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Assessment of combining ability for growth and survivability traits in mulberry using line × tester analysis

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Abstract
Combining ability in mulberry was assessed by using Line x Tester mating design. In the present study, five lines *viz*., MI-0543, MI-0615, MI-0651, MI-0685, MI-0718 and three testers *viz*., V_1, G_4, MI-0663 were crossed to obtain fifteen F_1 progenies. Growth and survivability traits were evaluated for the F_1 crosses through Analysis of variance (ANOVA), General combining ability (GCA) and Specific combining ability (SCA) for the parameters such as germination per cent, survivability per cent, plant height, number of leaves per branch, internodal distance and leaf moisture content. Among the eight parental genotypes MI-0543 (female) and V_1 (male) were the best combiners and MI-0685 x V_1 was the best cross for growth and survivability traits. Hence, both non- additive and additive gene actions are important for the mulberry improvement.

Keywords: Mulberry, line x tester analysis, combining ability, superior hybrids

Introduction
Mulberry is a cross pollinated heterozygous perennial plant of the family Moraceae. Sustainability and profitability of sericulture utterly depends on the quality and quantity of mulberry leaves, because of the monaphagous nature of silkworm. Mulberry exhibits high plasticity and acclimatize itself to various climatic conditions (Ashiru, 2002) [3]. Environmental conditions in India are most favourable for mulberry growth and development throughout the year.

Mulberry is a dioecious or sometimes monoecious plant. It exhibits different sex expressions, identifying the differences in sex expressions in mulberry is an enigmatic process. Since, sex of particular mulberry species is not static, it varies from season to season based on the environmental fluctuations, cultural practices and soil factors (Tikader *et al*., 1995) [29]. Genotype x Environment interaction complicates the screening of superior genotypes (Doss *et al*., 2012) [9].

New hybrids are produced by using various breeding techniques. Development of new hybrids with novel and desirable traits might be boosting Sericultural economy (Bedi, 1999) [4]. Selection of compatible parents is a pre-requisite for all breeding programs. Based on their phenotypic performance and intrinsic genetic values, parental genotypes should be selected for breeding programs (Bhalodiya *et al*., 2019) [8]. Among the various approaches, line x tester analysis is the fruitful approach for screening superior progenies and best combining parental genotypes. This method was introduced by Kempthrone (1957) [16]. Line x Tester analysis provides the information of general combining ability of parents and specific combining ability of the F_1 progenies and also additive, non-additive gene actions (Yehia and EI-Hashash, 2019) [35]. The ultimate goal of mulberry breeding is to develop high productive hybrids with superior leaf quality at less possible time and reasonable cost of production. Present study was designed to assess the combining ability for mulberry and to identify the suitable crosses through Line x Tester mating design.

Materials and methods
Mulberry accessions were procured from CSGRC, Hosur and combining ability studies were carried out at Department of Sericulture, Forest College and Research Institute, Mettupalayam. Aruna *et al*., (2018) screened twenty-four mulberry genotypes based on propagation

parameters. Among the screened accessions some genotypes were used as parents for the current study. Five lines *viz*., MI-0543, MI-0615, MI-0651, MI-0685, MI-0718 and three testers *viz*., V_1, G_4 and MI-0663 were used for line x tester analysis. Crossing was successfully done through several preliminary steps such as pruning, bagging and pollination. After a week, fully matured fruits were harvested from lines and seeds were extracted from matured fruits by soaking it in the water for overnight. Floating seeds were removed and sunken seeds were selected for sowing after shade drying (Mbora *et al*., 2008) [19].

Completely Randomized Design (CRD) with three replications was used for planting the F_1 progenies. Seeds were sown in polybags filled with Soil :Sand :FYM in 1:1:1 ratio. Observations pertaining to growth and survivability traits of mulberry were recorded on 60th, 75th and 90th day after sowing.

Survivability traits

Germination per cent (%)

Germination per cent was calculated from 10th day after sowing. Seed bags were maintained under greenhouse condition with regular watering. It was calculated using the formula;

$$\text{Germination per cent} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Survival per cent (%)

The survival per cent of seedlings was estimated by counting the available seedlings on 60th day after sowing in each tray.

$$\text{Surival per cent} = \frac{\text{Number of survived seedlings 60th DAS}}{\text{Total number of sowed seeds}} \times 100$$

Growth parameters

Plant height (cm)

Plant height was measured on 60th, 75th and 90th day after sowing and expressed in cm.

Number of branches per plant

Average number of branches per plant was calculated by using the formula;

$$\text{Number of branches per plant} = \frac{\text{Total number of branches}}{\text{Number of plants}}$$

Number of leaves per branch

Leaves in the individual branch of seedling were counted and average number of leaves per branch was calculated by using the formula;

$$\text{Number of leaves per branch} = \frac{\text{Total number of leaves}}{\text{Number of branches}}$$

Internodal distance (cm)

Space between two nodes of the plant was recorded by using meter scale and expressed in cm.

Single leaf area (cm²)

It was calculated by factor method and expressed in cm².

$$\text{Single leaf area} = \text{L} \times \text{B} \times 0.69$$

Where,

L= Length, B = Breadth, 0.69 = correction factor.

Mean correction factor for mulberry was reported by Singhal *et al*., (2003) [26]

Fresh leaf weight (g)

Fresh leaves were collected from three different portions *viz*., bottom, middle and top of the seedlings and weight was recorded using electronic weighing balance immediately and expressed in grams.

Leaf moisture content (%)

The moisture content of the leaf was estimated on dry weight basis. Leaves were collected in the morning and weighed immediately by using electronic weighing balance. The leaves were then dried in hot air oven at 60°C for 48 hours till the constant weight was obtained (Sivashankar, 2015) [27].

Leaf moisture content calculated as per the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

Moisture retention capacity (%)

It is the capacity of leaves to retain moisture after six hours from harvest which varies among the F_1 progenies. Fresh leaf samples were kept at room temperature and weighed after six hours of harvest to determine moisture retention capacity.

$$\text{Moisture retention capacity (\%)} = \frac{\text{Weight after 6 hours} - \text{Dry weight}}{\text{Fresh weight} - \text{Dry weight}} \times 100$$

The recorded data were uploaded to the software *viz*., TNAUSTAT for estimating the combining ability among the mulberry genotypes. This package was developed by Manivannan (2014) [18]. Results showed variance among the parents and progenies (ANOVA), general combining ability of the parents (GCA) and specific combining ability of the F_1 progenies (SCA).

Results and discussion

The results of the present study revealed that development of mulberry hybrids with novel desirable traits is much possible through systematic breeding programs. Most of the breeding studies were carried out on the development of pest and diseases resistant varieties, salt and drought tolerant varieties (Kumar *et al*., 1999, Vijayan *et al*., 2008, Gnanaraj *et al*., 2011) [17, 34, 11]. So far, only few studies in growth and yield related traits (Vijayan *et al*., 2004) [33]. Parental genotypes were crossed through line x tester mating design (Kempthrone, 1957) [16]. This method was used successfully in many other Agricultural crops like cotton (Bilwal *et al.*, 2018) [5], barley (Prasad *et al*., 2013) [22], wheat (Gowda *et al.*, 2010, Jain and Sastry, 2012) [12, 14], maize (Mohammad *et al*., 2013, Akhi *et al*., 2018, Zhou *et al*., 2018) [20, 1, 36] etc.

ANOVA

Results pertaining to analysis of variance for combining ability indicated that the mean squares due to testers were highly significant for number of branches per plant (11.8746), number of leaves per branch (199.4302), leaf moisture content (59.9772) and moisture retention capacity (240.3950) whereas significant variances among lines were recorded only for fresh leaf weight (0.6438). At the same time variances in line x tester interaction were observed to be highly significant for most of the parameters *viz*., germination per cent (382.6361), plant height (188.4747) and single leaf area (696.0493). The results revealed that all the mulberry genotypes showed variations with respect to the studied traits. Hence, there is a significant difference among the lines, testers and their interactions. And it shows the possibility to compute general combining ability for parents and specific combining ability for hybrids. (Vijayan *et al*., 1997, Mohammed *et al*., 2013) [31, 22].

The proportional contribution of lines, testers and their interactions to the total variance for the parameters under study are given in Table 2. Contribution of lines was higher compared to the contribution of testers for most of the traits. Lines contributed more for number of leaves per branch (39.5%) and testers contributed more for number of branches per plant (46.5%). Whereas, line x tester interaction contribution of crosses was found to be high for all the traits except number of leaves per plant and number of branches per plant. Among the traits, interactions contributed more to single leaf area (84.43%) and internodal distance (75.06%). Hence, lines and interactions afforded maximum contribution to the total variance (Table 2).

General combining ability effects

The estimates of GCA effects of parents for all the characters are given in Table 3. Among the parents the highest positive GCA effect were exhibited by the lines MI-0685 and MI-0615 (5.31 and 5.21 respectively) for germination per cent. Among the testers V_1 showed significant and positive GCA effect (1.97). Survivability is the capacity of a plant to withstand and survives under varied agro climatic conditions. It depends on genetic makeup and due to the influence of various environmental conditions (Honda, 1970 and Mvuyekure *et al*., 2017) [13, 24]. MI-0615 (line) and V_1 (tester) showed highly significant GCA effect, whereas MI-0718 recorded highly significant negative GCA value (-21.94). With respect to the plant height, significant and highest positive GCA effect was recorded for line MI-0543 (2.67) followed by MI-0615 (2.28) and tester V_1 showed highly significant GCA. On the other hand, MI-0718 showed highly significant negative GCA effects (-6.03) which indicates occurrence of dwarfness in seedlings.

Among the lines, MI-0685 recorded highly significant positive GCA value for number of branches per plant (.0.22) Whereas among the testers, V_1 showed highly significant positive GCA effect (0.31). MI- 0718 and G_4 showed negative and highly significant GCA effect (-0.22 and -0.39 respectively). For number of leaves per branch, MI-0543 showed highly significant and positive GCA (3.14), whereas MI-0718 recorded highly significant and negative GCA value (-5.78), which indicates very less number of leaves were produced per branch in the particular genotype. Among the three testers, V_1 recorded highest significant and positive GCA value (3.85). Conversely G_4 showed significant and negative GCA effect (-3.41).

Line MI-0718 and tester MI-0663 showed highly significant positive GCA effects for internodal distance (0.27 and 0.20 respectively) (Table 3). MI-0651 and V_1 were found to have highly significant and negative GCA value (-0.24 and -0.27 respectively). These results are similar to the findings of Rita Banerjee *et al*., (2007) [23], Ghosh *et al*., (2009) [10] and Peris Nderitu *et al*., (2014) [21]. Short internodal distance has been considered as desirable trait because less internodal distance would increase the number of leaves per unit length of the shoot. From the results, it is concluded that, MI -0651 and V_1 are desirable genotypes for growth related traits. For single leaf area, MI-0615 showed highly significant and positive GCA effect (6.65), at the same time MI-0543 showed negative GCA value (-7.85).

Out of five lines, MI 0543 was found to be exhibit highly significant and positive GCA effect for fresh leaf weight (0.44). It is an important character from yield point of view. Among the testers, V_1 showed positive significant GCA value (0.08). Similarly, Ghosh *et al.* (2009) [10], reported positive correlation between fresh leaf weight and leaf yield/plant. Leaf moisture is an essential parameter for mulberry. Based on the moisture content present in the leaves, it should be categorized and feed to different stages of silkworms. Among the lines, MI-0615 had highly significant and positive GCA effect (2.44) whereas, MI-0718 showed highest negative GCA value (-2.49). While, MI-0543 showed highly significant positive GCA followed by G_4 and V_1 (3.14, 2.34 and 2.28 respectively) for moisture retention capacity.

Specific combining ability effects

SCA estimates indicated that, at least one parent with good combining ability was required to produce superior hybrids. F_1 progenies with desirable SCA effects could be produced by crossing the parents with low x high, high x low, high x high GCA. Jolly and Dandin (1986) [15] opined that tropical mulberry varieties are good in sprouting. Among the fifteen crosses, five crosses showed highly positive significant SCA effects for germination per cent, MI-0718 x MI-0663 recorded highest SCA effect (14.13) followed by MI-0685 x V_1 (10.82), MI-0615 x G_4 (9.78), MI-0543 x G_4 (9.73) and MI-0651 x V_1 (7.76). Conversely, MI-0718 x V_1 had highly significant and negative SCA effect (-11.95). Chandrashekar *et al.* (2001) [7] proved that V_1, M_5, DD and S_{30} were the best mulberry genotypes for survivability rate compared to other genotypes. In the present study, MI-0543 x G_4, MI-0685 x V_1 and MI-0718 x MI-0663 were the best crosses for survivability rate (27.46, 21.43 and 18.10 respectively). MI-0651 x G_4 had significantly high negative SCA (-32.99) which was not suitable for survivability traits.

For plant height, MI-0718 x G_4 was the best one. It had highest significant SCA value (9.20). Vijayan *et al*., (1997) [31] observed that plant height was an important character and it was one of the major leaf yield component. On the other hand, MI-0718 x MI-0663 showed highly significant and negative SCA (-11.19). Among the fifteen crosses, MI-0615 x MI-0663 showed highest significant SCA value (0.42) for number of branches per plant followed by MI-0685 x V_1 and MI-5043 x V_1 (0.41 and 0.30 respectively) (Table 4). Number of leaves per branch indicated whether the particular genotype was suitable for leaf yield traits or not. MI- 0685 x V_1 (4.93), MI-0651x V_1(3.62) and MI-0718 x G_4 (3.44) showed highly significant positive SCA effects. Similarly, MI-0718 x V_1 showed negative and highly significant SCA (-5.66), which could bear only few number of leaves per branch.

Leaf yield is a multi-factorial trait and is positively correlated to the characters like height of the plant, number of shoots, and distance between the nodes. Breeding new mulberry hybrids with high leaf yield will be achieved by the improvement of leaf yield components (Tikader & Kamble, 2009 and Doss *et al*., 2012) [30, 9]. MI-0685 x MI-0663 recorded highly significant and positive SCA (0.85) for intermodal distance. Genotypes with shorter internodal distance were preferable because shoots with less nodal length contains more number of leaves.

Single leaf area of MI-0543 x V_1 had positively significant SCA (20.98) and fresh leaf weight of MI-0685 x MI-0663 showed highly positive SCA (0.37), Chaluvachari and Bongale (1995) [6] reported that high leaf moisture content and its retention capacity are considered as the important leaf quality parameters for better growth and development of silkworms. The results denoted that, MI-0543 x V_1 had high leaf moisture content. With respect to moisture retention capacity, MI-0685 x V_1, MI-0615 x G_4 and MI-0718 x MI-

0663 had highly significant positive SCA effect (5.39, 4.71 and 4.48 respectively) whereas, MI-0718 x V_1 showed significantly negative SCA for leaf moisture content (-5.25) and also for moisture retention capacity (-7.44).

Table 1: Analysis of variance for Line x Tester in mulberry

Source of variation	Df	G.P (%)	S.P (%)	P.H (cm)	No.of leaves/ branch	No.of branches/ plant	I.D (cm)	SLA (cm^2)	FLW (g)	LMC (%)	MRC (%)
Replication	2	7.1650	22.3857	2.1770	0.5686	0.0093	0.0603	90.0184	0.0059	43.6605	10.5757
Crosses	14	297.2438	1796.9104	152.1357	92.8321	0.5759	0.9201	471.1171	0.5152	48.8274	117.4816
Lines	4	253.1413	1919.9339	121.0818	128.3751	0.3245	0.3617	248.7799	0.6438	35.8481	89.2494
Testers	2	43.8795	844.0959	68.8877	199.4302	1.8746	0.8827	16.0627	0.0862	59.9772	240.3950
L x T	8	382.6361	1973.6022	188.4747	48.4110	0.3770	1.2086	696.0493	0.5582	52.5295	100.8693
Error	28	11.2014	5.9935	8.4415	2.0733	0.0066	0.0348	22.0555	0.0126	6.8933	14.0458
SED		2.7327	1.9989	2.3723	1.1757	0.0662	0.1523	3.8345	0.0917	2.1437	3.0600
CD (5%)		5.6020	4.0978	4.8632	2.4101	0.1356	0.3123	7.8608	0.1879	4.3946	6.2731
CD (1%)		7.5422	5.5170	6.5475	3.2449	0.1826	0.4204	10.5833	0.2530	5.9167	8.4457

G.P- Germination Per cent; S.P – Survivability Per cent; FLW- Fresh Leaf Weight; SLA – Single Leaf Area
P.H – Plant Height; LMC- Leaf Moisture Content; I.D – Internodal Distance; MRC – Moisture Retention Capacity

Table 2: Proportional contributions (%) of lines, testers and their interactions for total variance

S. No.	Characters	Lines	Testers	Line x Tester
1	Germination per cent (%)	24.33	2.11	73.56
2	Survival per cent (%)	30.53	6.71	62.76
3	Plant height (cm)	22.74	6.47	70.79
4	Number of leaves/ branch	39.51	30.69	29.80
5	Number of branches/ plant	16.10	46.50	37.40
6	Internodal distance(cm)	11.23	13.71	75.06
7	Single leaf area(cm^2)	15.09	0.49	84.43
8	Fresh leaf weight (g)	35.70	2.39	61.90
9	Leaf moisture content (%)	20.98	17.55	61.48
10	Moisture retention capacity (%)	21.71	29.23	49.06

Table 3: Estimation of General Combining Ability effects

Parents / Traits	G.P (%)	S.P (%)	P.H (cm)	No. of leaves/ branch	No. of branches/ plant	I.D (cm)	SLA (cm^2)	FLW (g)	LMC (%)	MRC (%)
Lines										
MI-0543	-2.78*	-0.47	2.67*	3.14**	0.17**	-0.07	-7.85**	0.44**	0.72	3.14*
MI-0615	5.21**	19.10**	2.28*	2.67**	-0.06	0.13*	1.20	-0.12**	2.44**	2.12
MI-0651	-0.74	0.87	-0.96	-1.85**	-0.11**	-0.24**	6.65**	0.04	-1.56	-1.72
MI-0685	5.31**	2.44**	2.04*	1.82**	0.22**	-0.09	1.29	-0.27**	0.90	1.06
MI-0718	-6.99**	-21.94**	-6.03**	-5.78**	-0.22**	0.27**	-1.29	-0.09*	-2.49**	-4.60**
SED	1.577	1.1541	1.3696	0.6788	0.0382	0.0879	2.2139	0.0529	1.2377	1.7667
CD (5%)	3.2343	2.3659	2.0877	1.3915	0.0783	0.1803	4.5384	0.1085	2.5372	3.6218
CD (1%)	4.3545	3.1853	3.7802	1.8734	0.1054	0.2427	6.1103	0.1460	3.4160	4.8761
Testers										
V_1	1.97*	8.15**	2.19**	3.85**	0.31**	-0.27**	1.18	0.08**	1.30	2.28*
G_4	-0.91	-1.54*	-2.10**	-3.41**	-0.39**	0.07	-0.45	-0.02	1.00	2.34*
MI-0663	-1.06	-6.61**	-0.09	-0.44	0.08**	0.20**	-0.74	-0.06*	-2.30**	-4.62**
SED	1.2221	0.8939	1.0609	0.5258	0.0296	0.0681	1.7149	0.0410	0.9587	1.3685
CD (5%)	2.5053	1.8326	2.1749	1.0779	0.0607	0.1397	3.5155	0.0840	1.9653	2.8054
CD (1%)	3.3730	2.4673	2.9281	1.4512	0.0817	0.1880	4.7330	0.1131	2.6460	3.7770

*significant at 5% level ($P < 0.05$); **significant at 1% level ($P < 0.01$)

Table 4: Estimation of Specific Combining Ability effects

S. No.	Crosses	G.P (%)	S.P (%)	P.H (cm)	No. of leaves/branch	No. of branch/plant	I.D	SLA	FLW	LMC	MRC
1	MI 0543 x V_1	-0.13	-2.88	6.08**	0.60	0.30**	0.61**	20.98**	-0.07	4.56**	4.44*
2	MI 0615 x V_1	-6.5**	-8.45**	-6.82**	-3.50**	-0.48**	-0.54**	-10.86**	-0.35**	-1.65	-5.01*
3	MI 0651 x V_1	7.76**	15.26**	-0.75	3.62**	0.08	-0.20	-8.85**	-0.27**	1.05	2.63
4	MI 0685 x V_1	10.82**	21.43**	-0.51	4.93**	0.41**	-0.21	-4.40	0.36**	1.28	5.39*
5	MI 0718 x V_1	-11.95**	-25.36**	1.99	-5.66**	-0.31**	0.35**	3.13	0.33**	-5.25**	-7.44**
6	MI 0543 x G_4	9.73**	27.46**	-4.50*	-1.58	-0.17**	-0.54**	-11.68**	0.07	-1.16	0.22
7	MI 0615 x G_4	9.78**	16.84**	5.09**	1.24	0.06	0.72**	9.24**	0.22**	2.54	4.71*
8	MI 0651 x G_4	-6.58**	-32.99**	-1.51	-1.13	0.11*	0.29*	16.83**	0.30**	-5.10**	-5.85*
9	MI 0685 x G_4	-10.75**	-18.57**	-8.27**	-1.97*	-0.22**	-0.63**	-13.86**	-0.73**	1.45	-2.05
10	MI 0718 x G_4	-2.18	7.26**	9.20**	3.44**	0.22**	0.16	-0.53	0.13	2.28	2.96
11	MI 0543 x MI 0663	-9.61**	-24.58**	-1.58	0.97	-0.13**	-0.07	-9.30**	-0.00	-3.40*	-4.66*
12	MI 0615 x MI 0663	-3.28	-8.39**	1.73	2.26*	0.42**	-0.17	1.63	0.12	-0.89	0.29
13	MI 0651 x MI 0663	-1.17	17.73**	2.26	-2.49**	-0.19**	-0.09	-7.98**	-0.03	4.05*	3.23

14	MI 0685 x MI 0663	-0.07	-2.86	8.78**	-2.96**	-0.19**	0.85**	18.26**	0.37**	-2.73	-3.34
15	MI 0718 x MI 0663	14.13**	18.10**	-11.19**	2.22*	0.09	-0.51**	-2.60	-0.46**	2.97	4.48*
	SED	2.7327	1.9989	2.3723	1.1757	0.0662	0.1523	3.8345	0.0917	2.1437	3.0600
	CD (5%)	5.6020	4.0978	4.8632	2.4101	0.1356	0.3123	7.8608	0.1879	4.3946	6.2731
	CD (1%)	7.5422	5.5170	6.5475	3.2449	0.1826	0.4204	10.5833	0.2530	5.9167	8.4457

*significant at 5% level ($P < 0.05$) ; **significant at 1% level ($P < 0.01$)

Conclusion

Selection of compatible parents with desirable GCA and crosses with superior SCA effects has a vital role in all successful breeding programmes. In the present study, variations were observed in all the eight parental genotypes and fifteen F_1 progenies of mulberry. Parents and crosses had significant amount of GCA and SCA respectively. Due to the variations, screening of better mulberry genotype is quite easy. Among the fifteen F_1 crosses, MI-0685 x V_1 was found to perform better than the remaining crosses and MI-0543 × V_1 was found to be the best combiners. Hence, these parents and F_1 progenies may be further used in breeding programs for mulberry crop improvement.

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