



Assessment of Gene Action and Genetic Variability for Seedling Growth Traits in Mulberry (*Morus* spp.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/acri/2025/v25i81395>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/140634>

Original Research Article

Received: 15/05/2025
Published: 28/07/2025

ABSTRACT

Information regarding the nature of gene action controlling the yield and its related traits is crucial for designing an effective breeding strategy. With this aim, a study was carried out to assess the genetic control and variation among lines and testers for seedling attributes in mulberry. The percentage contribution of testers, lines and their interactions to the total variation indicated that the female parents (lines) contributed more to the overall variability than the male parents (testers) for all traits. The variation due to lines was statistically significant for germination rate, seedling height at 60 and 90 days after sowing (DAS), branch number and internodal length, but not significant for the remaining traits. On the other hand, the variance attributed to testers was significant for germination rate and internodal length, but not for the other characteristics observed. The

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interaction effects between lines and testers were highly significant for germination rate, seedling height at 90 DAS, average leaf mass and leaf area, but not significant for plant height at 60 and 120 DAS, branch count and internodal distance. The variance due to hybrid combinations was highly significant for all traits except for internodal length. The study aimed to estimate the extent of general combining ability (GCA), specific combining ability (SCA) variances and determine the mode of gene action controlling these traits. Findings showed that the non-additive gene effects were predominant for most of the growth traits in this study.

Keywords: Mulberry; gene action; lines; testers; seedling traits; GCA & SCA variance.

1. INTRODUCTION

Mulberry (*Morus* spp.) 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, high-quality and productive mulberry leaves are crucial for ensuring the sustainability and profitability of the sericulture industry. Therefore, the development of novel mulberry hybrids with desirable traits is essential to enhance the sericultural economy (Bhuvana et al., 2020).

There is a scarcity of information on the genetic interactions that control the expression of numerous quantitative traits in mulberry. A strong affinity among different species of mulberry during interspecific hybridization has been observed (Das and Krishnaswami, 1965), which was further corroborated by crossability investigations (Dandin, 1987; Dwivedi, 1990). The selection of parents capable of transmitting desirable traits is essential for breeding superior cultivars. A rational breeding strategy involves identifying parents based on their combining ability, which reflects the underlying gene action controlling quantitative traits and aids in effective parent selection for hybridization programmes (Goyal & Kumar, 1991).

The success of any breeding programme depends on selecting compatible parents and applying appropriate breeding techniques. Parental genotypes should be chosen not only for their phenotypic performance but also for their intrinsic genetic potential (Bhalodiya et al., 2019). Among the various selection approaches, line x 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 F_1 progenies and also additive, non-additive gene actions (Yehia & 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. 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. MATERIALS 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 is 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 x 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 (Mborra et al., 2008). A completely randomized design (CRD) with three replications was employed for planting the twenty-four F_1 progenies (Table 2). Seeds were sown in polybags filled with a mixture of soil, sand and farmyard manure in a 1:1:1 ratio (Dandin et al., 2003; Ranjitha et al., 2023). Observations related to growth parameters of mulberry were recorded on the 30, 60 and 90 days after sowing (DAS).

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 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

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

Table 3. ANOVA for line x tester analysis

Source	Degrees of freedom	Mean sum of squares (MSS)	Expected Mean Squares
Replication	r - 1		
Lines	l - 1	M_l	$\sigma_e^2 + r [\text{COV}(\text{FS}) - 2\text{COV}(\text{HS})] + \text{tr COV}(\text{HS})$
Testers	t - 1	M_t	$\sigma_e^2 + r [\text{COV}(\text{FS}) - 2\text{COV}(\text{HS})] + \text{lr COV}(\text{HS})$

Source	Degrees of freedom	Mean sum of squares (MSS)	Expected Mean Squares
Lines X Testers	(l - 1) (t - 1)	M_{lt}	$\sigma_e^2 + r [\text{COV}(\text{FS}) - 2\text{COV}(\text{HS})]$
Error	(lt-1) (r-1)	M_e	σ_e^2
Total	(ltr-1)		

where,
 r = number of replications
 l = number of female parents (lines)
 t = number of male parents (testers)
 $\text{COV}(\text{FS})$ = Covariance of full sibs
 $\text{COV}(\text{HS})$ = Covariance of half sibs

From the mean sum of squares, covariance of full sibs and covariance of half sibs were estimated as follows:

$$\text{Co-variance of full sibs} = ((M_l - M_e) + (M_t - M_e) + (M_{lt} - M_e)) / 3r + ((6r \text{ Cov H.S.} - r (l+t) \text{ Cov H.S.}) / 3r)$$

$$\text{Co- variance of half sibs} = (M_l + M_t - 2 M_{lt}) / (r (l + t))$$

After estimating $\text{COV}(\text{HS})$ and $\text{COV}(\text{FS})$ the GCA variance of lines and testers and SCA variance of crosses were estimated as here under

$$\text{GCA variance for lines} = (M_l - M_{lt}) / R_t$$

$$\text{GCA variance for testers} = (M_t - M_{lt}) / r_l$$

$$\text{SCA variance for crosses} = (M_{lt} - M_e) / R$$

where,

M_l = MSS due to lines (females)
 M_t = MSS due to testers (males)
 M_{lt} = MSS due to lines x testers
 M_e = MSS due to error

2.2 Critical Difference (CD)

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

2.3 Proportional Contribution of Lines, Testers and Line x Tester

- Contribution of lines (%) = $(SS(f) / SS(c)) \times 100$
- Contribution of tester (%) = $(SS(m) / SS(c)) \times 100$

- Contribution of lines x tester (%) = $(SS(f \times m) / SS(c)) \times 100$

where,

$SS(c)$ = Sum of squares due to crosses
 $SS(f)$ = Sum of squares due to lines
 $SS(m)$ = Sum of squares due to testers
 $SS(f \times m)$ = Sum of squares due to lines x testers

3. RESULTS AND DISCUSSION

In this study, it was evident that the contribution of lines to the overall variance was recorded as higher than that of testers for all traits, including germination percentage, seedling height (cm) at 30, 60 and 90 days after sowing (DAS), number of leaves per plant, internodal length (cm), single leaf area (cm²) and fresh leaf weight per plant(g). This was depicted in Fig. 1.

The contribution of line x tester interactions to the overall variance was observed to be greater than that of the testers for seedling height (cm) at 30, 60 and 90 DAS, number of leaves per plant, internodal distance (cm), single leaf area (cm²) and fresh leaf weight per plant (g). Conversely, the contribution of line x tester interactions to the total variance was higher than that of the lines for internodal distance (cm) and single leaf area (cm²).

The contribution of lines is greater than that of testers to the overall variance for most of the traits examined. These findings are consistent with those of Banerjee et al. (2014), who observed that female parents contributed more significantly to the total variance for most traits in mulberry. Similarly, Bhuvana et al. (2020) reported that the line x tester interaction showed a high contribution to the total variance for all traits, except for the number of leaves per plant and the number of branches per plant. Among the evaluated traits, the highest interaction

contributions were recorded for single leaf area (84.43%) and internodal distance (75.06%). Hence, lines and line x tester interactions afforded maximum contribution to the total variance.

3.1 Analysis of Variance for Combining Ability

3.1.1 Variance due to lines, testers and lines x tester interaction

The variability attributed to lines, testers and the interaction between lines and testers concerning all the traits examined is illustrated in Table 4. The analysis of variance reveals notable and significant variability across all traits under investigation.

The variance attributed to the lines was notable and meaningful for the percentage of seeds that sprouted, the height of seedlings at 30, 60 and 90 DAS, number of leaves per plant, distance between nodes, single leaf area and fresh leaf weight per plant. The variance attributed to the testers was notable for the germination percentage and fresh leaf weight per plant, but non-significant for other traits examined.

The interaction variance between lines and testers was highly significant for the germination percentage, seedling height at 30, 60 and 90 DAS, number of leaves per plant, internodal distance (cm), single leaf area (cm²) and fresh leaf weight per plant (g). The variance attributed to the crosses was highly significant for all traits examined.

The present findings demonstrate considerable variation among the genotypes for majority of the traits analysed. The total sum of squares for these genotypes was subsequently partitioned into parental and cross contributions. The variance attributed to parents and crosses expressed significant differences among them, indicating genetic diversity that facilitates effective selection. The importance of variance due to lines, testers and the interaction between lines and testers highlighted substantial differences among the parental lines. These findings align with the studies conducted by Vijayan et al. (1997), Banerjee et al. (2014) and Pooja et al. (2016) Results pertaining to the analysis of variance for combining ability were consistent with the findings of Bhuvana et al.

(2020), who reported that the mean square values attributed to testers were highly significant for traits such as the number of branches per plant, the number of leaves per branch, leaf water content and the capacity to retain moisture whereas notable variation among lines was observed only for fresh leaf weight.

3.1.2 Variance components and nature of gene action

The variances (σ^2 GCA, σ^2 SCA) and the ratio (σ^2 GCA/ σ^2 SCA) are presented in Table 5.

For designing a breeding program, information regarding combining ability studies in terms of GCA and SCA variances is essential. Combining ability studies also provide insights into the gene action influencing a particular trait. Non-additive gene action can be inferred from the SCA variance, while the GCA variance reflects the extent of additive gene action for a specific trait. The ratio of non-additive to additive gene action should be calculated to determine the predominance of the type of genetic variation for a given character. If this ratio is less than one, it indicates that additive variance plays a major role in controlling the expression of the trait. Conversely, if the ratio is greater than one, it signifies the significance of non-additive variance (Gardner, 1963).

In this study, an effort was made to determine the extent of GCA and SCA variances, as well as the nature of gene action for the trait overall. The results revealed that the non-additive genetic component was predominant in the genetic variance for most growth parameters. This finding is consistent with Banerjee et al. (2014), who observed that non-additive genetic variance is more frequently involved in the inheritance of most yield traits in mulberry than additive variance. Similarly, Suresh et al. (2019) found that the dominance of non-additive genetic variance provides an opportunity to utilize heterosis in mulberry.

For all traits, the extent of SCA variance was greater than that of GCA variance, as shown by the analysis of variance, with the exception of germination percentage and fresh leaf weight per plant (g). The ratio of GCA to SCA variance was below one for all traits, with the exception of germination percentage and fresh leaf weight per plant (g), where the variance exceeded one.

Table 4. Analysis of variance for combining ability for different growth parameters at seedling stage in mulberry

Source	d.f.	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				
Replication	2	0.128	0.088	0.022	0.052	0.681	0.114	0.929	0.265
Crosses	23	247.790 **	2.170 **	16.262 **	97.196 **	5.966 **	0.514 **	981.954 **	9.788 **
Lines	5	763.112 **	5.344 *	40.497 *	230.239 **	11.472 *	1.125 *	1922.291 *	21.279 **
Testers	3	475.392 **	0.719	5.030	109.899	9.744	0.157	1039.109	23.074 **
Line × Tester	15	30.495 **	1.402 **	10.431 **	50.307 **	3.375 **	0.381 **	657.078 **	3.301 **
Error	46	0.451	0.065	0.113	0.592	0.015	0.002	2.042	0.007
Total	71	80.566	0.748	5.342	31.871	1.962	0.171	319.447	3.183

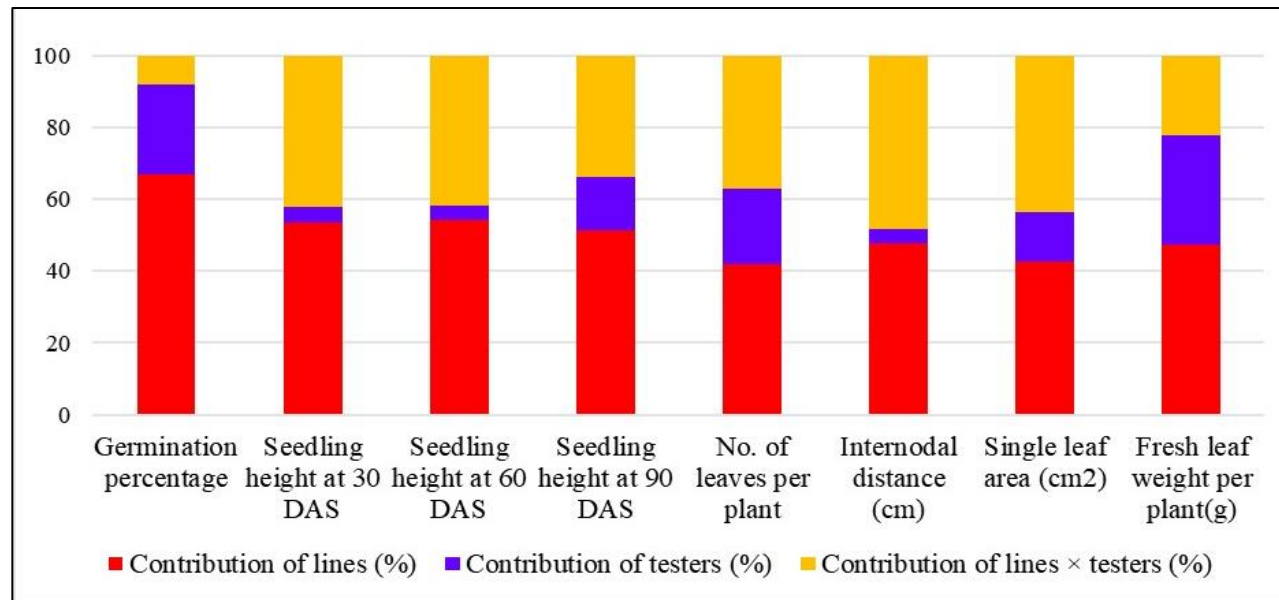
*Significant at $p = 0.05$ and ** significant at $p = 0.01$ **Fig. 1. Proportional contribution of lines, testers and their interaction to total variance**

Table 5. Estimation of variance components in respect of different growth parameters at seedling stage in mulberry

Sl. No.	Characters	σ^2 GCA	σ^2 SCA	σ^2 GCA/ σ^2 SCA
1.	Germination percentage	39.25**	10.02**	3.92
2.	Seedling height (cm) at			
	30 DAS	0.11**	0.45**	0.24
	60 DAS	0.82**	3.44**	0.24
	90 DAS	7.98**	16.62**	0.48
3.	No. of leaves per plant	0.48**	1.12**	0.43
4.	Internodal distance (cm)	0.02*	0.13**	0.14
5.	Single leaf area (cm ²)	54.91**	218.44**	0.25
6.	Fresh leaf weight/plant (g)	1.26**	1.10**	1.15

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

3.2 Germination Percentage

The GCA variance for germination rate was markedly significant and more influential compared to the SCA variance, as confirmed by the combining ability analysis. Additive genetic effects are influencing this trait, as the ratio of GCA to SCA variance exceeds one. This implies that recessive genes are primarily responsible for the trait, indicating that recessive alleles were more prevalent than dominant alleles in the parents and highlighting the significance of additive genetic effects in the inheritance of this characteristic. Same results were obtained by Kalpana et al. (2024) in germination of mulberry seeds.

3.3 Seedling Height (cm) at 30, 60 and 90 DAS

In the investigation, the ratio of general combining ability to specific combining ability variance was less than unity and the SCA variance was found to be significant, illustrating the prominence of dominant genetic effects on plant height at the 30, 60 and 90th days after sowing. These findings are consistent with those of Pooja et al. (2016) and Kalpana et al. (2024), who observed a variance ratio less than one for plant height.

3.4 Number of Leaves per Plant

In this experiment, SCA variance is greater than GCA variance for the number of leaves. The ratio of GCA to SCA is less than one, indicating the dominance of non-additive gene action in regulating this trait. Similar results were reported by Kalpana et al. (2024), who identified a variance of less than one for the number of leaves per plant in seedlings.

3.5 Internodal Distance (cm)

Studies on combining ability indicated a significant variance for general combining ability (GCA) and specific combining ability (SCA), with the GCA to SCA ratio being above one. This reflects the non-additive genetic effects for the trait. Comparable findings were reported by Vijayan et al. (1997), who confirmed the primary role of SCA variance for this trait, with a GCA/SCA ratio of less than one, signifying the predominance of non-additive over additive genetic influences.

3.6 Single Leaf Area (cm²)

Evaluation of the data for combining ability highlighted the importance of specific combining ability variance. The ratio of general combining ability to specific combining ability was below one, indicating the dominance of non-additive genetic effects for single leaf area. These results align with the observations made by Suresh et al. (2019) in mulberry.

3.7 Fresh Leaf Weight per Plant (g)

Analysis of the data for combining ability highlighted the significance of SCA variance. The ratio of GCA to SCA is less than one, which underscored the importance of non-additive gene action. The findings align with those of Suresh et al. (2019), who reported that the non-additive component also predominated for leaf weight in mulberry. Similar result was recorded by Kalpana et al. (2024) in mulberry seedlings.

4. CONCLUSION

The proportional contribution of lines to the overall variance was recorded as higher than that

of males for all traits. The contribution of female \times male interactions to the overall variance was observed to be greater than that of the testers for all characters. Conversely, the contribution of female \times male interactions to the total variance was higher than that of the females for internodal distance (cm) and single leaf area (cm²). The variance attributed to the lines (females) was notable and meaningful for the percentage of seeds that sprouted, the height of seedlings at 30, 60 and 90th DAS, number of leaves per plant, distance between nodes, single leaf area and fresh leaf weight per plant. The variance attributed to the testers (males) was notable for the germination percentage and fresh leaf weight per plant, but non-significant for other traits examined. The interaction variance between lines and testers was highly significant for the germination percentage, seedling height at 30, 60 and 90th DAS, no. of leaves per plant, internodal distance (cm), single leaf area (cm²) and fresh leaf weight per plant (g). The variance attributed to the crosses was highly significant for all traits examined. An attempt was made to determine the extent of GCA and SCA variance, as well as the overall pattern of gene action. In this experiment also, non-additive genetic effects represented the largest portion of genetic variance for most growth-related traits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares 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 this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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