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Combining Ability Studies on Growth and Improved Leaf Yield Traits in Mulberry, *Morus* spp.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to evaluate combining ability for growth, quality and yield parameters in mulberry at the field germplasm existed at the Department of Sericulture, UAS, GKVK, Bangalore. Twenty one F_1 hybrids was developed by crossing seven lines with three testers according to line x tester design. and assessed using randomized complete block design with three replications. Results revealed that analysis of general combining ability showed the higher magnitude of specific combining ability (SCA) variance than general combining ability (GCA) variance for most of the characters, which indicates the presence of non- additive gene action for most of the traits except for number of branches per plant, number of leaves per plant, and single leaf area. Among seven lines, *M. cathyana,* MI-47, *M. laevigata*, MI-494 were found to be best general combiners and in testers V1, MI-66 were found best general combiners with high GCA effects. Among twenty one crosses, BC-259 × MI-66, *M. indica* × MI-66, *M. laevigata* × V1 and *M. indica* × C-776 were found to be superior performers with high SCA effects.

*Keywords: Combining ability; F1 hybrids; general combining ability; line*tester interaction; specific combining ability.*

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1. INTRODUCTION

Mulberry is the principal host of the silkworm (*Bombyx mori* L.), which belongs to the family Moraceae and is commercially exploited [1]. Mulberry is responsible for more than 60% of the entire cost of cocoon production in commercial sericulture. Mulberry leaf output can be increased by generating new varieties with higher leaf yield and adaptability, which boosts sericulture productivity. The long-term goal of a breeder is to boost up the quality and quantity of leaf in mulberry through hybridization, selection, and mutation breeding. It is necessary to have sufficient knowledge of mulberry genetics in order to change the genetic constitution of plant [2].

Mulberry leaf output can be increased by generating new varieties with higher leaf yield and adaptability, which boosts sericulture productivity. The long- term goal of a breeder is to boost the quality and quantity of leaf in mulberry through hybridization, selection, and mutation breeding. Plant breeders face a significant challenge in developing a variety that performs equally well under different environmental conditions. It is necessary to have sufficient knowledge of mulberry genetics in order to change the genetic constitution of a plant [2].

Kempthorne's Line × Tester analysis is a useful method to assess genetic stock before using it in a hybridization programme in order to find good combiners that may be used to increase the population with favourable genes for successful yield improvement.

There is a scarcity of information on the genetic interactions which controls the expression of numerous quantitative features in mulberry. A strong affinity among different species of mulberry while attempting interspecific hybridization was discovered [3] which was corroborated by [4,5] through crossability investigations. Selection of parents capable of conveying desirable features is required when breeding for superior cultivars. A rational breeding strategy is to identify parents depending on their combining ability, that is characterized as the pattern of gene action in the expression of quantitative characters which provides knowledge for the selection of parents to include in a hybridization programme [6].

Line **×** tester analysis in mulberry (*Morus* spp.) in eight clonal varieties *viz.,* Berhampore-1, China white, MS-5, Mandalaya, Kosen, Assamjati, MS-1 and Kajli were studied. Combining ability studies were conducted for the plant parameters. Among the genotypes broad genetic variability was observed. While China white (line) and MS-1 (tester) were the good general combiners between the parents, Berhampore-1 and Kajli was the greatest cross combination for leaf yield [7,8].

The potentiality of a parent to create superior progenies when combined with another parent is referred to as good combining ability. The genetic variation between the parents and their selection is vital for producing F1 progeny with superior performance. Parents with high GCA are usually considered for population progress and for beginning of pedigree breeding as it is heritable and can be fixed. GCA consists of additive effects and additive × additive type of interactions. High heterosis can be produced by parents with high GCA/SCA (Specific combining ability) consists of dominant effects and non-additive effects, other interactions. Specific combining ability is not heritable and therefore it cannot be utilised in pure line breeding. To exploit hybrids commercially they should possess high SCA [9].

For planning the breeding program information regarding the combining ability studies in terms of GCA and SCA variances is needed. Combining ability studies also helps in knowing the gene action for a given trait. Non-additive gene action can be known from the SCA variance, GCA variance reflects the measure of additive gene action of a particular trait. Ratio of non-additive to additive gene action is to be measured to know the predominance of the type of genetic variation for a given character. If the ratio of non-additive to additive gene action is less than one indicates the major role of additive variance in controlling the expression of a character, whereas, more than one indicates the importance of non-additive variance [10].

For developing high yielding varieties in a crop sound breeding programme is essential. Information regarding the nature of gene action controlling the yield and yield contributing characters are essential for implementing good breeding program. In a good hybrid breeding programme important aspect is combining ability and its analysis is of special importance in cross pollinated crops like mulberry because it helps in identifying good parents which can be used for producing hybrids. Selection of parents in terms of performance of hybrid is provided by combining ability studies.

2. METHODOLOGY

For the current investigation, parents including seven lines and three testers were selected from the field germplasm existed at the Department of Sericulture, UAS, GKVK, Bangalore. The experimental plot is situated at an elevation of 931 m above mean sea level and has latitude of 12°58¢ N and longitude of 77°37¢ E. Seven lines and three testers were crossed according to line × tester mating design (Table 1). Twenty one F_1 crosses was developed and planted during 2019. All the crosses were assessed using randomized complete block design (RCBD) with three replications and 10 plants per treatment per replications were maintained (Table 2). The studies on combining ability and heterosis on different growth and yield attributing characters were studied in these mulberry crosses in the year 2020-21 and the crosses were evaluated at 60^{th} day after pruning for growth and yield parameters in two seasons *viz.,* winter and summer with all the recommended package of practices *viz.,* fertilizer application and weeding for mulberry crosses was given under rainfed conditions [11].

Table 1. List of lines and testers used in L × T study

Sl. No.	Parents
Female parents (Lines)	
1.	*M. laevigata*
2.	*M. multicaulis*
3.	MI-47
4.	BC- 259
5.	*M. cathyana*
6.	MI-494
7.	*M. indica* E-05
Male parents (Testers)	
8.	V1
9.	MI-66
10.	C-776

2.1 Combining Ability Analysis

Variances due to general combining ability (GCA) of parents and specific combining ability (SCA) of different cross combinations were worked out based on the procedures developed by [12] using means of each replication for the above seven characters recorded for twenty one crosses.

Table 2. List of mulberry crosses used in the study

Sl. No.	Crosses
1.	*M. laevigata* × V1
2.	*M. laevigata* × C-776
3.	*M. laevigata* × MI-66
4.	*M. multicaulis* × V1
5.	*M. multicaulis* × C-776
6.	*M. multicaulis* × MI-66
7.	MI-47 × V1
8.	MI-47 × C-776
9.	MI-47 × MI-66
10.	BC-259 × V1
11.	BC-259 × C-776
12.	BC-259 × MI-66
13.	*M.cathyana* × V1
14.	*M.cathyana* × C-776
15.	*M.cathyana* × MI-66
16.	MI-494 × V1
17.	MI-494 × C-776
18.	MI-494 ×MI-66
19.	*M.indica* × V1
20.	*M.indica* × C-776
21.	*M.indica* × MI-66

2.2 Estimation of Combining Ability Effects

Linear model given by [6] was used to estimate gca and sca effects which is as follows:

$$Xij = \mu + gi + gj + Sij + eijk \tag{1}$$

Where, μ = population mean
gi = gca effect of i^{th} female parent
gj = gca effect of j^{th} male parent
Sij = sca effect of ij^{th} combination
eijk = Error associated with the observation
i = Number of female parents
j = Number of male parents
k = Number of replications

The individual effects were estimated as indicated below:

2.3 Genetic Combining Ability Effects (GCA)

$$\text{Lines}: \ gl = \frac{Xi..}{tr} - \frac{X...}{Ltr} \tag{2}$$

$$\text{Testers}: \ gt = \frac{X\,j..}{lr} - \frac{X...}{Ltr} \tag{3}$$

Where, Xi = Total of i^{th} line over all testers and replications
Xj = Total of j^{th} testers over all lines and replications

2.4 Specific Combining Ability

$$\text{Sij} = \frac{\text{Xij}}{\text{r}} - \frac{\text{Xi..}}{\text{tr}} - \frac{\text{Xj..}}{\text{lr}} + \frac{\text{X...}}{\text{ltr}} \quad (4)$$

Sij = sca effect of ij^{th} combination
Xij = Total of ij^{th} combination over all replications

The critical difference values in each case were computed by multiplying their corresponding SE values with table 't' value at error degrees of freedom at 5 and 1 per cent level of significance.

3. RESULTS AND DISCUSSION

In the present experiment, an effort was made to know the information on the magnitude of GCA, SCA variance and nature of gene action for the trait as a whole.

The percentage of contribution of testers, lines and their interaction to total variance for the traits were given in Table 3. The contribution of lines towards total variance was registered higher than testers for all the characters *viz.,* plant height (cm), total shoot length (cm), number of leaves per plant, internodal distance (cm), single leaf area (cm^2), leaf yield/plant (g) except number of branches per plant where contribution of testers towards total variance was registered higher than lines. The contribution of lines × testers interaction towards the total variance was found to be higher than the testers for plant height (cm), total shoot length (cm), internodal distance (cm), single leaf area (cm^2) and leaf yield/plant (g), whereas the contribution of lines × testers interaction to the total variance was higher than the lines for internodal distance (cm) and leaf yield/plant (g) (Table 3).

The contribution of lines is higher than testers to total variance for most of the characters under study. These results are in accordance with [13] who evaluated crosses of mulberry clones to select good general combiners for resistance to Bacterial leaf spot [*Xanthomonas campestris pv. mori*] and to determine the genetics of important agronomic traits for mulberry improvement where significant negative gca effect of disease severity was found in genotype *Morus rotundiloba,* which is desirable for BLS resistance, whereas significant positive GCA for leaf yield was recorded in two parents, namely *Morus multicaulis* and C-2016 and revealed that female parents contributed higher to the total variance for most of the traits in mulberry.

3.1 Variances and Nature of Gene Action

In the present experiment, an effort was made to know the information on the magnitude of GCA, SCA variance and nature of gene action for the trait as a whole. In this experiment non-additive genetic component was the major part of genetic variance for most of the growth parameters.

For all character's magnitude of SCA variance was higher than that of GCA variance revealed by analysis of variance except for number of branches per plant, number of leaves per plant and single leaf area which recorded higher GCA variance during winter season. GCA and SCA variance ratio was less than one for all characters, except for number of branches per plant, number of leaves per plant, and single leaf area where GCA and SCA variance ratio was higher than unity in winter season.

Present study is in accordance with [14] who reported that non- additive genetic variance is more often evident in controlling the inheritance of majority of yield traits than additive components in mulberry [15] reported that predominance of non-additive genetic variance offers scope for exploitation of heterosis in mulberry.

Table 3. Proportional contribution of lines, testers and their interaction on total variance in mulberry

Sl. No.	Characters	Contribution of lines (%)	Contribution of testers (%)	Contribution of lines × testers (%)
1.	Plant height (cm)	45.94	14.54	39.50
2.	Number of branches per plant	39.65	36.77	23.57
3.	Total shoot length (cm)	48.21	3.62	48.15
4.	Number of leaves per plant	41.63	30.67	27.68
5.	Internodal distance (cm)	19.39	9.88	70.71
6.	Single leaf area (cm^2)	62.55	12.79	24.64
7.	Leaf yield/plant	40.85	17.01	42.12

Table 4. Estimates of variance components in respect of different growth and yield parameters in mulberry

Sl. No.	Characters	σ^2 GCA	σ^2 SCA	σ^2 GCA/ σ^2 SCA
1.	Plant height(cm)	143.03*	286.02***	0.50
2.	Number of branches per plant	0.68***	0.65***	1.05
3.	Total shoot length (cm)	46.51	175.33	0.26
4.	Number of leaves per plant	793.11***	561.79**	1.41
5.	Internodal distance (cm)	0.08	0.67***	0.12
6.	Single leaf area (cm^2)	103.72***	95.94**	1.08
7.	Leaf yield/plant (g)	2365.40*	5213.35***	0.45

** Significant at 5 % , **Significant at 1 % & *** Significant at 0.001 %*

3.2 Variance Due to Lines, Testers and Lines x Tester Interaction

The variances (σ^2 GCA, σ^2 SCA) and ratio (σ^2 GCA/σ^2 SCA) are given in Table 4.

Variance due to females, males and female × male interaction with respect to all the characters under study are presented in Table 5. The analysis of variance indicated that highly significant variability was recorded for all characters under study.

The variance due to lines (females) was significant for number of branches per plant, number of leaves per plant, and single leaf area but non- significant for the left-over characters studied. The variance due to testers (males) was significant for number of branches per plant and number of leaves per plant, but non-significant for other characters studied. The line × tester interaction variance was very much significant for plant height (cm), total shoot length (cm), number of leaves per plant, internodal distance (cm), single leaf area (cm^2), leaf yield/plant(g) but non-significant for number of branches per plant (Table 5).

The results illustrate significant variation among the genotypes for some of the traits studied. The sum of squares of genotypes for these characters was further divided into parents and crosses. The variance due to parents and crosses revealed significant differences among themselves indicating genetic variability for the efficient selection. Significance of variance due to lines, testers and between lines *vs* testers indicated the sufficient difference among the parents.

These results are in accordance with that of [15] who studied line **×** tester analysis in mulberry (*Morus* spp.) for eight clonal varieties for different plant parameters and the results of combining ability studies revealed broad genetic variability among the genotypes whereas China white (line) and MS-1 (tester) were found to be good general combiners between the parents, Berhampore-1 and Kajli was the greatest cross combination for leaf yield.

These results were also in accordance with that of [16] who carried out line × tester analysis in Mulberry (*Morus* spp.) to determine the combining ability for different growth parameters at seedling stage. The results of combining ability analysis revealed that estimate of SCA variances were higher than GCA variances, indicating predominance of non- additive gene action for most of the traits.

3.3 General Combining Ability (GCA)

Among seven lines *M. cathyana* is the best general combiner as it shows significant positive GCA for plant height(cm), total shoot length(cm) and single leaf area (cm^2). Whereas, MI-47 is best general combiner as it shows significant positive GCA for number of branches/plant and number of leaves per plant. *M. laevigata* showed significant positive GCA for leaf yield (g/plant) and MI-494 showed significant negative GCA for internodal distance (cm) which as been considered desirable as this would enhance the number of leaves per unit length of the stem thereby increasing the leaf yield per unit area [17] and [18].

Among the testers V1 was the best general combiner for plant height (cm), single leaf area(cm^2), internodal distance(cm). MI-66 was the best general combiner for number of branches per plant, number of leaves per plant and leaf yield/plant. Results revealed the predominant role of non-additive gene action for all characters studied by [19] (Table 6).

Table 5. Analysis of variance for combining ability for different growth and yield parameters in mulberry

Source	df	Plant height (cm)	Number of branches per plant	Total shoot length (cm)	Number of leaves per plant	Internodal distance (cm)	Single leaf area (cm^2)	Leaf yield/ plant (g)
Replicates	2	843.37**	0.38	560.40	3661.83	1.29	561.21	3788.35
Crosses	20	1542.64***	4.63***	941.78***	5780.79***	2.09***	997.17***	23910.85***
Line effect	6	2362.69	6.12*	1513.64	8023.71*	1.35	2079.26**	32561.74
Tester effect	2	2243.66	17.03**	341.58	17733.36*	2.07	1276.10	40696.05
L× T Eff.	12	1015.78***	1.82	755.89**	2667.23*	2.47***	409.63*	16787.88***
Error	40	151.99	1.03	281.96	1329.78	0.50	173.66	1611.93
Total	62	622.89	2.17	503.79	2840.81	1.04	451.81	8875.34

** Significant at 5 % , **Significant at 1 % & *** Significant at 0.001 %*

Table 6. Estimates of general combining ability effects of parents for different growth and yield parameters in mulberry

Parents	Plant height (cm)	Number of branches per plant	Total shoot length (cm)	Number of leaves per plant	Internodal distance (cm)	Single leaf area (cm^2)	Leaf yield/ Plant (g)
Lines							
M. laevigata	9.19*	0.08	5.37	14.36	0.44	11.16**	98.77***
M. multicaulis	-18.83***	0.67	-8.84	31.18**	0.09	-18.72***	6.73
MI-47	-1.69	1.37***	-1.29	39.84***	-0.31	-6.74	32.45**
BC-259	-4.73	-0.21	-8.51	-2.18	-0.31	-1.55	9.11
M. cathyana	27.93***	-0.25	21.71***	-14.33	0.33	28.06***	-3.91
MI-494	5.45	-1.18**	8.45	-35.33**	-0.54*	-6.83	-87.53***
M. indica	-17.32***	-0.47	-16.88**	-33.52**	0.31	-5.35	-55.61***
SE m±	4.18	0.33	5.05	10.44	0.22	3.67	11.29
CD at 5%	8.46	0.76	10.21	21.11	0.44	7.43	22.82
CD at 1%	11.32	1.02	13.66	28.24	0.59	9.94	30.54
Testers							
V1	11.67***	-0.98***	4.61	-26.45***	0.36*	8.95***	-22.88**
C-776	-3.67	0.21	-2.84	-4.65	-0.18	-5.29*	-27.86***
MI-66	-7.99**	0.77**	-1.76	31.10***	-0.17	-3.66	50.75***
SE m±	2.74	0.24	3.30	6.83	0.14	2.40	7.39
CD at 5%	5.53	0.50	6.68	13.82	0.29	4.86	14.94
CD at 1%	7.41	0.66	8.94	18.49	0.39	6.51	19.99

** Significant at 5 % , **Significant at 1 % & *** Significant at 0.001 %*

Table 7. Estimates of specific combining ability effects of crosses for different growth and yield parameters in mulberry

Crosses	Plant height (cm)	Number of branches per plant	Total shoot length (cm)	Number of leaves per plant	Internodal distance (cm)	Single leaf area (cm^2)	Leaf yield/ plant (g)
M. laevigata × V1	1.77	0.17	9.93	6.86	0.35	17.81**	1.14
M. laevigata × C-776	-0.21	0.19	-7.37	-29.05	-0.53	-10.74	-104.84***
M. laevigata × MI-66	-1.56	-0.36	-2.56	22.19	0.17	-7.06	103.69***
M. multicaulis × V1	-15.19*	-0.97	-19.16*	-19.73	-0.56	-5.00	-19.87
M. multicaulis × C-776	4.82	0.26	16.40	10.47	-0.18	-5.20	38.65
M. multicaulis × MI-66	10.36	0.71	2.76	9.26	0.75	10.20	-18.78
MI-47 × V1	8.54	0.21	9.38	12.82	-0.21	-10.08	65.41**
MI-47 × C-776	2.89	-0.10	-0.37	-1.75	-0.22	8.27	-15.68
MI-47 × MI-66	-11.44	-0.10	-9.01	-11.06	0.43	1.81	-49.73*
BC-259 × V1	-24.29**	-0.30	-23.17*	-38.70*	-0.49	-3.29	-80.18***
BC-259 × C-776	-4.72	-0.84	1.73	-12.26	0.16	1.93	-34.41
BC-259 × MI-66	29.02***	1.15	21.43*	50.96**	0.32	1.36	114.59***
M. cathyana × V1	18.58*	-0.04	17.83*	20.11	-0.47	11.14	-11.95
M. cathyana × C-776	-21.95**	1.07	-19.71*	23.21	-0.20	-0.89	45.98*
M. cathyana × MI-66	3.36	-1.03	1.88	-43.32*	0.67	-10.25	-34.03
MI-494 × V1	7.40	0.76	3.53	19.45	-0.10	0.90	17.55
MI-494 × C-776	-2.58	-0.65	-2.44	-10.67	0.39	9.95	16.99
MI-494 × MI-66	-4.82	-0.10	-1.08	-8.77	-0.28	-10.85	-34.55
M. indica × V1	3.18	0.17	1.64	-0.80	1.48***	-11.47	27.90
M. indica × C-776	21.75**	0.07	11.77	20.06	0.59	-3.32	53.29**
M. indica × MI-66	-24.93**	-0.25	-13.41	-19.25	-2.08***	14.79*	-81.19***
SE m±	7.25	0.65	8.75	18.09	0.38	6.37	19.56
CD at 5%	14.65	1.32	17.69	36.56	0.77	12.87	39.53
CD at 1%	19.60	1.76	23.67	48.92	1.03	17.23	52.89

These results were also in accordance with that of [16] who carried out line × tester analysis in Mulberry (*Morus* spp.) to determine the combining ability for different growth parameters at seedling stage. The results of combining ability analysis revealed that among the lines, MI-516 was good general combiner for germination per cent, plant height, number of branches and leaf moisture whereas MI-139 was good general combiner for number of leaves and internodal distance and ME-18 for single leaf area and fresh leaf weight. Among testers MI- 04 was general combiner for germination per cent, plant height, number of leaves, number of branches, growth rate, leaf moisture and internodal distance.

3.4 Specific Combining Ability (SCA)

Among the twenty one crosses, BC-259 × MI-66 (plant height(cm), number of leaves/plant, total shoot length(cm), leaf yield/plant(g)), *M. indica* × MI-66 (internodal distance (cm)), *M. laevigata* × V1 (single leaf area (g)) were best performers with high SCA effects.

The analysis of variance indicated that highly significant variability was recorded for all characters. The variance due to lines was significant for number of branches per plant, number of leaves per plant, and single leaf area but non- significant for the left-over characters studied. The variance due to testers was significant for number of branches per plant and number of leaves per plant, but non-significant for other characters.

The line × tester interaction variance was very much significant for plant height (cm), total shoot length (cm), number of leaves per plant, internodal distance (cm), single leaf area (cm^2), leaf yield/plant (g) but non- significant for number of branches per plant (Table 7).

These results were in accordance with that of [16] who carried out line × tester analysis in Mulberry (*Morus* spp.) to determine the combining ability for different growth parameters at seedling stage. The results of specific combining ability analysis revealed that the crosses MI-139 × C-776 (germination per cent, plant height, number of branches and leaf moisture), MI-516 × MI- 04 (plant height and number of branches) and ME-18 × MI-04 (leaf moisture) were good performers with high SCA effects at seedling stage.

The above results obtained were also in accordance with that of [20] carried out line × tester analysis to determine combining ability for growth and survivability traits in mulberry. The study revealed that growth and survivability of traits were evaluated for the F_1 crosses through Analysis of variance, General combining ability (GCA) and Specific combining ability (SCA) for the plant parameters such as germination per cent, survivability per cent, plant height, number of leaves per branch, internodal distance and leaf moisture content where parents and crosses had significant amount of GCA and SCA respectively. Among the eight parental genotypes MI- 0543 (female) and V1 (male) were the best combiners and MI-0685 x V1 was the best cross for growth and survivability traits. Among the F_1 crosses, MI-0685 x V1 was found to perform better than the remaining crosses and MI-0543 × V1 was found to be the best combiners. Hence, both non- additive and additive gene actions are important for the mulberry improvement.

4. CONCLUSION

Analysis of different growth and yield parameters of different crosses and parents indicated that significant differences were observed among all parents for general combining ability. Analysis of general combining ability showed the higher magnitude of SCA variance than GCA variance for most of the characters, which indicates the presence of non- additive gene action for most of the traits except for number of branches per plant, number of leaves per plant, and single leaf area. Present study is in accordance with [13] who reported that non-additive genetic variance is more often evident in controlling the inheritance of majority of yield traits than additive components in mulberry. The results illustrate significant variation among the genotypes for some of the traits studied. The sum of squares of genotypes for these characters was further divided into parents and crosses. The variance due to parents and crosses revealed significant differences among themselves indicating genetic variability for the efficient selection. Significance of variance due to lines, testers and between lines *vs* testers indicated the sufficient difference among the parents. These results are in accordance with those of [15,13,16]and [21].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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