

Assessment of general and specific combining ability for growth parameters at seedling stage in mulberry (*Morus* spp.)

Abstract

An investigation was conducted to assess combining ability for seedling growth parameters in mulberry, using six females and four males crossed in the line $\times$ tester mating design at the Department of Sericulture, University of Agricultural Sciences (UAS), Gandhi Krishi Vigyan Kendra (GKVK), Bengaluru during 2023-24. Seedling traits were evaluated using a CRD design with three replications. The study revealed that *Morus cathayana* (ME-03) expressed good general combining ability (GCA) for seedling height in cm (60 and 90 DAS), number of leaves per plant and fresh leaf weight per plant (g). Among the testers, *M. indica* (MI-0308) excelled in GCA for seedling height in cm (60 and 90 DAS), number of leaves per plant, internodal distance (cm) and fresh leaf weight per plant (g). Specific combining ability (SCA) of crosses indicated that *M. latifolia* (ME-0185) $\times$ *M. indica* (MI-0173) in germination percentage, *M. bombycis* (ME-18) $\times$ *M. indica* (MI-0308) for seedling height in cm (60 and 90 DAS), *M. multicaulis* (ME-06) $\times$ *M. alba* (MI-0423) for number of leaves per plant, single leaf area (cm²) and fresh leaf weight per plant (g) expressed high positive SCA.

Keywords: Mulberry; Seedling growth parameters; Combining ability; GCA; SCA; Line $\times$ tester design

1. Introduction

Mulberry is a cross pollinated heterozygous perennial plant, which belongs to family Moraceae. Mulberry exhibits high plasticity and acclimatizes itself to various climatic conditions (Ashiru, 2002). The foliage of mulberry serves as a sole source of food for monophagous silkworm, *Bombyx mori* L. and 60 per cent of total cost of cocoon production goes towards mulberry production alone. Hence, the productive quality leaves is utmost important for sustainability and profitability of sericulture industry. Therefore, development of new mulberry hybrids with novel and desirable traits boost sericultural economy (Bedi, 1999). Selection of compatible parents and appropriate breeding techniques are pre-requisites for any breeding programme. The parental genotypes should be selected based on their phenotypic performance and intrinsic genetic values for formulating successful breeding programs. Among the various selection approaches, line $\times$ tester analysis is fruitful for identification of best combining parental genotypes as it provides the information of general combining ability

(GCA) of parents and specific combining ability (SCA) of the F₁ progenies and also additive, non-additive gene actions (Yehia and El-Hashash, 2019). The primary objective of mulberry cultivation is to create highly productive hybrids with exceptional leaf quality in the shortest possible time and at a reasonable production cost. With this background the current investigation was designed to evaluate the combining ability for mulberry and to identify suitable crosses using a line x tester mating design.

2. Material and methods

For the present study, parental materials comprising six lines and four testers were chosen from the field germplasm available at the Department of Sericulture, UAS, GKVK, Bengaluru. The experimental site was located at an altitude of 931 m above sea level, with a latitude of 13.077492° N and longitude of 77.575778° E. The six lines and four testers were mated using a line × tester breeding design (Table 1). Successful crossing was achieved through several initial procedures including pruning, bagging and pollination. After one week, fully ripened fruits were collected from the lines and seeds were extracted by soaking the fruits in water overnight. Floating seeds were discarded, while the submerged seeds were selected for sowing after being shade-dried (Mbora *et al.*, 2008). A completely randomized design (CRD) with three replications was employed for planting the twenty-four F₁ progenies (Table 2). Seeds were sown in polybags filled with a mixture of soil, sand and farmyard manure in a 1:1:1 ratio. Observations related to growth parameters of mulberry were recorded on the 30, 60 and 90 days after sowing (DAS).

Table 1: List of lines and testers involved in study

Sl. No.	Scientific name	Accession number
LINES		
1.	<i>M. nigra</i>	ME-0008
2.	<i>M. latifolia</i>	ME-0185
3.	<i>M. cathayana</i>	ME-03
4.	<i>M. multicaulis</i>	ME-06
5.	<i>M. bombycis</i>	ME-18
6.	<i>M. sinensis</i>	MI-0025
TESTERS		
1.	<i>M. laevigata</i>	MI-0079
2.	<i>M. indica</i>	MI-0173
3.	<i>M. indica</i>	MI-0308
4.	<i>M. alba</i>	MI-0423

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 Kempthorne (1957) using means of each replication for six characters recorded for twenty-four crosses.

Table 2. List of mulberry crosses used in the study

Sl. No.	Crosses
1.	ME-0008×MI-0079
2.	ME-0008×MI-0173
3.	ME-0008×MI-0308
4.	ME-0008×MI-0423
5.	ME-0185×MI-0079
6.	ME-0185×MI-0173
7.	ME-0185×MI-0308
8.	ME-0185×MI-0423
9.	ME-03×MI-0079
10.	ME-03×MI-0173
11.	ME-03×MI-0308
12.	ME-03×MI-0423
13.	ME-06×MI-0079
14.	ME-06×MI-0173
15.	ME-06×MI-0308
16.	ME-06×MI-0423
17.	ME-18×MI-0079
18.	ME-18×MI-0173
19.	ME-18×MI-0308
20.	ME-18×MI-0423
21.	MI-0025×MI-0079
22.	MI-0025×MI-0173
23.	MI-0025×MI-0308
24.	MI-0025×MI-0423

2.2 Estimation of Combining Ability

Effects Linear model given by [Goyal and Kumar] was used to estimate GCA and SCA effects which is as follows:

$$X_{ij} = \mu + g_i + g_j + S_{ij} + e_{ijk}$$

Where,

μ = population mean

g_i = GCA effect of i^{th} female parent

g_j = GCA effect of j^{th} male parent

S_{ij} = SCA effect of ij^{th} combination

e_{ijk} = 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:

2.2.1 Genetic combining ability (GCA) effects for parents

$$\text{a) Lines: } g_l = \frac{X_{i..}}{tr} - \frac{X_{..}}{ltr} ; X = \sum_{j=1}^t \sum_{k=1}^r X_{ijk}$$

$$\text{b) Testers: } g_t = \frac{X_{.j.}}{lr} - \frac{X_{..}}{ltr} ; X = \sum_{i=1}^l \sum_{k=1}^r X_{ijk}$$

Where,

$X_{i..}$ = Total of i^{th} line over all testers and replications

$X_{.j.}$ = Total of j^{th} tester over all lines and replications

t = No. of testers

l = No. of lines

r = No. of replications

2.2.2 Specific combining ability (SCA) effects for crosses

$$S_{ij} = \frac{X_{ij.}}{r} - \frac{X_{i..}}{tr} - \frac{X_{.j.}}{lr} + \frac{X_{..}}{ltr}$$

Where,

S_{ij} = SCA effect of ij^{th} combination

X_{ij} = Total of ij^{th} combination over all replications

$$X = \sum_{k=1}^r X_{ijk}$$

t = no. of testers

l = no. of lines

r = no. of replications

2.3 Critical difference (CD)

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

The findings of the present study demonstrate that developing mulberry hybrids with novel and desirable traits is highly feasible through systematic breeding programs. Most of the breeding studies were carried out on the development of pest and disease-resistant varieties, as well as cultivars tolerant to salt and drought stresses (Kumar *et al.*, 1999; Vijayan *et al.*, 2008; Gnanaraj *et al.*, 2011). However, only a limited number of studies have targeted traits related to growth and yield (Vijayan *et al.*, 2004). In this research, parental genotypes were crossed using the line $\times$ tester mating design, as described by Kempthorne (1957). This method has been successfully applied in the improvement of several agricultural crops, including cotton (Bilwal *et al.*, 2018), barley (Prasad *et al.*, 2013), wheat (Gowda *et al.*, 2010; Jain and Sastry, 2012) and maize (Mohammad *et al.*, 2013; Akhi *et al.*, 2018; Zhou *et al.*, 2018).

3.1 General combining ability (GCA) effect for parents

Estimates of general combining ability (GCA) effects aid in selecting superior parents for developing high-quality genotypes by concentrating additive genes. The present study identified the lines and testers with high GCA effects across multiple traits, reflecting their genetic merit. Effective general combiners exhibit higher genetic potential (Table 3).

3.1.1 Germination percentage

The GCA effects for germination percentage among the lines ranged from -10.840 (ME-0008) to 9.868 (ME-06). ME-06 (9.868) exhibited a significant positive GCA effect. For the testers, GCA effects ranged from -7.188 (MI-0173) to 3.951 (MI-0423), with MI-0423 (3.951) demonstrating a highly significant positive GCA effect and MI-0173 (-7.188) a highly significant negative GCA effect (Table 3). This negative GCA indicates those genotypes are not good for germination of seeds. The present findings suggest that, ME-06 among the lines and MI-0423 among the testers are effective general combiners for germination percentage, as their high positive GCA effects indicate a strong potential for enhancing seed sprouting. These results align with the findings of Bhuvana *et al.* (2020) and Kalpana *et al.* (2024), who also reported significant positive GCA values for germination percentage in mulberry seedlings.

3.1.2 Seedling height at 30, 60 and 90 DAS (cm)

For seedling height, GCA effects for the testers ranged from -0.236 to 0.253, -0.386 to 0.775 and -3.056 to 2.928 at 30, 60 and 90 days after sowing (DAS), respectively. MI-0423 and MI-0308 consistently showed significant positive GCA, while MI-0079 and MI-0173 exhibited significant negative GCA, indicating dwarfness at 30 DAS (Table 3). Among all the parents, ME-03 line and MI-0308 tester are the most effective general combiner for increased plant height, showing significant positive values for seedling height at 60 and 90 DAS. In agreement with these results, Vijayan *et al.* (1997) also reported that, parents with highly significant positive GCA effects were effective combiners for developing tall mulberry plants. Similarly, ME-18 exhibited a positive GCA effect at the seedling stage, as observed by Pooja *et al.* (2016). Further, Kalpana *et al.* (2024) noted significant positive GCA effects in most of the parents for seedling height at 60, 90 and 120 DAS in mulberry.

3.1.3 Number of leaves per plant

For the number of leaves per plant, GCA effects among the lines ranged from -1.139 to 1.278, with ME-03 exhibiting a highly significant positive GCA and ME-0185 exhibiting significant negative GCA. For the testers, GCA effects ranged from -0.900 to 0.644, with MI-0308 displaying significant positive GCA, while MI-0079 and MI-0173 recorded significant negative GCA, indicating fewer leaves per plant in these genotypes (Table 3). In this study, ME-03 and MI-0308 were identified as the most effective general combiners for the number of leaves per plant due to their highly significant positive GCA values. The current findings are in line with those of Pooja *et al.* (2016), who observed that the parent genotype, MI-139 exhibited a significant positive GCA effect for the number of leaves per mulberry plant. Similarly, studies by Bhuvana *et al.* (2020), Sapna and Chikkalingaiah (2022) and Kalpana *et al.* (2024) also reported a significant positive GCA effects among parental genotypes for number of leaves in mulberry plants.

3.1.4 Internodal distance (cm)

The GCA effects for this trait in the lines ranged from -0.308 to 0.378, with significant positive GCA effects were observed in MI-0025, ME-0185 and ME-18, while ME-0008, ME-03 and ME-06 showed significant negative GCA effects. In the testers, GCA values ranged from -0.052 to 0.140, with MI-0173 exhibiting a significant positive GCA, while MI-0308, MI-0423 and MI-0079 showed significant negative GCA values (Table 3). ME-0008 and MI-0308 are the best general combiners for internodal distance, exhibiting high significant negative GCA values which indicating shorter internodal distance, which are desirable for increased leaf yield. Studies by Vijayan *et al.* (1997), Banerjee *et al.* (2014) and Bhuvana *et al.* (2020) reported significant negative GCA effects in mulberry parents for internodal distances, indicating their potential as effective combiners. These observations are consistent with the findings of the present study.

3.1.5 Single leaf area (cm²)

GCA effects for this trait ranged from -14.306 to 15.814 in lines and -4.040 to 11.388 in testers in which the line, ME-06 and the tester, MI-0423 were exhibited highly significant

positive GCA (Table 3), making them the best general combiners for this trait. In mulberry, larger single leaf area enhances photosynthesis, growth and biomass, boosting leaf yield which is essential for silkworm rearing. These findings are supported by Pooja *et al.* (2016) and Bhuvana *et al.* (2020), who identified parents with significant positive GCA as effective combiners for single leaf area in mulberry.

3.1.6 Fresh leaf weight per plant (g)

GCA effects for leaf weight ranged from -1.559 to 1.629 in lines and -1.283 to 1.378 in testers. Significant positive GCA was observed in ME-03, ME-06, MI-0025, MI-0308 and MI-0423, while ME-18, ME-0185, ME-0008, MI-0079 and MI-0173 showed significant negative GCA values (Table 3).

ME-03 and MI-0308 were identified as the best combiners for fresh leaf weight, due to high significant positive GCA. Higher fresh leaf weight in mulberry enhances leaf yield, supporting silkworm rearing by improving biomass and plant productivity. These findings are supported by Pooja *et al.* (2016), who identified parents with significant positive GCA for fresh leaf weight as effective combiners in mulberry, highlighting its importance for yield.

3.2 Specific combining ability (SCA) effect for crosses

3.2.1 Germination percentage

Out of 24 crosses, 11 crosses showed positive SCA effects, with ME-0185 $\times$ MI-0173 (3.146) demonstrating a significant positive effect, while 13 crosses exhibited negative SCA effects, including ME-06 $\times$ MI-0173 (-7.646), which showed a significant negative effect (Table 4). Similar findings were reported by Pooja *et al.* (2016), who observed significant positive SCA values for some crosses and significant negative values for others in relation to germination.

3.2.2 Seedling height at 30, 60 and 90 DAS (cm)

11 out of 24 crosses displayed positive values for SCA effects for seedling height at 30 DAS, among which ME-06 $\times$ MI-0173 registered highly significant positive SCA. At 60 DAS, ten crosses recorded positive values, while fourteen crosses showed negative values for SCA. The ME-18 $\times$ MI-0308 exhibited a significant positive SCA value for seedling height at 60 and 90 DAS (Table 4). The present findings regarding SCA for seedling height align with the results reported by Vijayan *et al.* (1997) and Suresh *et al.* (2019) and Sapna and Chikkalingaiah (2022).

3.2.3 Number of leaves per plant

In this trait, 13 crosses out of 24 exhibited a positive SCA effects. Among them, the cross, ME-06 $\times$ MI-0423 demonstrated a significant positive SCA effect, indicating its desirability for producing plants with a higher number of leaves. In contrast, the cross ME-03 $\times$ MI-0423 showed a significant negative SCA effect, which could bear only few number of leaves per plant (Table 4). These findings are consistent with the results of Pooja *et al.* (2016) and Bhuvana *et al.* (2020).

3.2.4 Internodal distance (cm)

13 crosses out of 24 displayed negative values concerning SCA effects, which are in a desirable direction. Among them, ME-03 $\times$ MI-0079 cross registered highly significant negative SCA effect (Table 4). These findings aligned with Bhuvana *et al.* (2020) and Sapna and Chikkalingaiah (2022) studies on combining ability for leaf yield attributing traits in mulberry.

3.2.5 Single leaf area (cm²)

Among 24 crosses, ME-06 $\times$ MI-0423 registered significant positive SCA for single leaf area. The cross, ME-06 $\times$ MI-0308 recorded a significant negative SCA for single leaf area (Table 4), indicating that the plant tends to produce leaves with a smaller leaf area, making it undesirable. Similar results were obtained by Pooja *et al.* (2016) and Suresh *et al.* (2019) in mulberry.

3.2.6 Fresh leaf weight per plant (g)

Positive SCA values were registered in 13 out of 24 crosses, among them, ME-06 $\times$ MI-0423 cross recorded significant positive SCA effect. Whereas ME-03 $\times$ MI-0079 exhibited significant negative SCA effect for fresh leaf weight (Table 4). Positive SCA effect indicated crosses are desirable for fresh leaf weight, this trait contributed to leaf yield parameter. Comparable findings were achieved by Suresh *et al.* (2019); Bhuvana *et al.* (2020); Sapna and Chikkalingaiah (2022), who observed both significant positive and negative SCA values for fresh leaf weight.

4. Conclusion

The selection of compatible progenitors with desirable GCA and hybrids with superior SCA effects plays a crucial role in all successful breeding programs. In the present investigation, variations for different growth parameters were noted among ten parental genotypes and twenty-four F₁ offspring of mulberry. Both the parents and the hybrids exhibited significant levels of GCA and SCA, respectively. These variations facilitate the identification of superior mulberry genotypes. ME-03 line and MI-0308 tester found to be the best combiners as indicated by their positive GCA effects. The cross of ME-06 $\times$ MI-0423 registered high SCA for number of leaves per plant, single leaf area and fresh leaf weight per plant. Similarly, ME-0185 $\times$ MI-0173 for germination percentage, ME-06 $\times$ MI-0173 for seedling height at 30 DAS, ME-18 $\times$ MI-0308 for seedling height at 60 & 90 DAS have registered high SCA. For internodal distance, ME-03 $\times$ MI-0079 was found to be best crosses as it registered negative SCA. Hence, these parents and F₁ progenies may be further used in breeding programs for mulberry crop improvement.

DISCLAIMER (ARTIFICIAL INTELLIGENCE):

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Table 3: Estimation of general combining ability effects of parents for different growth parameters at seedling stage in mulberry

Parents	Germination Percentage	Seedling height (cm) at			No. of leaves/ plant	Internodal distance (cm)	Single leaf area (cm ²)	Fresh leaf weight/plant (g)
		30 DAS	60 DAS	90 DAS				
Lines								
ME-0008	-10.840 **	-0.814 **	-2.608 **	-5.531 **	-1.139 **	-0.380 **	-13.449 **	-1.559 **
ME-0185	-3.757 **	-0.264 **	-1.467 **	-5.406 **	-0.556 **	0.208 **	-14.306 **	-1.557 **
ME-03	8.285 **	0.778 **	2.225 **	4.011 **	1.278 **	-0.292 **	10.879 **	1.629 **
ME-06	9.868 **	-0.464 **	-0.108	0.936 **	0.628 **	-0.113 **	15.814 **	1.014 **
ME-18	0.868 **	0.819 **	1.700 **	3.778 **	-0.856 **	0.199 **	5.348 **	-0.096 **
MI-0025	-4.424 **	-0.056	0.258 **	2.211 **	0.644 **	0.378 **	-4.287 **	0.568 **
SE m±	0.1912	0.0728	0.0898	0.1941	0.0361	0.0115	0.3832	0.0261
CD at 5 %	0.385	0.147	0.181	0.391	0.073	0.023	0.771	0.053
CD at 1 %	0.5138	0.1956	0.2414	0.5214	0.0969	0.0309	1.0296	0.0702
Testers								
MI-0079	-0.215	-0.236 **	-0.386 **	-0.383 *	-0.300 **	-0.038 **	-3.971 **	-0.432 **
MI-0173	-7.188 **	-0.008	-0.275 **	-3.056 **	-0.900 **	0.140 **	-4.040 **	-1.283 **
MI-0308	3.451 **	-0.007	0.775 **	2.928 **	0.644 **	-0.052 **	-3.377 **	1.378 **
MI-0423	3.951 **	0.253 **	-0.114	0.511 **	0.556 **	-0.049 **	11.388 **	0.337 **
SE m±	0.1561	0.0594	0.0734	0.1585	0.0294	0.0094	0.3129	0.0213
CD at 5 %	0.314	0.120	0.148	0.319	0.059	0.019	0.630	0.043
CD at 1 %	0.4195	0.1196	0.1971	0.4258	0.0791	0.0252	0.8407	0.0574

*Significant at p = 0.05 and ** significant at p = 0.01

Table 4: Estimation of specific combining ability effects of crosses for different growth parameters at seedling stage in mulberry

Crosses	Germination Percentage	Seedling height (cm) at			No. of leaves/plant	Internodal distance (cm)	Single leaf area (cm ²)	Fresh leaf weight/plant (g)
		30 DAS	60 DAS	90 DAS				
ME-0008×MI-0079	-1.576 **	0.419 **	0.286	-2.225 **	0.117	0.222 **	8.477 **	0.996 **
ME-0008×MI-0173	2.396 **	0.258	1.142 **	3.947 **	0.783 **	-0.190 **	11.374 **	0.308 **
ME-0008×MI-0308	-0.576	-0.642 **	-1.408 **	-3.036 **	-0.294 **	-0.098 **	-2.057 *	-0.810 **
ME-0008×MI-0423	-0.243	-0.036	-0.019	1.314 **	-0.606 **	0.066 **	-17.794 **	-0.494 **
ME-0185×MI-0079	-2.493 **	-0.064	0.178	1.717 **	0.467 **	0.067 **	1.856 *	0.450 **
ME-0185×MI-0173	3.146 **	0.042	-0.033	2.856 **	-0.333 **	0.006	12.505 **	0.634 **
ME-0185×MI-0308	-0.493	0.508 **	0.717 **	-4.728 **	0.122	-0.102 **	-2.706 **	-1.134 **
ME-0185×MI-0423	-0.160	-0.486 **	-0.861 **	0.156	-0.256 **	0.028	-11.656 **	0.049
ME-03×MI-0079	0.465	-0.106	1.819 **	4.889 **	0.967 **	-0.466 **	-10.150 **	-1.510 **
ME-03×MI-0173	2.938 **	-0.433 **	-1.458 **	-8.261 **	0.367 **	-0.401 **	-12.072 **	0.335 **
ME-03×MI-0308	-1.201 **	0.800 **	-3.108 **	-1.111 **	0.756 **	0.365 **	6.371 **	1.071 **
ME-03×MI-0423	-2.201 **	-0.261	2.747 **	3.372 **	-2.089 **	0.545 **	15.851 **	0.105
ME-06×MI-0079	3.049 **	0.269	-1.147 **	-3.758 **	-0.850 **	-0.045	-5.734 **	-0.019
ME-06×MI-0173	-7.646 **	0.908 **	1.742 **	0.214	-1.317 **	0.760 **	1.035	-1.065 **
ME-06×MI-0308	1.715 **	-0.925 **	-0.342	2.797 **	0.472 **	-0.315 **	-23.278 **	1.237 **
ME-06×MI-0423	2.882 **	-0.253	-0.253	0.747	1.694 **	-0.444 **	27.978 **	1.439 **
ME-18×MI-0079	2.880 **	-1.114 **	-2.189 **	-0.933 *	-1.100 **	0.226 **	1.025	-0.447 **
ME-18×MI-0173	-2.813 **	-0.242	-0.633 **	0.172	0.833 **	-0.102 **	-9.390 **	0.659 **
ME-18×MI-0308	1.549 **	0.658 **	3.383 **	6.000 **	-0.178 *	0.040	8.750 **	0.734 **
ME-18×MI-0423	-1.618 **	0.697 **	-0.561 **	-4.128 **	0.444 **	-0.163 **	-0.385	-0.946 **
MI-0025×MI-0079	-2.326 **	0.594 **	1.053 **	-0.800 *	0.400 **	-0.003	4.526 **	0.530 **
MI-0025×MI-0173	1.979 **	-0.533 **	-0.758 **	1.072 **	-0.333 **	-0.031	-3.452 **	-0.872 **
MI-0025×MI-0308	-0.993 *	-0.400 **	0.758 **	1.189 **	-0.878 **	0.110 **	12.920 **	-1.098 **
MI-0025×MI-0423	1.340 **	0.339 *	-1.053 **	-1.461 **	0.811 **	-0.076 **	-13.994 **	-0.154 **
SE m±	0.3824	0.1456	0.1797	0.3881	0.0721	0.0230	0.7664	0.0523
CD at 5 %	0.770	0.293	0.362	0.781	0.145	0.046	1.543	0.105
CD at 1 %	1.0276	0.3912	0.4828	1.0429	0.1938	0.0618	2.0593	0.1405

*Significant at p = 0.05 and ** significant at p = 0.01