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Diallel analysis of growth trait inheritance in mulberry (*Morus* sp.)

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Abstract

Aim: To assess the combining abilities of four mulberry genotypes to identify genetic variations that can enhance growth traits and improve hybridization outcomes. Ultimately, the study aims to optimize parental selection for better production in mulberry and bringing advancements in mulberry breeding.

Methodology: Griffing approach of full diallel mating design (Model 1 and Method 1) was used where parents, F1 hybrids and F1 hybrids reciprocals were included.

Results: V1 and MR2 excelled in general combining ability (GCA) for growth traits. Positive specific combining effects (SCA) were observed in V1×G4 and G4×V1 crosses. Higher specific combing ability variance and significant reciprocal combing ability (RCA) indicate strong non-additive gene action and maternal inheritance.

Interpretation: The study highlights V1 and MR2 as superior combiners with high positive GCA, emphasizing the importance of parent interactions. Non-additive gene action, indicated by higher SCA variance, suggests focusing on these interactions for breeding. The crosses V1×G4 and G4×V1 showed strong SCA effects, offering promise for improving growth and mulberry productivity in sericulture.

Key words: Additive gene effect analysis, Diallel, Growth traits, Mulberry genotypes, Non- additive gene effect

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Introduction

Sericulture is the practice of cultivating mulberry trees and breeding silkworms to produce silk fibers (Bhuvana *et al.,* 2022). It is a vital source of employment in India and other Asian nations. India is unique in hosting silkworms that produce all four silk types, with mulberry silk making up the majority of production (Kiruthika *et al.,* 2024). *Bombyx mori,* which feeds solely on mulberry leaves, makes leaf quality and quantity per unit area as a key factor in sericulture, directly affecting cocoon yield (Sahan *et al.,* 2020). Variable climatic conditions and regional challenges contribute to productivity disparities in sericulture (Ramya *et al.,* 2022). To improve mulberry leaf production, breeders aim to develop new varieties with higher yields, better adaptability and enhanced resistance to biotic and abiotic stresses. The aim of parent selection is to maximize the probability of generating offspring that surpass the best current variation (Dobhal *et al.,* 2019). But choosing parental lines only based on phenotypic performance is not a good way to guarantee that the progeny will have superior genotypes (Banerjee *et al.,* 2014). This is because any genetic advantage derived from the selected parents will be attributed to their phenotypic traits, which can be unpredictable and non-repeatable (Bertan *et al.,* 2007) and result in subpar recombinants in the segregating generations. Therefore, selecting parents based on their capacity to combine traits (combining ability) is essential to create better recombinants that are more prevalence (Banerjee *et al.,* 2012).

In biometrical genetics, two forms of combining abilities - General Combing Ability (GCA) and Specific Combining Ability (SCA) are assessed. SCA is used when a specific cross performs noticeably better or worse than would be predicted based on the average performance of the line involved, and GCA is the average performance of a line in hybridization (Sprague and Tatum, 1942; Ramya *et al.,* 2024). SCA and GCA both indicate the significance of genes with non-additive effects and additive effects. The diallel is capable of providing estimates of genetic parameter in addition to information on a population's ability to combine (Bharathi *et al.,* 2024). This study is distinct from other studies as diallel mating design is a highly controlled system in which all possible crosses are made between a predetermined set of genotypes. Typically, it involves F_1 crosses from selected parents to estimate combining ability for different parameters (Neshagaran *et al.,* 2016).

However, there are significant concerns over the use of diallel analysis as a source of data which supports a breeding program (Aguila *et al.,* 2021). Griffing (1956b) provided two models, while selecting parents based on their performance, Model-I (fixed sample) was employed as the criterion to compare the parent's combining capacity and identify better hybrid combinations; however, if parents, F1 hybrids, and F1 reciprocals are taken into account, Method-I is used. The type of information needed and the availability of physical resources both affect how many parents to include in a diallel experiment. It takes at least four parents to assess in combining abilities (Ramya *et al.*, 2024). Accordingly, the primary objectives of this research were to perform diallel analysis for determining the genetic interaction of four mulberry genotypes in the expression of various traits, evaluate F_1 hybrids for survival and growth traits and to assess combining ability studies (GCA and SCA) on the selected parents and hybrids for improved hybridization outcomes, ultimately optimizing parental selection for better production using diallel (He *et al*., 2023).

Materials and Methods

The research was carried out during the year 2021 – 2022 in both kharif and rabi seasons at the Department of Sericulture, Forest College and Research Institute, Mettupalayam. The experimental site is situated at a 11.20° N latitude and 76.56° E longitude, 320 m above mean sea level, with an annual average rainfall of 922.0 mm.

Parental selection: Selection of the congenial parents per crossing is one of the important key for a successful hybridization programme and it is done by identifying the genotypes with pledging and desirable agronomical traits for plant breeding. (Bertan *et al.,* 2007). The four mulberry parents *viz.* V1, G4, S36 and MR2 were selected and maintained at the Department of Sericulture, Forest College and Research Institute, Mettupalayam as they were the popular grown varieties locally. V1 variety was resistant to leaf spot and high yielding; G4 variety was known for high yielding potential:S36 variety was tolerant to leaf spot and ideal for raising chawki worms whereas MR2variety had succulent and soft leaves, resistant to powdery mildew (Ramya *et al.,* 2022).

Experimental diallel mating design: Griffing's approach– Model I & Method I; Method I – This method incorporates all possible crosses and parents (Hallauer and Carena, 2010).

Model I – Fixed Effect Model - A set of fixed genotypes and varieties are included in the experimental material. Such a set of genotypes is considered as a population and inferences are drawn about individual variety (Choudhary *et al.,* 2004). Full diallel analysis with parents (Table 1). Number of crosses: P^2 =16 (Parents, Direct and Reciprocal crosses).

p^2 Combinations divided into three groups

(1) the *p* parental lines themselves

(2) one set of ½ *p* (*p*-1) F_1's,

(3) the set of ½ *p* (*p*-1) reciprocal F_1's. (Griffing, 1956a; Choudhary *et al.,* 2004).

Hybridization programme: In December 2021, cuttings were taken from selected male and female plants. After 45 days, the plants were pruned to synchronize flowering, and catkins appeared 10 days later. Female catkins with undeveloped buds were enclosed in 15x10 cm butter paper bags with aeration holes. The top branches were trimmed to prevent bag rupture due to

Table 1: Parents in Full Diallel mating design

♀ \ ♂		Parents (Male)			
		V1	**G4**	**S36**	**Mr2**
Parents (Female)	V1	@	#	#	#
	G4	×	@	#	#
	S36	×	×	@	#
	MR2	×	×	×	@

@: Parents; #: Direct crosses; x: reciprocals

stem growth. Catkins of similar size and age were bagged together for uniform hybridization. Manual pollination involved collection of pollens from male catkins with the help of a paint brush. Anther dehiscence occurred between 10:00-11:30 AM and 3:00-4:00 PM (Khanduri, 2011). Pollination was initiated 10 days after the emergence of female catkins (Chowdhuri *et al.,* 2009) and continued for 4 days which is until 14th day for optimal seed setting. Cross-pollinated catkins were immediately bagged to prevent spontaneous pollination. Once fertilized, the white stigma of female flower turned brown and dried. After bagging, the plants were tagged with details like pollination date and parentage. Fruits (sorosis) ripened after 19–23 days of pollination. Reddish-black sorosis were carefully harvested from tagged plants and seeds were extracted separately from each cross (Doss *et al.,* 2012). Mature fruits were soaked in water for 24 hr and mashed. Seeds were separated by the flotation method. The viable seeds (Yellowish- brown, sunken) were identified from non – viable seeds (floating on the water surface) (Wani *et al*.,2019; Mbora *et al*.,2008).

Experimental design: The seeds were planted in poly bags containing mixture of fine soil, farm yard manure and vermiculite (1:1:1) (Vijayan *et al.,* 2009). Three replications, each with five plants, were maintained. A completely randomized design (CRD) was used to analyse sixteen F1 progenies. Observations relevant to mulberry growth traits were recorded on 45, 60, 75 and 90 DAS. The 16F1 crosses obtained from diallel mating design are as follows: T_1- P1×P1 (parents) - V1×V1; T_2 - P1×P2 (reciprocal cross) - V1×S36; T_3 - P1×P3 (reciprocal cross) - V1×G4; T_4 - P1×P4 (reciprocal cross) - V1×MR2; T_5- P2×P1 (direct cross) - S36×V1; T_6- P2×P2 (parents) - S36×S36; T_7 - P2×P3 (direct cross) - S36×G4; T_8- P2×P4 (reciprocal cross) - S36×MR2; T_9 - P3×P1 (direct cross) -G4×V1; T_{10}- P3×P2 (reciprocal cross) - G4×S36; T_{11}- P3×P3 (parents) - G4×G4; T_{12} - P3×P4 (reciprocal cross) - G4×MR2; T_{13} - P4×P1 (direct cross) - MR2×V1; T_{14}- P4×P2 (direct cross) - MR2×S36; T_{15} - P4×P3 (direct cross) - MR2×G4; T_{16} - P4×P4 (parents) - MR2×MR2.

Survival per cent: The survival rate was calculated by counting the available seedlings at 60DAS.

$$\text{Survival per cent} = \frac{\text{Number of survived seedlings at 60 DAS}}{\text{Total number of seeds sown}} \times 100$$

Growth rate: The height of the offspring was assessed between the 60th and 75th days after sowing, as well as between 75th and 90th days. The following formula was used to compute the growth rate, which was given in centimetres per day.

$$\text{Growth rate} = \frac{W_2 - W_1}{T}$$

W_1= first measurement; W_2 = second measurement; T = time interval between W1 and W

Plant height (cm): The ecological strategy of a plant species is determined by its height. Because of relationships between plant height and features such as leaf mass fraction, leaf area ratio, leaf nitrogen per area, leaf mass per area, and canopy size, which are all yield indices, it is a primary predictor of a plant's capacity to compete for light (Moles *et al.,* 2009). Plant height was determined by measuring the length of the longest branch from the base of the plant to the tip of the largest glossy leaf. The measurements were taken on the 30th, 60th,75th and 90th days after sowing and were expressed in centimetres (Bhuvana *et al*., 2020b).

Statistical Analysis for combining ability studies: The data recorded for F_1 progenies were subjected to the following statistical analysis. Completely randomized design was used to estimate the variance among the crosses (Kirk, 1995). The methodology outlined by Griffing (1956a) was used to quantify the variance related to parents' general combining ability (GCA) and F_1 progenies' specific combining ability (SCA). The GCA and SCA values were calculated using TNAU stat software.

Results and Discussion

To estimate the overall ability of crosses, the mean performance of sixteen F1 progenies were done. The survivability was highest in treatment T9 (62.027%), which was on par with T_4 (61.341%), T_3 (60.001%), T_6 (60.666%), T_{12} (57.412%) and T_{13} (59.312%) treatments. Cross T_{10} (47.869%) showed the least survivability which was on par with T_{14} (49.023%),T_5 (50.184%), T_7 (51.354%) and T_8 (51.943%) treatments. The plant height at 60 DAS was maximum in the crosses *viz*. T_{10}, T_{11} and T_{15} (Table 2). T_{10} (11.50 cm) followed by T_{11} (11.40 cm) and T_{15} (11.10 cm), which were on par with each other whereas the poorest performance were recorded in crosses T_{12} (7.40 cm), T_4 (7.50 cm), T_3 (7.90 cm) and T_{13} (8.20 cm). However, the maximum plant height at 75 DAS was recorded in the cross T_5 (16.20 cm) and was on par with T_1 (15.40 cm), T_8 (14.70 cm), T_{10} (15.90 cm) and T_{15} (16.00 cm). The least performance was observed in cross T_3 (12.30 cm). Plant height at 90 DAS was maximum for T_1 treatment (22.10 cm) and minimum (18.20 cm) in T_{13}. The mean performance of the sixteen F1 progenies are shown in Table 2. Cross T_5 (0.500 cm per day) recorded the highest growth rate at 60-75 days interval and treatment T_6 (0.166 cm per day) showed the least growth rate which was on par with treatment T_{11} (0.186 cm per day). Whereas the growth rate at 75-90 days interval was highest in treatment T_4

Table 2: Mean performance of 16 F1 crosses for growth traits

Treatment	Crosses	Survivability (%)	Plant height at 60 DAS (cm)	Plant height at 75 DAS (cm)	Plant height at 90 DAS (cm)	Growth rate at 60-75 days interval (cm/day)	Growth rate at 75-90 days interval (cm/day)
T_1	V1×V1	53.724^{cde}	10.40^{b}	15.40^{ab}	22.10^{a}	0.333^{ad}	0.446^{d}
T_2	V1 ×G4	50.767^{def}	9.50^{c}	14.40^{bc}	20.30^{bc}	0.326^{d}	0.393^{e}
T_3	V1×S36	60.001^{ab}	7.90^{fg}	12.30^{g}	19.90^{bcd}	0.293^{e}	0.506^{ab}
T_4	V1×MR2	61.341^{a}	7.50^{g}	13.60^{cdef}	21.70^{a}	0.406^{bc}	0.540^{a}
T_5	G4×V1	50.184^{ef}	8.70^{cdef}	16.20^{a}	20.70^{abc}	0.500^{a}	0.300^{f}
T_6	G4×G4	60.666^{a}	10.50^{b}	12.90^{eg}	18.80^{cde}	0.166^{f}	0.393^{e}
T_7	G4×S36	51.354^{def}	8.80^{cde}	13.50^{cdef}	19.10^{cde}	0.313^{de}	0.373^{e}
T_8	G4×MR2	51.943^{def}	8.50^{def}	14.70^{ab}	21.50^{ab}	0.413^{b}	0.453^{cd}
T_9	S36×V1	62.027^{a}	9.20^{cd}	15.5b^{c}	18.30^{de}	0.420^{b}	0.186^{g}
T_{10}	S36×G4	47.869^{f}	11.50^{a}	15.90^{a}	19.10^{cde}	0.293^{e}	0.213^{g}
T_{11}	S36×S36	53.130^{cde}	11.40^{a}	14.20^{cd}	18.40^{de}	0.186^{f}	0.280^{f}
T_{12}	S63×MR2	57.417^{abc}	7.40^{g}	13.10^{defg}	19.70^{bcde}	0.384^{c}	0.440^{d}
T_{13}	MR2×V1	59.342^{ab}	8.20^{efg}	12.50^{fg}	18.20^{e}	0.286^{e}	0.380^{e}
T_{14}	MR2×G4	49.023^{ef}	9.50^{c}	14.50^{bc}	20.50^{abc}	0.333^{d}	0.400^{e}
T_{15}	MR2×S36	55.550^{bcd}	11.10^{ab}	16.0^{a}	22.00^{a}	0.3206^{d}	0.400^{e}
T_{16}	MR2×S36	60.666^{a}	9.00^{cde}	13.90^{cde}	21.20^{ab}	0.320^{6d}	0.486^{bc}
SEd		2.380	0.4184	0.54	0.79	0.014	0.019
CD (0.05)		4.848	0.8523	1.12	1.62	0.029	0.039
Fvalue		*	*	*	*	*	*

*Significant at 5%

(0.540 cm per day) followed by treatment T_3 (0.506 cm per day) and the least was recorded by treatment T_9 (0.186 cm per day). The results showed the traits studied were significant at 5% as supported by Hermann (2007).

A combined analysis of variance was performed to estimate the variability for characters among the parents, their F_1 direct and F_1 reciprocals. ANOVA for mulberry through diallel is given in Table 3. The variance for combining ability indicated that GCA effects were highly significant ($p<0.001$) for survivability (0.074), plant height at 60 DAS (2.184), 75 DAS (0.178) and 90 DAS (3.003), growth rate 60-75 days interval (0.007), 75-90 days interval (0.019), SCA was highly significant ($p < 0.001$) for all the traits, except plant height at 90 DAS (1.315), which was highly significant at $p < 0.01$. Reciprocal effects (RCA) for survivability, plant height and growth rate were highly significant at $p< 0.001$. The findings reveal that there are chances to analyse combining ability for recognizing the good combiners and pledging hybrids for growth and survivability traits (Dos *et al.,* 2016). Similarly, Chakrabarty *et al.* (2019). He stated that the variations between the genotypes are mandatory for all breeding programmes.

A low GCA with either positive or negative values indicates a small difference between the mean of one parent in crossing with the other from the general mean of the crosses. Conversely, a high GCA value indicated that the parental mean is better or worse than the general mean (Yao *et al.,* 2011). This provides strong evidence of advantageous gene flow from parents to offsprings at a high level and provides details on the concentration of predominantly additive genes (Aslam *et al.,* 2015). High GCA also indicated higher heredity with fewer environmental effect. Less gene interaction and higher success rates in selection may potentially be the outcomes (Bassuony *et al.,* 2021). The estimates of GCA effects of parents for the traits are given in Table 4.

The GCA effect for survivability ranged from -0.094(V1) to 0-106 (MR2) with no significant difference among all the four parents. Plant height at 60 DAS was significant and highest positive GCA effect was recoded for S36 (0.519), while V1 (-0.344) exhibited highly negative significance (Table 4). Four parents showed no significant difference for plant height at 75 DAS. For plant height at 90 DAS, MR2 (0.656) exhibited highest and positive significance, while S36 (-0.731) exhibited a negative significant. Results of the present study showed a small variation in the height of the seedlings during 60 to 90 days after sowing, which was in conformity with the findings of Prasad *et al.* (1992) whose findings showed maximum difference in the height of the seedlings before 60 DAS, as photosynthetic rate is higher during first 60 DAS (Rukmangada *et al.,* 2020).

Growth rate during first year ranged between 10 and 30 cm reported by Suresh (2020). Highest significant estimates of GCA for growth rate at 60-75 days interval was positive for V1 (0.031) and negative for G4 (-0.019). Estimates of GCA ranged from 0.061 (MR2) to-0.052 (S36) for growth rate at 75-90 days

Table 3: Analysis of Variance for Combining Ability

Source	Degree of freedom	Survivability (%)	Plant height at 60 DAS (cm)	Plant height at 75 DAS (cm)	Plant height at 90 DAS (cm)	Growth rate at 60–75 days interval (cm/day)	Growth rate at 75-90 days interval (cm/day)
GCA	3	0.074 ***	2.184***	0.178***	3.003***	0.007***	0.019 ***
SCA	6	0.871 ***	1.272***	1.834***	1.315**	0.008***	0.0013***
RCA	6	0.015***	2.066***	1.987***	1.772***	0.006***	0.014***
ERR	30	0.043	0.092	0.150	0.339	0.0001	0.0002

*** significant at 0.1% level(p< 0.001); ** significant at 1% level (p< 0.01); *significant at 5% level(p< 0.05);significant at 10% level (p<0.1)

Table 4: Estimation of General Combining Ability effects for Parents

Progenitor	Survivability Percent	Plant height at 60DAS (cm)	Plant height at 75 DAS (cm)	Plant height at 90 DAS (cm)	Growth rate at 60–75 days interval (cm/day)	Growth rate at 75-90 days interval (cm/day)
P1-(V1)	-0.094	-0.344**	0.012	0.319	0.031**	0.013**
P2 – (G4)	-0.069	0.369**	0.200	-0.244	-0.019**	-0.022**
P3 – (S36)	0.056	0.519**	-0.062	-0.731**	-0.031**	-0.052**
P4–(MR2)	0.106	-0.544**	-0.150	0.656**	0.019**	0.061**
SEd	0.104	0.152	0.194	0.291	0.005	0.007

*** significantat 0.1% level (p<0.001); ** significant at 1% level (p<0.01); *significant at 5% level (p<0.05); significant at 10% level (p<0.1)

which was highest significant positive and negative. When the increase or decrease in the growth rate is considered MR2 showed the increased trend from 0.019 at 60-75 days interval to 0.061 at 75- 90 days interval which was highly significant positive. The results corroborates with the findings of Christie *et al.* (2010). It shows that, during 75-90 days interval the maximum variation in the growth occurred. This increase in growth rate may be due to high photosynthetic rate and increased plant height during first 60 DAS. The parent MR2 can be included in the crossing programme to improve the growth rate. These results are in line with the findings of Bhuvana *et al.* (2022).

If the cross shows a greater deviation from the general mean, it has higher SCA and is good for the breeding programmes. According to the report by Thorat *et al.* (2017), good pairs are obtained using the SCA estimate, which results in the generation of useful hybrids (Ahmad *et al.,* 2011). Observations of the performance of different crossings have been used to draw conclusions regarding gene action (Table 5). The results indicated that minimum one parent with good combining ability was required to produce superior hybrids. The results align with the findings of El-Rawy *et al.* (2014) and Aslam *et al.* (2017). The results exhibited slight variations in the survivability, which is similar to the findings of Ljubicic *et al.* (2017). Among the crosses, T7 having highest positive SCA (0.756) indicated that this particular combination can grow well by adopting itself. T8 had significantly high negative SCA (-0.594) which was not suitable for survivability traits. No positive significant difference in SCA values for plant height at 60 DAS was recorded, however, the highest negative significant SCA was recorded in T14 (-0.500). Variation in plant height was observed at 75 DAS which increased at 90 DAS. Plant height at 75 DAS showed highly significant positive SCA effect for T2 (0.825), while T3 (-0.863) showed highly significant negative SCA. At 90 DAS, T13 exhibited a highly positive and significant SCA value of 1.750 for plant height. These findings are in accordance with the report of Bhuvana *et al.* (2020b) and Sapna *et al.* (2022). T13- MR2×V1 had highest SCA (1.750) effect for plant height at 90 DAS. This high SCA was manifested by good × poor (MR2×V1) general combiner parents. The positive additive effects of good general combining parent MR2, and the epistatic impact of poor general combiner satisfies this favourable plant characteristics. Growth rate, an imperative character is closely associated with plant height, as plant height is a major determinant of a plant's ability to compete for light (Moles *et al.,* 2009).

All the crosses showed wide variation for growth rate at both 60-75 and 75-90 days. Among the crosses, growth rate at 60-75 days interval was highly significant, positive SCA for T2 (0.069) and T4 exhibited highest negatively significant SCA (-0.035). Growth rate at 75-90 days interval for T9-S36 × V1 showed highly positive significant SCA (0.160) followed by MR2 × V1 (0.080). V1 (0.013) had good GCA for growth rate but GCA for S36 (-0.052) was negatively significant (Table 5). This high SCA effect for growth rate may be due to dominant effect of good combiner (V1), however, S36 × V1 showed non- significant SCA for plant height, both S36 and V1 had negative and non-significant GCA, resulting in non-significant SCA for plant height. This result is contrary to the above statement where plant height was closely associated with growth rate and in line with the findings Qulmamatora *et al.* (2022).

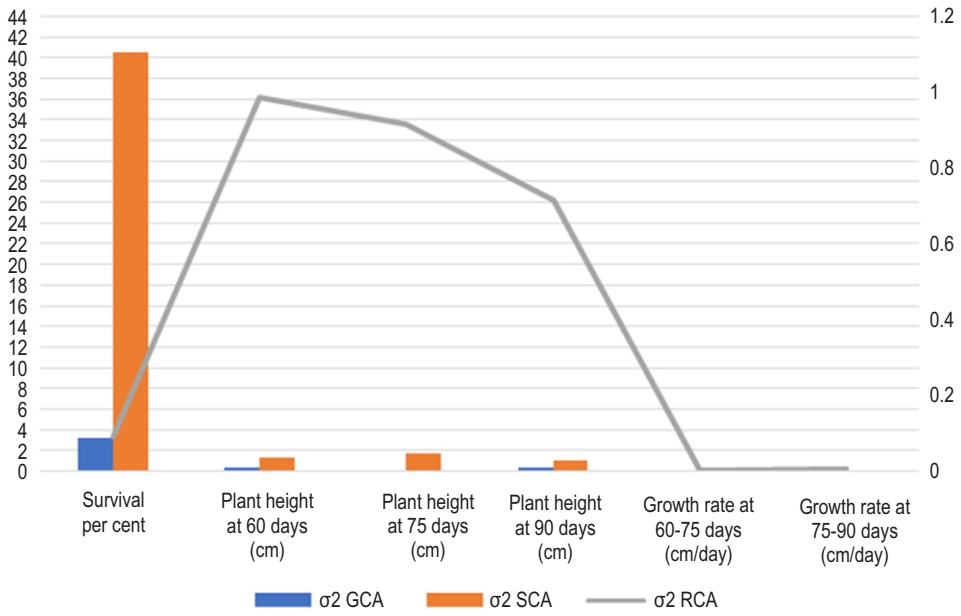

Fig. 1: Estimation of genetic components of parents.

Table 5: Estimation of Specific Combining Ability effects for F_1 progenies

Crosses	Survivability (%)	Plant height at 60DAS (cm)	Plant height at 75 DAS (cm)	Plant height at 90 DAS (cm)	Growth rate at 60-75 days interval (cm/day)	Growth rate at 75-90 days interval (cm/day)
V1×G4	-0.144	-0.244	0.825**	0.331	0.069**	-0.031**
V1×S36	-0.818**	-0.944**	-0.863**	-0.581	0.025**	-0.001
V1×MR2	0.031	-0.581**	-1.075**	-1.119**	-0.035**	-0.001
G4×V1	0.050	0.399	-0.900**	-0.200	-0.087**	0.047**
G4×S36	0.756**	-0.056	0.300	-0.019	0.022**	0.079**
G4×MR2	-0.594**	-0.144	0.638**	0.494	0.042**	0.026*
S36×V1	-0.100	-0.649**	-1.050**	0.800	-0.064**	0.160**
S36×G4	0.100	-1.349**	-1.200**	-0.0002	0.009	0.079**
S63×MR2	0.631**	-0.044	0.500*	0.831*	0.035**	0.024**
MR2×V1	0.100	-0.349	0.550	1.750**	0.060	0.080**
MR2×G4	-0.100	-0.500*	0.450	0.500	0.040**	0.026*
MR2×S36	0.050	-1.850**	-1.450**	-1.149**	0.027**	0.020
Sed	0.233	0.339	0.433	0.651	0.0113	0.016

**significant at1% level (p< 0.01);*significant at 5% level (p<0.05)

In the MR2 × V1 cross, both parents are strong general combiners for growth rate, resulting in a high SCA of 0.800. Additionally, MR2 exhibited a highly significant GCA for plant height, leading to a high SCA of 1.750 for plant height at 90 DAS in the MR2×V1 cross as well. Therefore, MR2 × V1 was considered as the best cross for plant height and growth rate. The larger SCA variation (40.603) over GCA variance (3.123) (Fig. 1) for trait survival demonstrated the dominant role of non-additive gene action (Yao *et al.,* 2011). At 60 DAS, SCA variance (1.180) was observed with high value over GCA variance (0.261) for plant height. The ratio for plant height at 75 DAS was 0.002, which was less than one. SCA variance (0.975) at 90 DAS was greater than GCA variance (0.333), and the GCA to SCA ratio was less than unity. The combining ability variances are given in the Fig. 1. The GCA variance (0.0008) at 60-75 days interval for growth rate was lower than SCA variance (0.0082). The GCA variance (0.002) for growth rate at 75-90 days interval was higher over SCA variance (0.001). The GCA to SCA ratio was greater than one (2.0499). The collective analysis of variance indicated that the progenies displayed considerable and adequate variation among

Table 6: Ratio of variances

Traits	σ2GCA/σ2SCA
Survival percent	0.077
Plant height at 60 days (cm)	0.221
Plant height at 75 days (cm)	0.002
Plant height at 90 days (cm)	0.341
Growth rate at 60-75 days (cm/day)	0.105
Growth rate at 75-90 days (cm/day)	2.045

themselves. (Moles *et al.,* 2009). Except for trait growth rate at 75-90 days interval, all the other traits recorded SCA variance greater than GCA variance and their ratio was less than unity (Table 6). This indicated that most of the characters in mulberry were governed by non-additive genes. Since there were enough genetic variations among the genotypes, it was possible to quantify the combining ability effects (Dos *et al.,* 2016).

The study found that among the four parents, V1 and MR2 had highly significant positive GCA effects for various traits. V1 showed the highest positive GCA for growth rate at 60-75 days, while MR2 excelled in survival percentage, plant height at 90 DAS, and growth rate at 75-90 days. These two varieties were identified as the best genotypes for growth traits and are suitable for mulberry hybrid development (Bassuony *et al.*, 2021).SCA estimates showed that crosses V1×G4 (plant height, growth rate) and its reciprocal G4×V1 (growth rate at 75-90 days) had significant SCA effects. Other crosses, such as S36×V1 (growth rate) and MR2×V1 (plant height), also showed high positive SCA. All high SCA crosses had V1 as the parent with the best GCA. These combinations can be used for breeding specific traits. The results align with Aslam *et al.* (2017), and high RCA indicates maternal inheritance, as noted by Christie *et al.* (2010).

The analysis showed significant genetic variation among mulberry genotypes, with non-additive gene effects dominating most traits, suggesting greater improvement in advanced generations. The best survivability was seen in the V1×G4 combination and its reciprocal (G4×V1), followed by MR2×V1. V1 and MR2 were identified as top combiners. These crosses are recommended for genetic enhancement and cultivar development. Reciprocal effects highlight the importance of maternal inheritance, stressing careful selection of both parents.

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