economical and ecological importance, a lot of work has been done on cultivation aspects of poplar but a little systematic effort has been made on the genetic improvement specially control breeding in India. Selecting superior poplar genotypes (clones) and their mass multiplication can increase its productivity. Similarly, significant variation could be expected through control crosses such as heterosis breeding. It has been reported that crossing among trees from different sources can result in superior inter racial  $F_1$  families (Sharma and Sharma 2018; Sharma et al. 2018). Genetic markers are now days routinely used to monitor the efficiency of various tree improvement activities and are necessary for the construction of genetic maps for aiding breeding (White et al. 2007). Many microsatellites in forest tree species such as *Populus* have been reported (Cervera et al. 2005). Molecular markers can help to characterize populations, estimate genetic variability within and among wild or generated populations and can identify individuals at a young age that will express a trait at maturity.

The various species of *Populus* both indigenous and exotic, have assumed great importance in breeding programme. Basic genetic studies such as crossability pattern, estimate of genetic parameters of traits of interest, right from growth, adaptability and productivity to produce are very much needed. The combination of traditional breeding fortified with advances in molecular research could advance genetic improvement of the genus. More variation can also be created artificially by interspecific and intra-specific hybridization.

## Materials and methods

### Experimental site

The study site is located at an elevation of 1200 m above mean sea level in north-west of Himalaya and lies between 30°51' N latitude and 76°11' E longitude. The experimental area is hilly, marked with elevations, depressions and has a gentle slope towards the south-eastern aspect. The area experiences a wide range of temperature with a minimum of 2 °C in winters to a maximum of 32.6 °C in the summers. The soil is well drained, sandy-loam type with pH of

7.2. The experiment was conducted in the nursery, whereas, assessment of paternity verification using molecular marker (Simple Sequence Repeats) techniques was carried out in the forest genetics laboratory.

### Experimental material

The flowering branches of the females and males were obtained from Haldwani and Shyampur in month of December 2012 and February 2013, respectively. These materials were utilized for the different studies and crosses among male and female were made in 2013 to develop hybrids in the germplasm block of Naganji nursery, Department of Tree Improvement and Genetic Resources, College of Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan, (H.P.). Flowering branches of male clones are kept in the water buckets to get abundant pollen for hybridization work. Flowering branches of female clones were grafted separately on grown root stock of *Populus deltoides*. The grafted plants are kept moist by intermittent irrigation/watering.

### Experimental methodology

For investigation on the reproductive biology of each male and female cutting material of *Populus deltoides* clones were selected and marked in the glass house and field respectively in 2013. Each cutting was tagged with metallic tag and data recorded on the following characters; Colour and size of male and female bud, Flowering, Pollen studies i.e. Pollen Collection, Pollen morphology (size).

- (a) *Control crossing (Hybridization)* The flowering branches of the female and male clones of *Populus deltoides* were used for control crossing (hybridization). Male catkins were removed for collection of pollen at anthesis time to accomplish artificial pollination. All the female clones were crossed with each of male clones by hand pollination at stigma receptivity stage. Pollen used in controlled crossing was tested for in vitro pollen viability. Every controlled cross involves single pollen and no pollen mixture was used. After pollination the flowers were bagged and tagged. Seeds of controlled crosses were harvested when mature and sowed immediately. Observations were recorded on following parameters. Capsule size (mm), Number of seeds set per capsule, Successful crosses (%), Germination percentage, Survival per cent.

$$\text{Successful crosses(\%)} = \frac{\text{Successful crosses}}{\text{Total crosses}} \times 100$$

$$\text{Germination(\%)} = \frac{\text{Germinated seeds}}{\text{Total number of seed sown}} \times 100$$

$$\text{Survival(\%)} = \frac{\text{Survived seedling}}{\text{Total number seeds germinated}} \times 100$$

- (b) *Nursery Studies* Survival percent was calculated as the number of seedlings that survived after it reached 4 leaves stage out of number of germinated seedlings and expressed in percentage.

The nursery study was carried out in two stages i.e. for stage one and stage two. During stage one, the seedlings were raised in the glass house and were shifted to nursery in March 2014. The  $F_1$  population of the successful crosses was grown in the nursery under uniform environmental conditions. During this stage; 5 plants of each cross were taken randomly for recording on observations on morphological characters in 2014.

In stage two, best individuals of  $F_1$  were selected and the progeny was cloned. The cuttings of all selected individuals were raised in the RBD during February, 2015 in 3 replications at the experimental field of Naganji nursery. The five plants were selected from each hybrid and from each plants nine cutting were taken and three cutting per replication of the full sib progeny were used for recording observations on physiological characters in June-July and morphological and wood characters in September to December, 2015. For experimental material, sixteen  $F_1$  hybrids were needed for Line  $\times$  Tester ( $4 \times 4$  factorial) mating design using 4 males and 4 females but twelve  $F_1$  hybrids survived for evaluation in the nursery trial in stage two (Table 1).

The nursery growth was undergone for morphological characterization by using following characteristics; Stem characteristics (Plant height (cm), Collar diameter (mm), Internodal length (cm), Stem colour at sun side, Stem shape; Curved, Slightly Curved, Straight and Stem felt; Present, Absent), Branch characteristics (Number of leaves/plant and Branch nature; Curved Upward, Horizontal, Curved Downward), Leaf characteristics (Colour of leaf, Apex angle ( $^\circ$ ), Shape of tip, Leaf blade altitude; Upward,

Horizontal, Downward, and Leaf base; Wavy, Slightly Wavy and flat, Leaf area ( $\text{cm}^2$ ), Length of lamina (cm), Maximum width of leaf (cm), Ratio of Length of lamina/maximum width of leaf, General shape of base, Angle between the mid rib and 2nd lower lateral mid rib ( $^\circ$ ), Distribution of anthocyanin coloration of mid rib; Base, Base to midrib, Whole midrib), Petiole characteristics (Petiole length (cm), Colour intensity of petiole at junction, Colour of petiole, Shape of junction with petiole), Shoot bark thickness (mm) (digital caliper), Root bark thickness (mm) (digital caliper) (electronic top pan balance), Fresh shoot weight (g) (electronic top pan balance), Dry shoot weight (g) (electronic top pan balance), Fresh root weight (g) (electronic top pan balance), Dry root weight (g) (electronic top pan balance), Dry root shoot ratio (dividing dry root weight with dry shoot weight), Root length (cm) (measuring tape), Total fresh weight (g) (summing up fresh shoot weight and fresh root weight), Total dry weight (g) (summing up dry shoot weight and dry root weight).

Physiological Parameters to be studied under following; Internal  $\text{CO}_2$  concentration, Photosynthetic rate, Transpiration rate, Stomatal conductance, Water use efficiency by using formula:

$$\text{WUE} = \frac{\text{Number of molecules of } \text{CO}_2 \text{ fixed}}{\text{Number of molecules of } \text{H}_2\text{O} \text{ transpired}}$$

Wood density and fibre length was studied under wood Characters. For wood density ( $\text{g}/\text{cm}^3$ ), the specific gravity of wood of selected hybrids was determined by the maximum moisture content method (Smith, 1954). The green weight of sample was taken at maximum moisture content. Then these samples are kept in oven at  $105 \pm 3^\circ\text{C}$  for drying till it attains constant weight. The specific gravity is determined for these samples separately as per the following formula:

$$\text{Specific gravity} = \frac{1}{(\text{Mm} - \text{Mo})/\text{Mo} + 1/\text{GS}}$$

where, Mm represent Green weight of the sample having moisture, Mo was Oven dried constant weight of the sample and GS was Average density of wood substance (a

**Table 1** List of clones involved in line  $\times$  tester design

Sr. No.	Clones	Sex	Source country/Originally developed
1.	G-48	Female	Australia
2.	S <sub>1</sub>	Female	India (Shyampur, Haridwar Forest Division)
3.	S <sub>7</sub> C <sub>8</sub>	Female	USA
4.	L-62/84	Female	India (Lalkuan Selection)
5.	S <sub>7</sub> C <sub>11</sub>	Male	USA
6.	L-124/86	Male	India (Lalkuan Selection)
7.	L-17/92	Male	India (Lalkuan Selection)
8.	S <sub>7</sub> C <sub>1</sub>	Male	USA

constant having value 1.53). Whereas, for fibre length one year old hybrids were taken for measurements from which small segments were removed from the wedges. Fibres were macerated in a mixture having equal parts of 10% chromic acid and 10% nitric acid for 24 to 48 h. The pulp was thoroughly washed with water, stained with 2% aqueous safranin, teased in 10% glycerine and mounted in glycerine jelly with coverslip. The measurements were made with the help of ocular micrometer standardized with the help of stage micrometer.

### Verification of F<sub>1</sub> hybrids as well as parents using molecular marker

For evaluation of each hybrid & parental lines in the above mentioned plant sources, DNA based marker i.e. Simple Sequence Repeats (SSRs) was used. Leaf samples were collected from both F<sub>1</sub> hybrids as well as from parents growing in the field. Genomic DNA was extracted from young healthy leaves using the CTAB method with slight modification (Sharma et al. 2019). A total 18 SSR primers were custom synthesized from Eurofins genomics (GeNei<sub>TM</sub>) (Table 2). The PCR reaction was performed with final reaction volume of 20 µl consisting of 1 mM dNTP, 1U Taq DNA polymerase, 1 × Taq Buffer A (with 75 mM MgCl<sub>2</sub>), 10 pico-moles of each forward and reverse primers, and 50 ng DNA in ProFlex<sup>TM</sup> Thermal Cycler (Applied Biosystem, Inc.) (Singh et al. 2019). The PCR conditions followed for amplification was as follow: initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, extension at 72 °C for 2 min followed by final extension at 72 °C for 5 min (Samriti et al. 2017). The amplified product was electrophoresed in 2.0% agarose gel after mixing with 6 × loading dye and visualized through gel documentation system (Syngene International Ltd.).

### Statistical analysis

The data obtained for crosses (progenies) was subjected to statistical analysis as per the design. The statistical analysis for each parameter was carried out on mean values and the analysis of variance (ANOVA) table was set up as under:

#### Analysis of variance (ANOVA)

For working out the analysis of variance, the data were analyzed by using the following model as suggested by Panse and Sukhatme (1967).

$$Y_{ij} = \mu + g_i + b_j + e_{ij}$$

$$i = 1, 2, \dots, g, j = 1, 2, \dots, r$$

**Table 2** Details of primers used in present study

S. No.	Primer names	Sequences
1.	WPMS-03	FP-TTTACATAGCATTTAGCCTTTAGA RP-TTATGATTTTGGGGGTGTTATGGA
2.	WPMS-05	FP-TTCTTTT CAA CTG CCT AACCT RP-TGATCCAATAACAGACAGAACA
3.	ORPM-015	FP-CGTGAGTTTGTAGGCCATTT RP-CATGGAAAGGATCACCCACT
4.	PMGC-451	FP-AATTACAACCACTTTAGCATATTC RP-TGCCGACACATCACACATACC
5.	PMGC-325	FP-CGATTTATGACAGACAGCTTG RP-GTACCGTTGAGGTGGCTAG
6.	PMGC-333	FP-CTTAGTGGTGAAGTATTC RP-GAG TGG GTG CTGATTCATCC
7.	PMGC-409	FP-ACGTATATGAAGTTCTTGATTGC RP-GACAGATCATTATGATTACTACAG
8.	PMGC-420	FP-ATGGATGAGAAATGCTTG RP-ACTGGCACACGCTTTAACTGG
9.	PMGC-422	FP-AACCTCGAATTAAGAATAACCC RP-GTCTCGGTTAAGGTATTGTGCG
10.	PMGC-433	FP-GCAGCATTGTAGAATAATAAAAG RP-AAGGGGTCTATTATCCACG
11.	ORPM-026	FP-GCTGCAGTCAAATCCAAAA RP-CGAGCGTCTTCTTCATGGAT
12.	PMGC-562	FP-TTTTGGGAGGGGAGTCGAG RP-ACAACCTCTCAACTTCCTAAC
13.	PMGC-571	FP-CTGGTACCGATGGAGAAGAC RP-CAAACCAACAACCTACCGTAC
14.	PMGC-2020	FP-TAAGGCTCTGTTTGTAGTCAG 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

where,  $Y_{ij}$  represent phenotypic observation of  $i$ th genotype and  $j$ th replication,  $\mu$  was general mean of the population,  $g_i$  was effect of  $i$ th genotype,  $b_j$  was effect of  $j$ th replication and  $e_{ij}$  was error component.

#### Critical difference (CD)

The significance the treatment means was tested by 'F' test. Wherever 'F' test was found significant, (\*) critical

**Table 3** The variation in bud size and its colour, and also variation in first and last date of male flowering, pollen collection, catkin colour and pollen size among different male clones

Sr. No.	Male clones	Bud colour	Bud length (cm)	Bud breadth (cm)	Floral bud burst (collection of bud cuttings: 21/02/2013)	Floral (catkin) colour	Pollen collection	Pollen length (μ)	Pollen breadth (μ)
1	82-42-5	Greyed Orange 173 (Group A)	2.62 ± 0.05	6.40 ± 0.19	27/02/2013	Greyed Yellow 160 (Group A)	02/03/2013	33.34 ± 2.09	32.49 ± 2.13
2	UD-88	Greyed Green 189 (Group A)	3.80 ± 0.20	9.93 ± 0.44	04/03/2013	Greyed Green 194 (Group A)	08/03/2014	35.05 ± 1.39	32.49 ± 1.14
3	L-124/86	Greyed Green 189 (Group A)	3.24 ± 0.11	9.65 ± 0.26	04/03/2013	Greyed Purple 185 (Group A)	07/03/2013	33.77 ± 2.42	32.06 ± 2.48
4	39-N	Greyed Green 191 (Group A)	3.36 ± 0.06	10.30 ± 0.35	03/03/2013	Greyed Green 194 (Group A)	08/03/2013	36.76 ± 1.71	33.34 ± 1.89
5	S <sub>7</sub> C <sub>11</sub>	Greyed Orange 174 (Group A)	3.24 ± 0.15	9.21 ± 0.28	04/03/2013	Greyed Purple 185 (Group A)	08/03/2013	33.22 ± 0.89	32.56 ± 0.78
6	25-N	Greyed Green 189 (Group A)	2.14 ± 0.16	7.38 ± 0.73	05/03/2013	Greyed Yellow 162 (Group A)	11/03/2013	35.48 ± 2.21	33.77 ± 2.33
7	26-N	Greyed Yellow 160 (Group A)	3.32 ± 0.09	8.90 ± 0.30	02/03/2013	Greyed Green 194 (Group A)	07/03/2013	36.33 ± 1.46	32.91 ± 1.43
8	S <sub>7</sub> C <sub>1</sub>	Greyed Green 189 (Group A)	3.26 ± 0.11	9.46 ± 0.54	04/03/2013	Greyed Yellow 160 (Group A)	08/03/2013	36.76 ± 2.22	37.62 ± 1.89
9	S <sub>7</sub> C <sub>4</sub>	Greyed Red 189 (Group A)	1.96 ± 0.12	6.56 ± 0.45	03/03/2013	Greyed Yellow 160 (Group A)	07/03/2013	32.34 ± 0.66	31.90 ± 0.49
10	L-17/92	Greyed Yellow 160 (Group A)	3.50 ± 0.07	10.52 ± 1.02	04/03/2013	Greyed Green 193 (Group A)	08/03/2013	34.62 ± 1.48	33.77 ± 0.99
11	G-3	Greyed Green 189 (Group A)	3.74 ± 0.26	7.46 ± 0.33	06/03/2013	Greyed Yellow 162 (Group A)	10/03/2013	41.89 ± 1.99	40.18 ± 1.82

difference was calculated to test the significance between any two or more treatment means.

The critical difference (CD) was calculated as under:  
 $CD = SE_d \times t_{0.05}$  (error degree of freedom) where  $SE_d$  = Standard error of difference:

$$SE_d = \sqrt{\frac{2Me}{r}}$$

$t_{0.05}$  error degree of freedom = t (table) value at 5 per cent level of significance

## Results and discussion

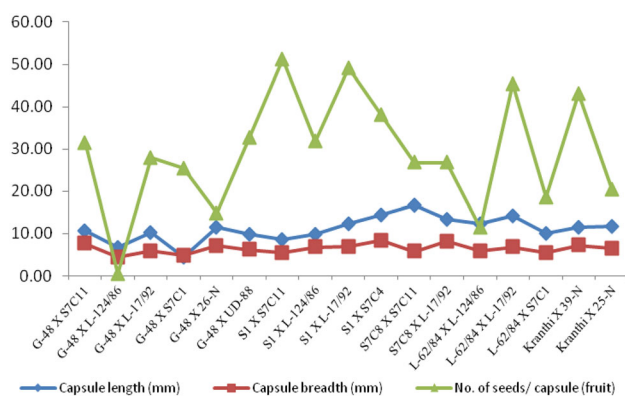
The present investigations entitled “Line × Tester analysis in *Populus deltoides* Bartr.” was undertaken with a view to assess the nature of gene action among different hybrids developed from selected clones of *Populus deltoides* in 4 × 4 (Line × Tester) crosses and to analyze their paternity pattern using SSR molecular marker. An attempt has been made to understand the genetic system to control inheritance of yield and its components from a Line × Tester mating design analysis. The data collected on different characters were subjected to statistical and biometrical analysis.

### Analysis of means with standard error

Information on reproductive biology is useful for understanding genetic and taxonomic relationships between species and as such data are pre-requisite for initiating a meaningful breeding programme. The male clones of *Populus deltoides* were evaluated for length, breadth and colour of the buds (Table 3). A perusal of the data in Table 3 indicated that the Greyed Green 189 (Group A) colour in buds was observed in maximum number of male clones. The maximum bud length (3.80 cm) was recorded in UD-88 male clone followed by G-3 male clone (3.74 cm) and least value (1.96 cm) was recorded in S<sub>7</sub>C<sub>4</sub> male clone. Similarly, maximum (10.52 cm) bud breadth was recorded in L-17/92 male clone this was followed by 39-N male clone (10.30 cm) and least value (6.40 cm) was recorded in 82-42-5 male clone (Table 3).

The observations on date of floral (catkin) bud burst and floral colour for various male clones are presented in Table 3. A scrutiny of data in Table 2 reflected that earliest flowering was observed in 82-42-5 clone on date 27/02/2013. Similarly, flowering after 27/02/2013 in male clones cutting were also observed in UD-88, L-124/86, S<sub>7</sub>C<sub>11</sub>, S<sub>7</sub>C<sub>1</sub> and L-17/92 clones. The latest flowering was observed in G-3 male clone, on date 06/03/2013. The catkin colour was Greyed Green 193 (Group A) which was found only in L-17/92. Similarly, earliest pollen collection





**Fig. 1** Variation in capsule length, breadth and number of seeds per capsule among the hybrids of *Populus deltoides*

was observed in 82–42–5 (02/03/2013) followed by L-124/86 male clones on date 07/03/2013. The maximum length (41.89 $\mu$ ) and breadth (40.18 $\mu$ ) of pollen was found in G-3 male clone and minimum length (32.34 $\mu$ ) and breadth (31.90 $\mu$ ) of pollen was found in S<sub>7</sub>C<sub>4</sub> clone. In general, the period between initiation and flowering is correlated with growth habit of the tree, which is in turn governed by climatic range of species (Sedgley and Grifftin, 1989). Knowledge of the development of flower buds is essential in achieving successful breeding programme. Similar trends of findings were reported in *Dalbergia sissoo* (Chauhan et al. 2004) *Grewia optiva* (Pant et al. 2003) and *Bauhinia variegata* (Wani, 2005). In *P. ciliata*, it took 15 days after the floral bud break with a receptivity period of the female catkins being extended from 2 to 3 days (Khurana 2000). The delay in bud swelling and its bursting may be attributed to the climatic factors as such as temperature and rainfall.

The Table 4 revealed the length, breadth and colour of female bud clones of *Populus deltoides*. It was evident from the table that maximum number of clones were seen having Greyed Yellow 160 (Group A) colour of the buds. The bud length was recorded maximum (2.20 cm) in L-62/84 female clone, which was however, followed by PIP-201 female clone giving values of 2.18 cm, while minimum (1.36 cm) bud length was recorded in S<sub>1</sub> clone. Likewise, bud breadth was observed maximum (4.59 cm) in PIP-201 female clone, which was followed by G-48 female clone with the value of 3.93 cm, while the minimum value (2.58 cm) of bud breadth was observed in 82–33–3 female clone.

An overview of data in Fig. 1 showed length & breadth of capsule with number of seeds/capsule resulted from various crosses of *Populus deltoides*. The data showed that maximum (16.76 mm) capsule length was recorded in S<sub>7</sub>C<sub>8</sub> × S<sub>7</sub>C<sub>11</sub>, which is followed by S<sub>1</sub> × S<sub>7</sub>C<sub>4</sub> (14.46 mm) of hybrid of *Populus deltoides*. The minimum

**Table 4** The variation in bud size and its colour, and also in first and last date of cross pollination, number of days involved in capsule maturation, capsule size and number of seeds/capsule among the crosses among different female clones

Sr. No.	Female clones	Bud colour	Bud length (cm)	Bud breadth (cm)	Date of cross pollination	No. of days involved in capsule maturation	Capsule length (mm)	Capsule breadth (mm)	Number of seeds/capsule
1	G-48	Greyed Red 180 (Group A)	1.62 ± 0.03	3.93 ± 0.28	30/03/2013	38 days (08/05/2013)	10.74 ± 0.33	7.66 ± 0.32	31.4 ± 7.80
2	S <sub>7</sub> C <sub>8</sub>	Greyed Orange 167 (Group A)	2.12 ± 0.07	3.87 ± 0.25	09/04/2013	36 days (16/05/2013)	6.66 ± 0.29	4.42 ± 0.37	0.4 ± 0.24
3	S <sub>7</sub> C <sub>15</sub>	Greyed Orange 170 (Group A)	2.12 ± 0.07	3.87 ± 0.25	04/04/2013	30 days (05/05/2013)	10.25 ± 0.42	5.87 ± 0.09	28.0 ± 1.92
4	L-62/84	Greyed Orange 167 (Group A)	2.20 ± 0.12	3.78 ± 0.23	23/03/2013	45 days (08/05/2013)	4.48 ± 0.32	4.83 ± 0.62	25.4 ± 2.15
5	PIP-201	Greyed Orange 171 (Group A)	2.18 ± 0.08	4.59 ± 0.16	28/03/2013	35 days (03/05/2013)	11.49 ± 0.40	7.20 ± 0.24	14.8 ± 2.95
6	82–33–3	Greyed Yellow 160 (Group A)	2.12 ± 0.05	2.58 ± 0.56	30/03/2013	38 days (08/05/2013)	9.82 ± 0.13	6.20 ± 0.17	32.8 ± 2.74
7	D-121	Greyed Orange 164 (Group A)	1.82 ± 0.08	3.71 ± 0.50	28/03/2013	41 days (09/05/2013)	8.57 ± 0.29	5.44 ± 0.31	51.2 ± 1.35
8	Kranthi	Greyed Orange 173 (Group A)	1.58 ± 0.07	4.31 ± 0.17	09/04/2013	23 days (03/05/2013)	9.78 ± 0.72	6.84 ± 0.73	31.8 ± 3.77
9	L-39	Greyed Yellow 162 (Group A)	1.76 ± 0.06	3.49 ± 0.27	28/03/2013	42 days (10/05/2013)	12.37 ± 0.85	6.98 ± 0.14	49.2 ± 2.55
10	PIP-221	Greyed Yellow 160 (Group A)	1.90 ± 0.18	3.06 ± 0.46	28/03/2013	35 days (03/05/2013)	14.46 ± 0.43	8.36 ± 0.15	38.2 ± 3.52
11	S <sub>1</sub>	Greyed Yellow 160 (Group A)	1.36 ± 0.13	2.85 ± 0.37	01/04/2013	31 days (03/05/2013)	16.76 ± 2.86	5.79 ± 0.27	26.8 ± 2.55
12	S <sub>2</sub>	Greyed Yellow 160 (Group A)	1.62 ± 0.10	2.98 ± 0.64	28/03/2013	42 days (10/05/2013)	13.36 ± 0.66	8.13 ± 0.28	26.8 ± 4.36
13	PIP-219	Greyed Yellow 162 (Group A)	1.50 ± 0.08	3.58 ± 0.54	04/04/2013	30 days (05/05/2013)	12.32 ± 0.14	5.93 ± 0.52	11.4 ± 2.78

capsule length of 4.48 mm was however, recorded in  $G-48 \times S_7C_1$ . It was apparent from data in Fig. 1 that variation in capsule breadth was observed among the hybrids of *Populus deltoides*. The maximum (8.36 mm) breadth of capsule was obtained in  $S_1 \times S_7C_4$ , which is followed by  $S_7C_8 \times L-17/92$  (8.13 mm), while minimum (4.42 mm) capsule breadth was recorded in  $G-48 \times L-124/86$  hybrid. The effect of various crosses between of *Populus deltoides* on number of seed/capsule has been shown in Fig. 1. It is seen from data that maximum (51.2) number of seed/capsule was recorded in  $S_1 \times S_7C_{11}$  hybrid. This was however, followed by cross combination  $S_1 \times L-17/92$  (49.2) while the minimum number (0.4) of number of seed/capsule was observed in  $G-48 \times L-124/86$  hybrid of *Populus deltoides*.

The present finding lends supported to the results of Dhiman and Gandhi (2012) who reported similar observations in *Populus* species. Similarly, Khurana and Nar-khede (1995) in *Populus ciliata* reported that the fruits ripen in May–June with catkins elongating up to 20–30 cm in size. Four valved capsules dehisce on maturity thereby releasing the tiny seed.

### Nursery characters

The five Seedlings of hybrids obtained by crossing parents were evaluated for morphological characters. The mean performance and range of variation for nursery characteristics among 17 hybrids for stage one (2014) (Table 5). The analysis of mean revealed that greatest plant height among hybrids was observed in  $G-48 \times S_7C_1$  (408 cm) whereas, minimum value of plant height was observed in  $L-62/84 \times L-124/86$  (191 cm). It is evident from the Table 5 that maximum collar diameter was observed in  $S_1 \times L-124/86$  (26.10 mm). The minimum value for this trait was recorded in  $L-62/84 \times L-124/86$  (12.45 mm). For internodal length, maximum value was depicted by  $G-48 \times S_7C_{11}$  (8.36 cm), whereas minimum value by  $G-48 \times 26-N$  (3.88 cm). The maximum average number of leaves per plant among hybrids was observed in  $G-48 \times S_7C_1$  (118.0). The minimum average number of leaves per plant was recorded in  $L-62/84 \times L-124/86$  (37.8). For leaf area, maximum value was depicted by  $G-48 \times S_7C_{11}$  (270.52 cm<sup>2</sup>), whereas minimum leaf area recorded in  $Kranthi \times 25-N$  (149.78 cm<sup>2</sup>). The maximum average apex angle among hybrids was observed in  $G-48 \times S_7C_1$  (45°). The minimum value was recorded in  $L-62/84 \times L-124/86$  (38.2°). For length of lamina, maximum value was depicted by  $Kranthi \times 25-N$  (20.88 cm), whereas minimum value by  $L-62/84 \times L-124/86$  (13.38 cm). The maximum width of leaf maximum among hybrids was observed in  $Kranthi \times 25-N$  (22.56 cm). The minimum value was recorded in  $L-62/84 \times L-124/86$

(13.41 cm). For ratio of length of lamina/maximum width of leaf maximum value was depicted by  $S_1 \times L-17/92$  (1.02), whereas minimum value by  $Kranthi \times 39-N$  (0.84). The mean performance for petiole length was recorded maximum in  $Kranthi \times 25-N$  (13.15 cm). However, the minimum value was observed in  $L-62/84 \times L-124/86$  (6.97 cm).

For angle between the midrib and 2nd lower lateral mid rib, maximum value was depicted by  $L-62/84 \times L-17/92$  (60°), whereas minimum value by  $G-48 \times 26-N$  (41.6°). The data in the Table 5 clearly indicated that maximum number of branches/plant was recorded in  $S_1 \times S_7C_{11}$  (8.4). The minimum value for the trait was observed in  $G-48 \times UD-88$  (1.0). For branch angle, maximum value was depicted by  $Kranthi \times 25-N$  (70°), whereas minimum value by  $G-48 \times UD-88$  (30°). The data in the Table 5 clearly indicated that maximum branch length was recorded in  $L-62/84 \times L-124/86$  (47.05 cm). The minimum value for the trait was observed in  $Kranthi \times 25-N$  (6.70 cm). Our results are in agreement with the findings of Ceulemans et al. (1992) who observed superiority of  $F_1$  over their parents for height and diameter growth, stem volume production, leaf phenology, leaf number and number of branches in cross between *Populus deltoides*  $\times$  *P. trichocarpa*. Similarly, Wu and Stettler, (1997) also reported that the growth difference in the two  $F_2$  families of *Populus trichocarpa* and *Populus deltoides* hybrids had a significant impact of genetic component.

### Analysis of means and variance

A cursory glance at the data presented in Fig. 2 revealed significant differences for crossability parameters viz., successful crosses (%), germination percentage and survival percentage among the clones of *Populus deltoides*. The range of successful crosses (Table 6) lied between 13.35 to 66.64 per cent. Successful cross percentage was highest in  $S_1 \times L-17/92$  (66.64%) followed by,  $S_1 \times S_7C_4$  (50%) and  $S_1 \times L-124/86$  (41.65%). The minimum of successful cross percentage was obtained in  $S_7C_8 \times S_7C_{11}$  (13.35%).

The range of germination per cent lied between 14.71 to 96.00 per cent. Significant differences were observed among crosses for germination percent (Fig. 2). Maximum germination percent (96.00%) was recorded between  $Kranthi \times 39-N$ , followed by  $G-48 \times L-17/92$  (93.33%) and  $S_1 \times S_7C_{11}$  (88.24%) were recorded. Minimum value for germination was recorded in  $G-48 \times L-124/86$  (14.71%).

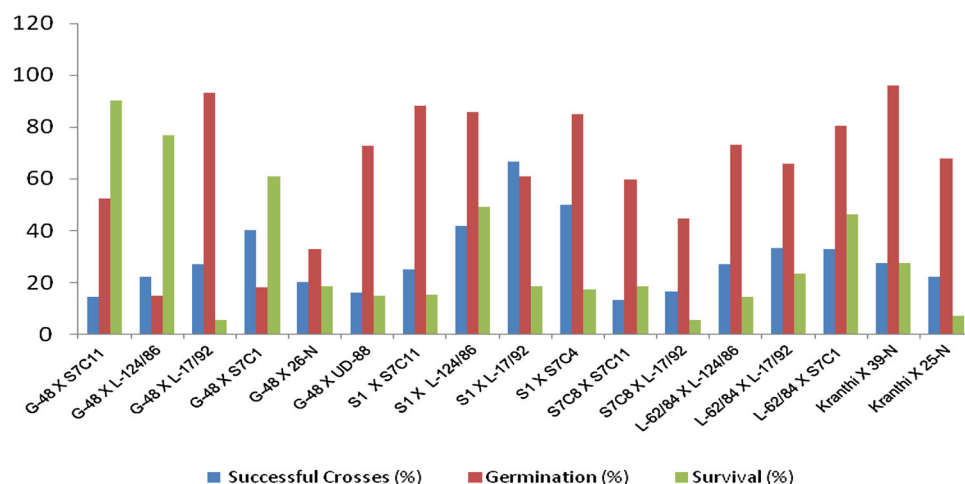
Survival is one of the important factors in successful establishment of a species and is the best indicator of species with respect to adaptation and growth. The range for survival per cent was between 5.44 to 90.44 per cent

**Table 5** The variation in different morphological characters among seventeen hybrids of *Populus deltoides*

Sr. No.	Crosses	Plant height (cm)	Collar diameter (mm)	Internodal length (cm)	No. of leaves/plant	Leaf area (cm <sup>2</sup> )	Apex angle (°)	Length of lamina (cm)	Maximum width of leaf (cm)	Ratio of length of lamina/maximum width of leaf	Petiole length (cm)	Angle between the midrib and 2 <sup>nd</sup> lower lateral mid rib (°)	No. of branches/plant	Branch angle (°)	Branch length (cm)
1	G-48 × S <sub>7</sub> C <sub>11</sub>	340	21.59	8.36	44.8	270.52	43.3	15.85	17.41	0.91	8.76	51.6	5.0	55.0	15.40
2	G-48 × L-124/86	296	18.56	4.84	63.6	203.92	44.1	14.77	15.92	0.92	9.52	53.2	2.4	54.8	16.12
3	G-48 × L-17/92	314	23.99	4.34	56.0	180.44	44.0	15.16	15.75	0.99	8.35	55.0	2.0	45.0	12.50
4	G-48 × S <sub>7</sub> C <sub>1</sub>	408	25.27	4.46	118.0	182.98	45.0	15.47	15.60	0.98	9.19	48.4	6.3	49.0	38.00
5	G-48 × 26-N	382	23.57	3.88	95.0	244.86	43.2	15.52	17.14	0.89	9.84	41.6	6.4	37.0	25.08
6	G-48 × UD-88	385	22.43	4.70	66.8	222.76	42.5	16.66	16.65	0.95	8.10	48.0	1.0	30.0	35.80
7	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	400	22.82	4.66	102.6	231.78	43.8	16.31	17.97	0.90	11.75	56.0	8.4	55.0	21.50
8	S <sub>1</sub> × L-124/86	372	26.10	4.74	47.6	253.25	41.5	17.48	19.29	0.90	10.75	53.6	6.2	51.6	21.22
9	S <sub>1</sub> × L-17/92	310	19.32	4.78	72.6	244.15	41.5	16.41	16.07	1.02	9.51	56.8	4.8	53.0	15.22
10	S <sub>1</sub> × S <sub>7</sub> C <sub>4</sub>	308	19.49	4.38	58.6	263.80	42.4	15.48	16.58	0.93	10.76	52.4	7.2	66.75	19.77
11	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	378	22.29	5.22	76.8	216.65	44.2	15.58	17.69	0.87	10.77	54.4	7.0	45.0	26.42
12	S <sub>7</sub> C <sub>8</sub> × L-17/92	338	19.71	5.08	88.0	203.26	42.3	14.62	16.38	0.88	9.64	49.6	4.0	60.0	25.00
13	L-62/84 × L-124/86	191	12.45	4.02	37.8	161.75	38.2	13.38	13.41	0.98	6.97	51.2	2.5	55.0	47.05
14	L-62/84 × L-17/92	310	19.40	5.98	50.0	217.92	43.6	16.90	16.76	1.00	9.14	60.0	6.0	50.0	14.90
15	L-62/84 × S <sub>7</sub> C <sub>1</sub>	362	20.57	6.20	63.2	234.61	41.9	17.18	17.22	0.99	10.38	54.4	2.0	40.0	40.85
16	Kranthi × 39-N	354	22.28	4.74	69.8	214.33	43.5	14.28	16.82	0.84	10.74	57.2	5.6	60.0	16.90
17	Kranthi × 25-N	235	17.82	4.80	54.0	149.78	42.5	20.88	22.56	0.90	13.15	50.0	2.0	70.0	6.70
SE		13.99	0.78	0.25	5.28	8.32	0.37	0.40	0.45	0.01	0.35	1.04	0.54	2.47	2.68
CV		17.26	15.37	20.88	31.76	15.79	3.62	10.34	11.09	5.65	14.82	8.20	48.73	19.78	47.25



**Fig. 2** Variation in successful crosses (%), germination (%) and survival (%) in hybrids of *Populus deltoides*



**Table 6** The variation in different crossability parameters of various intra-specific hybrids of *Populus deltoides*

Sr. No.	Crosses	Successful crosses (%)	Germination (%)	Survival (%)
1	G-48 × S <sub>7</sub> C <sub>11</sub>	14.41 (22.83)	52.33 (46.33)	90.44 (71.99)
2	G-48 × L-124/86	22.22 (28.77)	14.71 (6.67)	76.67 (66.14)
3	G-48 × L-17/92	27.22 (31.62)	93.33 (75.28)	5.56 (1.43)
4	G-48 × S <sub>7</sub> C <sub>1</sub>	40.00 (39.23)	18.16 (9.78)	60.85 (51.60)
5	G-48 × 26-N	20.00 (26.57)	32.89 (34.98)	18.66 (25.33)
6	G-48 × UD-88	16.13 (24.53)	72.89 (58.63)	14.74 (6.66)
7	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	25.00 (27.21)	88.24 (70.31)	15.12 (6.99)
8	S <sub>1</sub> × L-124/86	41.65 (44.61)	85.99 (68.46)	49.17 (39.36)
9	S <sub>1</sub> × L-17/92	66.64 (61.62)	60.98 (52.02)	18.54 (25.39)
10	S <sub>1</sub> × S <sub>7</sub> C <sub>4</sub>	50.00 (45.00)	84.89 (68.74)	17.34 (9.07)
11	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	13.35 (21.73)	59.56 (50.66)	18.47 (25.44)
12	S <sub>7</sub> C <sub>8</sub> × L-17/92	16.66 (25.03)	44.67 (41.88)	5.44 (1.35)
13	L-62/84 × L-124/86	27.31 (31.72)	73.33 (59.61)	14.55 (21.18)
14	L-62/84 × L-17/92	33.39 (36.26)	65.79 (55.60)	23.49 (27.74)
15	L-62/84 × S <sub>7</sub> C <sub>1</sub>	33.00 (36.21)	80.65 (64.77)	46.42 (42.72)
16	Kranthi × 39-N	27.37 (31.72)	96.00 (78.57)	27.65 (31.23)
17	Kranthi × 25-N	22.18 (28.69)	68.00 (56.03)	7.06 (2.26)
CD (0.05)		7.87	13.44	15.67

\*Values in parenthesis are arcsine values

among the crosses. Cross G-48 × S<sub>7</sub>C<sub>11</sub> recorded highest survival (90.44%) among all other crosses followed by G-48 × L-124/86 (76.67%) and G-48 × S<sub>7</sub>C<sub>1</sub> (60.85%). Whereas, cross S<sub>7</sub>C<sub>8</sub> × L-17/92 (5.44%) was recorded minimum survival. Our results find support from the observation of Pichot and Teissier (1988) in clones of *Populus nigra* which produced twenty-one full sib families. The present investigations are also in agreement with the findings of Singh, (2002) in poplar. The results are in conformity with the findings of Dhiman and Gandhi, (2012) whom has reported the crossability relationships among poplars.

### Controlled pollination (hybridization) under line × tester mating design

Controlled pollination (hybridization) is a vital means of combining genes of two or more varieties/clones/species to produce progenies, the evaluation and selection of which leads to the recommendation of new varieties that have gene combinations superior to the parent population. The objective of hybridization is to capture a sufficiently inclusive and unbiased genetic representation and to generate large hybrid families. There are three main motives for hybridization viz., (i) combine desirable characters from different species/varieties (ii) capture heterosis or

hybrid vigor and (iii) to obtain increased developmental homeostasis i.e. greater phenotypic stability in varied environment (Stettler et al. 1996). Intra specific controlled crossing was carried out during February to mid May in 2013. The sixteen  $F_1$  hybrids were needed for Line  $\times$  Tester ( $4 \times 4$  factorial) mating design using 4 males and 4 females but twelve  $F_1$  hybrids survived to obtain appropriate and most promising combination for growth, biomass and wood characters.

### Morphological characters

Table 7 showed the visual morphological traits in *Populus deltoides* hybrids. It was observed that among various colour of stem at sun side, Yellow Green 148 (Group A) colour was observed in maximum number of hybrids, which is followed by Yellow Green 148 (Group B) colour in hybrids of *Populus deltoides*. The stem shape was observed straight in maximum number of hybrids. The stem felt was found absent in maximum number of hybrids, while it was present in two hybrids ( $G-48 \times S_7C_{11}$  and  $S_7C_8 \times S_7C_{11}$ ) only. The branch nature was found straight in maximum number of hybrids, while, leaf blade altitude was observed downward in all the hybrids.

The Table 7 also revealed that Green 138 (Group A) colour in the leaves was observed only in  $S_1 \times L-17/92$  and  $S_7C_8 \times L-17/92$  hybrids. The shape of leaf tip of all the hybrids was narrow long acuminate etroit, long, acumine schmal langzugespitzt, while leaf base was observed slightly wavy to wavy in number of hybrids (Plate 3). Most of the hybrids depicted weakly cordate legerement cordiforme leicht herzformig general shape of the leaf base. The distribution of anthocyanin coloration from base to midrib was observed in maximum number of hybrids except  $G-48$  cross combinations hybrids. The Greyed-Red 179 (Group A) colour intensity of petiole at junction was found in maximum number of hybrids. On the other hand, only two hybrids i.e.  $G-48 \times S_7C_{11}$  and  $S_7C_8 \times L-17/92$  showed Green 143 (Group D) colour of petiole, while rest of the hybrids showed distinctive colour. The widely wedge shaped cuneiforme large breit keilformig was the shape of junction with petiole which was found in maximum number of hybrids, followed by Steep profunde steil shape, while Shallow peu profunde flach shape was found in minimum number of hybrids.

The average plant height of hybrids was found 255.69 cm, whereas average plant height of controls was found is 209.90 cm. Analysis of variance revealed that, among the hybrids, maximum plant height was observed in  $G-48 \times L-17/92$  (316.11 cm) followed by  $L-62/84 \times S_7C_1$  (286.03 cm) and  $L-62/84 \times L-17/92$  (280 cm) whereas, the minimum plant height (210.45 cm) was recorded in  $G-48 \times S_7C_{11}$ . Average collar diameter of hybrids was

found 16.93 mm, whereas average collar diameter of controls was found 14.67 mm. It is evident from Table 8 (Fig. 3) that maximum collar diameter among hybrids (21.22 mm) was observed for  $G-48 \times L-17/92$  which was at par with  $G-48 \times L-124/86$  (18.58 mm) and  $L-62/84 \times S_7C_1$  (18.52 mm). The minimum collar diameter (13.68 mm) was observed in  $L-62/84 \times L-124/86$  hybrid. Average internodal length of controls was found 4.16 cm, whereas average internodal length of hybrids was found 4.05 cm. Among hybrids maximum internodal length of 4.58 cm was found in  $G-48 \times L-17/92$  which was at par with  $L-62/84 \times L-124/86$  (4.47 cm) and  $L-62/84 \times L-17/92$  (4.43 cm), whereas minimum value (3.42 cm) observed in  $G-48 \times S_7C_{11}$ .

Average number of leaves/plant was found 41.51 in the hybrids, whereas average number of leaves/plant of controls was found 30.47. Analysis of variance revealed that maximum number of leaves/plant among hybrids was observed in  $S_1 \times S_7C_{11}$  (50.41) followed by  $G-48 \times S_7C_1$  (48.65) and  $G-48 \times L-124/86$  (47.00) whereas, the minimum average number of leaves/plant was recorded in  $L-62/84 \times L-124/86$  (29.66).

Average petiole length of hybrids was found 8.30 cm, whereas average petiole length of controls was found 7.45 cm. Petiole length among hybrids was found to be maximum in  $S_1 \times S_7C_{11}$  (9.42 cm) followed by  $S_7C_8 \times L-17/92$  (9.01 cm) and  $S_7C_8 \times S_7C_{11}$  (8.67 cm) whereas, the minimum in  $G-48 \times S_7C_{11}$  (6.55 cm). Average leaf area of hybrids was found 160.87 cm<sup>2</sup> whereas, average leaf area of controls was found 147.28 cm<sup>2</sup>. Analysis revealed that among hybrids, leaf area was found to be maximum in  $S_1 \times L-17/92$  (231.50 cm<sup>2</sup>) followed by  $L-62/84 \times S_7C_1$  (211.99 cm<sup>2</sup>) &  $G-48 \times L-17/92$  (199.80 cm<sup>2</sup>) whereas, the minimum value was observed in  $G-48 \times S_7C_{11}$  (109.11 cm<sup>2</sup>). Average apex angle of hybrids was found 36.19° whereas average plant apex angle of controls was found 34°. It is evident from Table 8 that among hybrids apex angle was found to be maximum (39.66°) in  $S_1 \times L-124/86$  and minimum in  $L-62/84 \times L-17/92$  (28.66°), respectively. Average length of lamina of hybrids was found 14.15 cm, whereas average length of lamina of controls was found 13.25 cm. It is evident from Table 8 (Fig. 3) that maximum length of lamina among hybrids (16.42 cm) was observed for  $L-62/84 \times L-17/92$  which was at par with  $L-62/84 \times L-124/86$  (15.33 cm) and  $L-62/84 \times S_7C_1$  (14.85 cm). The minimum value for length of lamina (11.76 cm) was observed in  $G-48 \times S_7C_{11}$  hybrid.

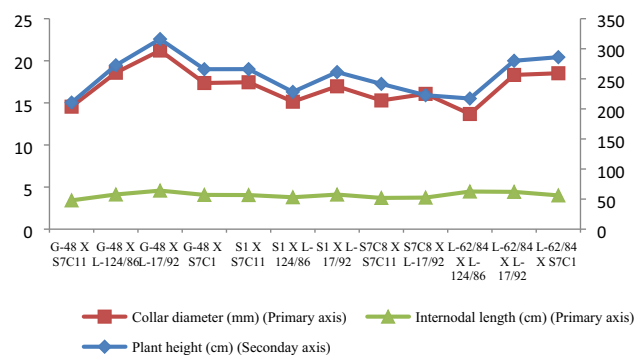
Average maximum width of leaf of hybrids was found 14.68 cm, whereas average maximum width of leaf of controls was found 12.65 cm. It is evident from Table 8 that among hybrids, width of leaf was found to be maximum (15.71 cm) in  $G-48 \times L-17/92$  followed by  $L-62/$

**Table 7** The variation in different visual morphological characters among twelve hybrids of *Populus deltoides*

Sr. No.	Crosses	Stem colour at sun side	Stem shape	Stem felt	Branch nature	Leaf blade altitude	Colour of leaf	Shape of leaf tip	Leaf base	General shape of leaf base	Distribution of anthocyanin coloration of mid rib	Colour intensity of petiole at junction	Colour of petiole	Shape of junction with petiole
1	G-48 × S <sub>7</sub> C <sub>11</sub>	Green N-138 (Group-B)	Slightly curved	Present	Slightly curved	Downward	Yellow-Green 147 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Medium cordate moyennement cordiforme mittel herzformig	Base	Red 47 (Group B)	Green 143 (Group D)	Steep profonnde steil
2	G-48 × L-124/86	Yellow-Green 148 (Group-B)	Slightly curved	Absent	Straight	Downward	Green-N 137 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Strongly cordate forment cordiforme deutlich herzformig	Base	Greyed-Red 179 (Group A)	Yellow Green 144 (Group B)	Widely wedge shaped cuneiforme large breit keilformig
3	G-48 × L-17/92	Yellow-Green 148 (Group-B)	Curved	Absent	Straight	Downward	Green 137 (Group C)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Wavy	Weakly cordate legerement cordiforme leicht herzformig	Base	Red 45 (Group B)	Yellow 17 (Group C)	Widely wedge shaped cuneiforme large breit keilformig
4	G-48 × S <sub>7</sub> C <sub>1</sub>	Yellow-Green 148 (Group-A)	Curved	Absent	Straight	Downward	Yellow-Green 151 (Group B)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Strongly cordate forment cordiforme deutlich herzformig	Base	Yellow - Green 154 (Group D)	Yellow Green 154 (Group D)	Steep profonnde steil
5	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	Yellow-Green 148 (Group-A)	Straight	Absent	Straight	Downward	Green 143 (Group B)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Weakly cordate legerement cordiforme leicht herzformig	Base to midrib	Greyed-Red 179 (Group A)	Greyed-Yellow 160 (Group A)	Widely wedge shaped cuneiforme large breit keilformig
6	S <sub>1</sub> × L-124/86	Green 138 (Group-A)	Straight	Absent	Slightly straight	Downward	Yellow-Green 146 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Weakly cordate legerement cordiforme leicht herzformig	Base to midrib	Red 47 (Group A)	Yellow-Green 144 (Group D)	Shallow peu profonnde flach
7	S <sub>1</sub> × L-17/92	Yellow-Green 148 (Group-A)	Straight	Absent	Straight	Downward	Green 138 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Wavy	Medium cordate moyennement cordiforme mittel herzformig	Base to midrib	Red 45 (Group A)	Yellow 11 (Group C)	Widely wedge shaped cuneiforme large breit keilformig
8	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	Yellow-Green 148 (Group-A)	Straight	Present	Slightly straight	Downward	Green 137 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Weakly cordate legerement cordiforme leicht herzformig	Base to midrib	Red 45 (Group B)	Red 45 (Group C)	Shallow peu profonnde flach
9	S <sub>7</sub> C <sub>8</sub> × L-17/92	Yellow-Green 148 (Group-C)	Straight	Absent	Straight	Downward	Green 138 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Wavy	Weakly cordate legerement cordiforme leicht herzformig	Base to midrib	Green 143 (Group C)	Green 143 (Group D)	Widely wedge shaped cuneiforme large breit keilformig

Table 7 continued

Sr. No.	Crosses	Stem colour at sun side	Stem shape	Stem felt	Branch nature	Leaf blade altitude	Colour of leaf	Shape of leaf tip	Leaf base	General shape of leaf base	Distribution of anthocyanin coloration of mid rib	Colour intensity of petiole at junction	Colour of petiole	Shape of junction with petiole
10	L-62/84 × L-124/86	Yellow-Green 146 (Group-D)	Straight	Absent	Straight	Downward	Green 143 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Straight droite gerade	Base to midrib	Red 45 (Group A)	Yellow-Green 145 (Group A)	Widely wedge shaped cuneiforme large breit keilförmig
11	L-62/84 × L-17/92	Yellow-Green 148 (Group-A)	Straight	Absent	Straight	Downward	Green 143 (Group C)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Wavy	Medium cordate moyennement cordiforme mittel herzformig	Base to midrib	Red 42 (Group A)	Red 42 (Group A)	Steep profonnde steil
12	L-62/84 × S <sub>7</sub> C <sub>1</sub>	Yellow-Green 148 (Group-A)	Straight	Absent	Slightly Curved	Downward	Yellow-Green 144 (Group A)	Narrow long acuminate etroit, long,acumine schmal langzugespitzt	Slightly wavy	Weakly cordate legerement cordiforme leicht herzformig	Base to midrib	Greyed-Red 179 (Group A)	Yellow 11 (Group -B)	Widely wedge shaped cuneiforme large breit keilförmig

Fig. 3 Variation in plant height, collar diameter and intermodal length among the hybrids of *Populus deltoides*

84 × L-17/92 (15.70 cm) and S<sub>1</sub> × L-124/86 (15.57 cm) and minimum in G-48 × S<sub>7</sub>C<sub>11</sub> (12.44 cm). Average ratio of length of lamina/maximum width of leaf of controls was found 1.05, whereas average ratio of length of lamina/maximum width of leaf of hybrids was found 0.97. Among hybrids, ratio of length of lamina/maximum width of leaf was found greatest in L-62/84 × L-17/92 (1.04) which was found at par with L-62/84 × S<sub>7</sub>C<sub>1</sub> (1.02) and S<sub>1</sub> × L-17/92 (1.01), whereas minimum value (0.87) observed in G-48 × L-17/92. Average angle between the mid rib and 2nd lower lateral mid rib of controls was found 72.67° whereas average angle between the mid rib and 2nd lower lateral mid rib of controls was found 16.57°. Angle between the mid rib and 2nd lower lateral mid rib was found maximum in G-48 × S<sub>7</sub>C<sub>1</sub> (80°) followed by G-48 × S<sub>7</sub>C<sub>11</sub> (77.66°) and G-48 × L-124/86 (76.66°) and minimum in S<sub>7</sub>C<sub>8</sub> × L-17/92 (57°) among the hybrids (Table 8).

Average shoot bark thickness of hybrids was found 0.65 mm whereas, average shoot bark thickness of controls was found 0.60 mm. Shoot bark thickness among hybrids was found maximum in S<sub>7</sub>C<sub>8</sub> × L-17/92 (1.45 mm) followed by G-48 × L-124/86 (0.78 mm) and G-48 × L-17/92 (0.73 mm) and minimum in G-48 × S<sub>7</sub>C<sub>1</sub> (0.30 mm). Average root bark thickness of controls was found 1.35 mm, whereas average root bark thickness of hybrids was found 0.94 mm. Root bark thickness among hybrids was found maximum in S<sub>7</sub>C<sub>8</sub> × L-17/92 (1.36 mm) followed by L-62/84 × L-124/86 (1.17 mm) and G-48 × L-124/86 (1.13 mm) and minimum in S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub> (0.43 mm) (Table 9). Average fresh shoot weight of hybrids was found 268.34 g, whereas average fresh shoot weight of controls was found 172.16 g. Fresh shoot weight among hybrids was found maximum in S<sub>1</sub> × L-17/92 (338.12 g) followed by G-48 × L-17/92 (326.75 g) & L-62/84 × S<sub>7</sub>C<sub>1</sub> (324.99) and minimum in L-62/84 × L-124/86 (154.16 g). Average dry shoot weight of hybrids was found 189.87 g, whereas average dry shoot weight of controls was found 128.79 g. Dry shoot weight among hybrids was

**Table 8** The variation in different morphological characters among twelve hybrids of *Populus deltoides*

Sr. No.		Plant height (cm)	Collar diameter (mm)	Internodal length (cm)	Number of leaves/plant	Petiole length (cm)	Leaf area (cm <sup>2</sup> )	Apex angle (°)	Length of lamina (cm)	Maximum width of leaf (cm)	Ratio of length of lamina/maximum width of leaf	Angle between the mid rib and 2nd lower lateral mid rib (°)
<b>Crosses</b>												
1	G-48 × S <sub>7</sub> C <sub>11</sub>	210.45	14.55	3.42	41.66	6.55	109.11	38.33	11.76	12.44	0.94	77.66
2	G-48 × L-124/86	272.52	18.58	4.12	47.00	7.55	122.33	39.00	14.50	15.51	0.93	76.66
3	G-48 × L-17/92	316.11	21.22	4.58	41.44	8.41	199.80	38.66	13.51	15.71	0.87	59.80
4	G-48 × S <sub>7</sub> C <sub>1</sub>	265.98	17.36	4.07	48.65	8.22	128.93	39.00	13.76	14.02	0.98	80.00
5	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	266.12	17.45	4.05	50.41	9.42	119.40	37.33	13.91	14.86	0.93	68.00
6	S <sub>1</sub> × L-124/86	228.25	15.14	3.79	36.63	8.53	187.89	39.66	14.59	15.57	0.94	72.33
7	S <sub>1</sub> × L-17/92	261.21	16.97	4.11	45.55	8.42	231.50	30.66	14.19	13.94	1.01	68.00
8	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	241.56	15.29	3.71	39.78	8.67	137.71	35.66	13.62	14.82	0.91	63.33
9	S <sub>7</sub> C <sub>8</sub> × L-17/92	222.77	16.07	3.75	40.55	9.01	146.35	36.00	13.38	13.39	0.99	57.00
10	L-62/84 × L-124/86	217.23	13.68	4.47	29.66	7.82	176.04	35.33	15.33	15.56	0.99	69.66
11	L-62/84 × L-17/92	280.00	18.32	4.43	35.58	8.56	159.35	28.66	16.42	15.70	1.04	68.66
12	L-62/84 × S <sub>7</sub> C <sub>1</sub>	286.03	18.52	4.01	41.17	8.46	211.99	36.00	14.85	14.55	1.02	73.66
	Mean	255.69	16.93	4.05	41.51	8.30	160.87	36.19	14.15	14.68	0.97	69.57
<b>Controls</b>												
1	G-48	224.62	15.32	4.11	33.11	7.50	195.91	34.33	13.87	12.93	1.07	73.33
2	6P	195.18	14.01	4.20	27.83	7.39	98.65	33.67	12.63	12.38	1.03	72.00
	Mean	209.90	14.67	4.16	30.47	7.45	147.28	34.00	13.25	12.65	1.05	72.67
	CD control v/s crosses	29.61	1.89	NS	5.81	0.58	NS	NS	NS	0.96	0.04	2.43
	CD between crosses	54.83	3.51	0.56	10.77	1.08	44.22	5.04	2.12	1.77	0.08	4.51
	CD between control	NS	NS	NS	NS	NS	44.22	NS	NS	NS	NS	NS



**Table 9** The variation in different morphological characters among twelve hybrids of *Populus deltoids*

Sr. No.		Shoot bark thickness (mm)	Root bark thickness (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Dry root shoot ratio	Root length (cm)	Total fresh weight (g)	Total dry weight (g)
Crosses											
1	G-48 × S <sub>7</sub> C <sub>11</sub>	0.63	1.09	181.03	148.85	95.96	59.15	0.47	40.08	264.05	189.61
2	G-48 × L-124/86	0.78	1.13	213.94	171.25	112.04	63.67	0.28	39.49	311.85	207.92
3	G-48 × L-17/92	0.73	0.95	326.75	237.51	117.50	77.87	0.29	36.20	444.25	308.86
4	G-48 × S <sub>7</sub> C <sub>1</sub>	0.30	0.21	234.03	191.45	99.49	58.54	0.37	33.59	335.35	251.16
5	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	0.49	0.43	323.89	226.75	138.30	78.92	0.39	42.39	462.19	299.00
6	S <sub>1</sub> × L-124/86	0.64	1.11	235.31	151.20	145.84	71.741	0.66	42.23	381.15	236.56
7	S <sub>1</sub> × L-17/92	0.53	1.08	338.12	198.10	174.73	102.10	0.55	39.50	512.85	300.21
8	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	0.57	0.82	287.51	202.88	115.97	67.83	0.36	39.45	403.49	272.21
9	S <sub>7</sub> C <sub>8</sub> × L-17/92	1.45	1.36	308.38	208.50	119.83	77.03	0.46	34.45	395.75	290.90
10	L-62/84 × L-124/86	0.59	1.17	154.16	130.39	88.92	47.52	0.30	31.13	239.84	148.89
11	L-62/84 × L-17/92	0.36	0.94	291.92	194.64	133.50	78.31	0.39	34.25	425.43	272.25
12	L-62/84 × S <sub>7</sub> C <sub>1</sub>	0.67	0.88	324.99	216.84	161.64	93.43	0.51	41.06	486.64	305.57
	Mean	0.65	0.94	268.34	189.87	125.31	73.01	0.42	37.82	388.57	256.93
Controls											
1	G-48	0.70	1.98	221.69	150.07	105.90	53.21	0.45	36.50	321.61	213.64
2	6P	0.49	0.72	122.63	107.50	75.17	61.75	0.67	31.79	170.98	167.50
	Mean	0.60	1.35	172.16	128.79	90.53	57.48	0.56	34.15	246.29	190.57
	CD control v/s crosses	NS	0.32	56.20	34.51	15.59	9.68	0.09	2.40	67.19	43.84
	CD between crosses	0.20	0.49	84.96	52.17	23.57	14.63	0.14	3.63	101.59	66.29
	CD between control	NS	0.49	NS	NS	23.57	NS	0.14	3.63	101.59	NS

found maximum in G-48 × L-17/92 (237.51 g) followed by S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub> (226.75 g) and L-62/84 × S<sub>7</sub>C<sub>1</sub> (216.84 g) while, minimum in L-62/84 × L-124/86 (130.39 g). Average fresh root weight of hybrids was found 125.31 g, whereas average fresh root weight of controls was found 90.53 g. The fresh root weight among hybrids was recorded maximum in S<sub>1</sub> × L-17/92 (174.73 g) which was at par with L-62/84 × S<sub>7</sub>C<sub>1</sub> (161.64 g) & S<sub>1</sub> × L-124/86 (145.84 g) whereas, minimum fresh root weight (88.92 g) was recorded in L-62/84 × L-124/86. Average dry root weight of hybrids was found 73.01 g whereas average dry root weight of controls was found 57.48 g. The maximum dry root weight was recorded in S<sub>1</sub> × L-17/92 (102.10 g) which was at par with L-62/84 × S<sub>7</sub>C<sub>1</sub> (93.43 g) & L-62/84 × L-17/92 (78.31 g) whereas, minimum value (47.52 g) of dry root weight was recorded in L-62/84 × L-124/86 among hybrids. Average dry root shoot ratio of controls was found 0.56 whereas average dry root shoot ratio of hybrids was found 0.42. The maximum dry root shoot ratio was recorded in S<sub>1</sub> × L-124/86 (0.66) which

was at par with S<sub>1</sub> × L-17/92 (0.55) & L-62/84 × S<sub>7</sub>C<sub>1</sub> (0.51) whereas, minimum dry root shoot ratio (0.28) was recorded in G-48 × L-124/86 among hybrids.

Average root length of hybrids was found 37.82 cm whereas average root length of controls was found 34.15 cm. The root length was recorded maximum in S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub> (42.39 cm) which was at par with S<sub>1</sub> × L-124/86 (42.23 cm) & L-62/84 × S<sub>7</sub>C<sub>1</sub> (41.06 cm) whereas, root length (31.13 cm) was recorded in L-62/84 × L-124/86. Average total fresh weight of hybrids was found 388.57 g whereas average total fresh weight of controls was found 246.29 g. The total fresh weight among hybrids was recorded maximum in S<sub>1</sub> × L-17/92 (512.85 g) which was at par with L-62/84 × S<sub>7</sub>C<sub>1</sub> (486.64 g) & S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub> (462.19 g) whereas, minimum value (239.84 g) total fresh weight was recorded in L-62/84 × L-124/86. Average total dry weight of hybrids was found is 256.93 g whereas average total dry weight of controls was found is 190.57 g. Maximum total dry weight among hybrids was recorded in G-48 × L-17/92 (308.86 g) which was at par with L-62/

84 × S<sub>7</sub>C<sub>1</sub> (305.57 g) & S<sub>1</sub> × L-17/92 (300.21 g) whereas, minimum value (148.89 g) for the trait was recorded in L-62/84 × L-124/86 (Table 9). Similarly variations with respect to hybrid performance have also been reported by Chaudhary, 2011; on willows. The results are in conformity with the findings of Krstinic and Vidakovic, (1988) who reported better performance of intra-specific hybrids of *S. alba* as compared to their inter-specific hybrids with *S. fragilis*. Ozel et al. (2010) confirmed better performance of intra-specific crosses of eastern cottonwood (*Populus deltoides*) and black poplar (*P. nigra*). In conformity with the present findings, Satoh et al. (2011) in a study on above ground biomass yield of willow clones reported significant differences in the productivity of different clones.

Singh et al. (2013) also reported that the characters significantly varied between the families except leaf width and leaf area hybrids of *Populus* species. Our results are in line with the finding of Dhir and Mohn, (1976) that observed significant differences between populations of eastern cottonwood developed by crossing two sources for plant height, stem diameter, number of internodes and petiole length. Similar findings on *P. tremula* reported by Vaario et al. (2011) and they observed that significant height and mean number of leaves was obtained in families by controlled crossing between four male and three female trees at two different soil types. Similarly, variation with respect to physiological traits among clones, families and seed sources of *Populus* and *Salix* has earlier been reported by Aasamaa et al. (2010) and Bouman and Sylliboy, (2012). The productivity is the result of genetic and environment regulation of the physiological process of plant growth and morphological development. For wood traits the results are in conformity with the findings of Gupta et al. (2014) and Raja (2014) in *Salix*.

### Physiological parameters

Average internal CO<sub>2</sub> concentration of hybrids was found 13,954.76 ppm whereas average internal CO<sub>2</sub> concentration of controls was found 12,920.30 ppm. The L-62/84 × S<sub>7</sub>C<sub>1</sub> hybrid recorded maximum value (23,601.68 ppm) for internal CO<sub>2</sub> concentration among hybrids and it was found to be at par with G-48 × S<sub>7</sub>C<sub>11</sub> (19,282.53 ppm) whereas it was observed to be minimum (7275.84 ppm) in G-48 × L-124/86. Average rate of photosynthesis of hybrids was found 16.71 μmol m<sup>-2</sup> s<sup>-1</sup> whereas average rate of photosynthesis of controls was found 14.82 μmol m<sup>-2</sup> s<sup>-1</sup>. The L-62/84 × S<sub>7</sub>C<sub>1</sub> (21.35 μmol m<sup>-2</sup> s<sup>-1</sup>) hybrid recorded the maximum value for rate of photosynthesis among hybrids and it was found to be at par with L-62/84 × L-124/86

(18.40 μmol m<sup>-2</sup> s<sup>-1</sup>) whereas it was observed to be minimum (13.92 μmol m<sup>-2</sup> s<sup>-1</sup>) in G-48 × L-124/86.

Average transpiration rate of controls was found 5.11 μmol m<sup>-2</sup> s<sup>-1</sup>, whereas, average transpiration rate of hybrids was found 5.01 μmol m<sup>-2</sup> s<sup>-1</sup>. The G-48 × L-124/86 (5.86 μmol m<sup>-2</sup> s<sup>-1</sup>) hybrid recorded the maximum value for transpiration rate among hybrids and it was found to be at par with G-48 × S<sub>7</sub>C<sub>11</sub> (5.80 μmol m<sup>-2</sup> s<sup>-1</sup>) whereas it was observed to be minimum (3.67 μmol m<sup>-2</sup> s<sup>-1</sup>) in S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub>. Average stomatal conductance of hybrids was found 4.53 mol m<sup>-2</sup> s<sup>-1</sup> whereas average stomatal conductance of controls was found 4.24 mol m<sup>-2</sup> s<sup>-1</sup>. It is evident from Table 10 that G-48 × S<sub>7</sub>C<sub>11</sub> recorded the maximum stomatal conductance (7.06 mol m<sup>-2</sup> s<sup>-1</sup>) which was at par with G-48 × S<sub>7</sub>C<sub>1</sub> (5.20 mol m<sup>-2</sup> s<sup>-1</sup>) among hybrids. The minimum value for stomatal conductance was found in S<sub>7</sub>C<sub>8</sub> × L-17/92 (3.54 mol m<sup>-2</sup> s<sup>-1</sup>).

Average water use efficiency of hybrids was found 3.69 μmol CO<sub>2</sub>/μmol H<sub>2</sub>O whereas average water uses efficiency of controls was found 2.94 μmol CO<sub>2</sub>/μmol H<sub>2</sub>O. Water use efficiency among hybrids was found to be maximum in S<sub>1</sub> × S<sub>7</sub>C<sub>11</sub> (5.66 μmol CO<sub>2</sub>/μmol H<sub>2</sub>O) which was at par with L-62/84 × L-124/86 (4.18 μmol CO<sub>2</sub>/μmol H<sub>2</sub>O) and it was recorded minimum in G-48 × L-124/86 (2.41 μmol CO<sub>2</sub>/μmol H<sub>2</sub>O). Average wood density of both hybrids and controls were found 0.41 g/cm<sup>3</sup>. Maximum wood density (0.4168 g/cm<sup>3</sup>) among hybrids was found in L-62/84 × L-17/92 and minimum (0.4110 g/cm<sup>3</sup>) in S<sub>7</sub>C<sub>8</sub> × L-17/92. Average fibre length of hybrids was found 0.49 mm whereas average fibre length of controls was found 0.42 mm. Maximum fibre length (0.6415 mm) among hybrids was found in L-62/84 × L-124/86 and minimum (0.3794 mm) in S<sub>1</sub> × L-124/86. Results also find supports from findings of Singh, (2002) who also reported intra-specific variation in hybrids performance of poplars. On the other hand, Villar et al. (1987) reported interspecific incompatibility reactions between *P. nigra* and *P. alba*.

### Estimation of genetic components of variance

Variability for morphological characters was estimated in terms of mean, range, genotypic and phenotypic coefficient of variation. Genetic parameters were worked out with regards to estimate heritability (broad sense), genetic advance and genetic gain as per cent of mean. Variability estimated and genetic parameters were calculated for growth, morphological and physiological traits.

Among all the morphological characters, total fresh weight showed widest range of values (239.84–512.85 g and mean 388.57 g), followed by fresh shoot weight (154.16–338.12 g and 268.34 g) indicating the extent of

**Table 10** The variation in different physiological wood characters among twelve hybrids of *Populus deltoides*

Sr. No.		Internal CO <sub>2</sub> concentration (ppm)	Rate of photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Rate of transpiration ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ )	Water use efficiency ( $\mu\text{mol CO}_2/\mu\text{mol H}_2\text{O}$ )	Wood density ( $\text{g/cm}^3$ )	Fibre length (mm)
<b>Crosses</b>								
1	G-48 × S <sub>7</sub> C <sub>11</sub>	19,282.53	17.48	5.80	7.06	3.15	0.4134	0.5267
2	G-48 × L-124/86	7275.84	13.92	5.86	4.21	2.41	0.4164	0.5588
3	G-48 × L-17/92	9913.51	16.45	5.15	4.08	3.46	0.4149	0.4229
4	G-48 × S <sub>7</sub> C <sub>1</sub>	15,545.96	17.13	5.73	5.20	3.25	0.4136	0.5204
5	S <sub>1</sub> × S <sub>7</sub> C <sub>11</sub>	12,405.15	17.57	3.67	3.55	5.66	0.4139	0.4954
6	S <sub>1</sub> × L-124/86	14,119.10	17.16	4.93	4.29	3.78	0.4165	0.3794
7	S <sub>1</sub> × L-17/92	12,544.15	13.99	4.46	4.58	3.29	0.4144	0.4260
8	S <sub>7</sub> C <sub>8</sub> × S <sub>7</sub> C <sub>11</sub>	10,384.28	14.44	4.31	4.00	3.67	0.4126	0.4872
9	S <sub>7</sub> C <sub>8</sub> × L-17/92	10,692.98	15.87	5.29	3.54	3.21	0.4110	0.5061
10	L-62/84 × L-124/86	16,592.78	18.40	4.18	3.96	4.81	0.4144	0.6415
11	L-62/84 × L-17/92	15,099.09	16.69	5.30	4.95	3.48	0.4168	0.4268
12	L-62/84 × S <sub>7</sub> C <sub>1</sub>	23,601.68	21.35	5.37	4.96	4.03	0.4156	0.5208
	Mean	13,954.76	16.71	5.01	4.53	3.69	0.41	0.49
<b>Controls</b>								
1	G-48	17,863.37	14.71	5.29	3.83	2.76	0.41	0.45
2	6P	7977.22	14.93	4.92	4.65	3.12	0.41	0.38
	Mean	12,920.30	14.82	5.11	4.24	2.94	0.41	0.42
	CD control v/s crosses	NS	NS	NS	NS	NS	NS	0.0101
	CD between crosses	NS	NS	1.14	1.29	1.47	NS	0.0153
	CD between control	NS	NS	NS	NS	NS	NS	0.0153

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%). The phenotypic coefficient of variation values were found slightly higher for all of the parameters than genotypic coefficient of variation (GCV), which indicated that the traits were greatly influenced by the environment.

High heritability and genetic gain were recorded for angle between the mid rib and 2nd lower lateral mid rib & shoot bark thickness respectively. Highest genetic gain (67.07%) was recorded for shoot bark thickness followed by leaf area (36.88%) and fresh root weight (24.84%) among morphological characters suggesting that additive genetic effects are important in the determination of these characters and therefore, selection would be effective for

these traits. Among all the physiological characters, internal CO<sub>2</sub> concentration showed widest range of values (7275.84–23,601.68 ppm and mean 13,954.76 ppm), followed by stomatal conductance (3.54–7.06 mol m<sup>-2</sup> s<sup>-1</sup>, & mean 4.53 mol m<sup>-2</sup> s<sup>-1</sup>) indicating the extent of variation existing in the plants. Phenotypic coefficient of variation (PCV) was found to be maximum for internal CO<sub>2</sub> concentration (51.34%) followed by water use efficiency (31.16%). The phenotypic coefficient of variation values were found slightly higher for all of the parameters than genotypic coefficient of variation (GCV) which indicated that the traits were greatly influenced by the environment.

Both high heritability and genetic gain were recorded for stomatal conductance. Highest genetic gain (26.35%) was

recorded for stomatal conductance followed by water use efficiency (19.88%) and transpiration rate (13.82%) among physiological characters suggesting that additive genetic effects are important in the determination of these characters and therefore, selection would be effective for these traits.

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 *Populus deltoides*. The findings reported by Huse (2004) on willow clones are also in agreement to our study.

### Estimation of genetic components of variances and 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. For these purposes the present work was carried out to assess the gene action for certain quantitative characters in twelve hybrids developed by crossing eight parents.

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. Highly significant variances were observed among crosses for most of the characters. There was higher magnitude of specific combining ability variances as compared to general combining ability variances for all the traits and as such ratio of general combining ability to specific combining ability was found less than unity. The dominance variance was more than the additive variance as such the ratio of additive genetic variance/dominance genetic variance was less than unity in all the parameters studied (Tables 11, 12, 13).

The proportional contribution of lines ranged from 6.63% (dry shoot weight) to 61.58% (length of lamina), whereas for testers it ranged from 0.92% (dry root shoot ratio) to 56.71% (total dry weight). However, the proportional contribution of line  $\times$  tester interaction ranged from 2.08% (angle between the mid rib and 2nd lower lateral mid rib) to 58.84% (dry root shoot ratio) indicating the importance of combination of specific parents. The proportional contribution of lines interaction was higher than individual contribution of testers or line  $\times$  tester

**Table 11** Effect of variance components on morphological characters in *Populus deltoides*

Sr. No.	Variance components	Plant height (cm)	Collar diameter (mm)	Internodal length (cm)	No. of leaves/plant	Petiole length (cm)	Leaf area (cm <sup>2</sup> )	Apex angle (°)	Length of lamina (cm)	Maximum width of leaf (cm)	Ratio of length of lamina/maximum width of leaf	Angle between the mid rib and 2nd lower lateral mid rib (°)
1	Variances of GCA ( $\delta^2_g$ )	18.38	0.04	0.002	0.80	0.004	32.30	0.39	0.08	0.01	$7.81 \times 10^{-5}$	3.62
2	Variances of SCA ( $\delta^2_s$ )	662.60	2.96	0.07	21.00	0.40	1388.41	8.72	0.81	0.72	0.001	48.21
3	Additive variance (D)	73.55	0.19	0.009	3.23	0.01	129.21	1.59	0.35	0.04	0.0003	14.50
4	Dominance variance (H)	2650.42	11.86	0.31	84.00	1.60	5553.64	34.91	3.25	2.88	0.006	192.85
5	Contribution of lines	14.98	15.63	32.09	46.05	47.61	27.61	39.98	61.58	16.65	46.42	44.46
6	Contribution of testers	28.86	32.38	33.95	22.44	12.12	38.85	34.93	33.40	31.48	27.45	53.45
7	Interactions (Line $\times$ Tester)	56.15	51.97	33.94	31.50	40.25	33.53	25.08	5.00	51.86	26.11	2.08

**Table 12** Effect of variance components on morphological characters in *Populus deltoids*

Sr. No.	Variance components	Shoot bark thickness (mm)	Root bark thickness (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Dry root shoot ratio	Root length (cm)	Total fresh weight (g)	Total dry weight (g)
1	Variances of GCA ( $\delta^2_g$ )	– 0.0005	0.0009	122.75	12.02	11.73	3.74	– 0.0002	0.14	150.01	78.46
2	Variances of SCA ( $\delta^2_s$ )	0.0767	0.0603	2656.51	613.31	597.42	193.17	0.0092	11.64	5623.41	1910.00
3	Additive variance (D)	– 0.0023	0.0037	491.02	48.08	46.93	14.97	– 0.0011	0.56	600.04	313.84
4	Dominance variance (H)	0.3068	0.2412	10,626.04	2453.27	2389.68	772.70	0.0370	46.57	22,493.66	7640.01
5	Contribution of lines	35.8867	9.2483	19.62	6.63	50.85	25.61	40.2353	36.94	27.29	15.30
6	Contribution of testers	14.4800	50.6733	53.61	54.45	13.75	38.59	0.9242	23.63	39.27	56.71
7	Interactions (Line $\times$ Tester)	49.6332	40.0782	26.76	38.91	35.38	35.78	58.8403	39.41	33.42	27.97

interaction except for plant height, collar diameter, internodal length, leaf area, maximum width of leaf, angle between the mid rib and 2nd lower lateral mid rib, shoot bark thickness, root bark thickness, fresh shoot weight, dry shoot weight, dry root weight, dry root shoot ratio, root length, total fresh weight, total dry weight, stomatal conductance and fibre length where interactions contribution was less.

Cameron et al. (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. 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. Xiaomei et al. (2011) while conducting a progeny test of *Pinus massoniana* using nested mating design reported that DBH and wood basic density were primarily controlled by additive effect and then by dominant effect, whereas height, individual volume and stem fullness were almost completely controlled by additive effects and the progeny performance could be predicted by parents performance.

### Paternity verification with SSR markers

In this study, 18 primer pairs of SSR's markers associated with each hybrid and parental lines were assessed on 3.5 per cent agarose. 'The hybrids obtained after the completion of hybridization programme were tested for their paternity using SSR marker. Among 18 SSR markers, fifteen markers WPMS-03, WPMS-05, ORPM-015, PMGC-325, PMGC-333, PMGC-409, PMGC-420, PMGC-422, PMGC-433, ORPM-026, PMGC-562, PMGC-571, PMGC-2140, PMGC-2143 and PMGC-2163) 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. The amplification products were consisting of one or combination of the two bands for all the samples of *P. deltoides*. 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 SSR markers (Plate-6). The identified SSR in  $F_1$  hybrids showed complementary banding pattern of 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



**Table 13** Estimation of variance components for physiological characters in *Populus deltoides*

S. No.	Variance components	Internal CO <sub>2</sub> concentration (ppm)	Photosynthetic rate ( $\mu\text{ mol m}^{-2}\text{ s}^{-1}$ )	Transpiration rate ( $\mu\text{ mol m}^{-2}\text{ s}^{-1}$ )	Stomatal conductance ( $\text{mol m}^{-2}\text{ s}^{-1}$ )	Water use efficiency ( $\mu\text{ mol CO}_2/\mu\text{ mol H}_2\text{O}$ )	Wood density ( $\text{g/cm}^3$ )	Fibre length (mm)
1	Variances of GCA ( $\delta^2\text{g}$ )	788,724.20	0.16	0.01	– 0.01	– 0.0009	$1.59 \times 10^{-7}$	$-1.231 \times 10^{-5}$
2	Variances of SCA ( $\delta^2\text{s}$ )	7,058,951.00	0.32	0.34	0.71	0.4557	$9.22 \times 10^{-7}$	0.005
3	Additive variance (D)	3,154,897.00	0.65	0.06	– 0.04	– 0.0038	$6.39 \times 10^{-7}$	$-4.924 \times 10^{-5}$
4	Dominance variance (H)	28,235,803.00	1.29	1.37	2.86	1.8228	$3.68 \times 10^{-6}$	0.020
5	Contribution of lines	40.57	41.63	53.95	30.87	39.8091	58.22	27.634
6	Contribution of testers	37.32	35.45	20.47	15.88	13.9303	28.14	25.471
7	Interactions (Line $\times$ Tester)	22.09	22.91	25.57	53.24	46.2605	13.63	46.894

hybrids. 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.

In conclusion, 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.

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