# Evaluation of the Combining Ability in Mulberry (*Morus* spp.)

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***Authors' contributions***

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## ABSTRACT

The research aimed to comprehend the patterns of gene action influencing the inheritance and expression of quantitative features in mulberry by studying combing ability. Four parents *viz.*, V1, G4, MR2 and S36 were used in mating design. Estimates of SCA depicted that crosses V1 × G4 (shoot diameter and chlorophyll - b) and it's reciprocal cross G4 × V1 (chlorophyll – a, total chlorophyll) showed high significant SCA effects. V1 × MR2 for shoot diameter and lowest positive SCA for internodal distance. Overall, the crosses with the highest SCA values for specific traits consistently involved V1 as one of the parents. This observation highlights that V1, which possesses the best general combining ability (GCA) effect, contributes significantly to the high SCA observed in these crosses for the traits studied. V1 and MR2 were the best general combiners, with high GCA for growth traits. Crosses G4×V1 and V1×G4 demonstrated positive SCA effects for most traits followed by S36 × V1 and MR2 × V1. Notably, SCA variance was greater than GCA variance for most traits, indicating the dominance of non-additive gene effects.

## 1. INTRODUCTION

Sericulture is the practice of growing mulberry trees and rearing silkworms to produce silk, a significant source of employment in India and other Asian countries [1]. India is distinct in its ability to produces all four types of silk *viz.*, mulberry, eri, tasar and muga. Among these, mulberry silk is the most prominent, making the quality and quantity of mulberry leaves essential for feeding silkworms and, thereby, for maximizing cocoon yield [2].

Since the 1960s, India has focused on developing improved mulberry cultivars to enhance sericulture [3]. Identifying the best parent lines for breeding remains challenging, as relying solely on phenotypic traits is unreliable due to random genetic gains [4]. Instead, evaluating combining ability—General Combining Ability (GCA) and Specific Combining Ability (SCA) —(remove this) is more effective. GCA assesses average performance in hybrids, while SCA evaluates specific cross performance [5]. Diallel crosses, as developed by Sprague and Tatum [6] and refined by Hayman [7] and Griffing [8], are used to assess combining ability. Griffing's methods, particularly Model-I and Method-1 help in selecting superior parents and hybrids, requiring at least four parents for a thorough analysis [9].

## 2. MATERIALS AND METHODS

The research was carried out during the kharif and rabi seasons at the Department of Sericulture, Forest College and Research Institute, Mettupalayam for studying Combining ability in Mulberry.

**Materials used:** Four genotypes, Polybags, Butter paper bags, tags, thread, needle, petri plate, paint brush for dusting pollen.

## 2.1 Parental Selection

Selecting suitable parents for crossing is crucial for a successful hybridization program. This process involves identifying genotypes with promising and desirable agronomic traits for plant breeding [10]. The four parents -V1, G4, S36, and MR2 were selected and are maintained at the Department of Sericulture, Forest College and Research Institute, Mettupalayam.

## 2.2 Experimental Design

### 2.2.1 Mating design

In plant breeding, three key biometrical methods - diallel, partial diallel, and line tester analysis are commonly used for parent selection. Diallel analysis is particularly efficient for gathering extensive data on the genetic contributions of

parent plants by focusing on the parents and their F1 offspring. Chaudhary [11] notes that diallel crosses are widely utilized to assess the value of parent lines and analyse gene action across different traits.

**Diallel mating design - 4× 4**

**Griffing's approach – Model I & Method I**

**Method I** – Method I involves including all possible crosses as well as the parent lines.

**Model I** – Fixed Effect Model - This model involves using a specific set of fixed genotypes and varieties in the experiment. These genotypes are treated as a population, and conclusions are drawn about each individual variety within this set.

**Complete diallel analysis with parents:**

> Count of cross obtained: $P^2$ =16 (Direct, Parents and Reciprocal crosses)

$p^2$ combinations categorized into three groups

(1) the $p$ parental lines themselves,
(2) one set of $\frac{1}{2}p(p-1)$ F$_1$ hybrids,
(3) the set of $\frac{1}{2}p(p-1)$ reciprocal F$_1$ hybrids [12].

## 2.3 Working Procedure (Hybridization programme)

Cuttings from selected male and female parent plants were collected from well-established specimens in December. To synchronize flowering, all plants were pruned 45 days after planting. Catkin development was noted 10 days following the pruning.

### 2.3.1 Bagging

Female catkins with immature flower buds were enclosed in 15 x 10 cm butter paper bags, with holes made for air circulation. Branch tops were trimmed to prevent bag rupture from stem growth. Catkins of similar size and age were bagged together for uniform hybridization. Male catkins were also bagged to ensure pollen purity and prevent contamination from other plants [13].

### 2.3.2 Crossing

#### 2.3.2.1 Pollen collection

Manual pollination involved gathering pollen from male catkins. With the Peak Period of Anther

dehiscence (PPA) occurring between 10:00 AM and 11:30 AM and 3:00 PM and 4:00 PM, anther dehiscence in Morus sp. was diurnal, occurring throughout the day and lasting only one to four days [14]. In order to collect pollen sterile petri plant and paint brush were used to.

#### 2.3.2.2 Pollination

In order to achieve greater seed setting and higher seed germination, pollination was started on the tenth day after the female catkin emerged. The process of pollination was carried out until the fourteenth day of emergence to guarantee competent mating. To avoid accidental cross-pollination and protect the catkins from external factors, they were bagged as soon as they crossed each day. The white stigma becomes brownish in colour and eventually dries up after the female flowers have been fertilized [15].

#### 2.3.2.3 Tagging

Once the catkins were encased, they were marked using rectangular tags measuring 3 × 2 cm. The tags beneath the catkin were fastened with thread. The following details were on the tags.

✓ Date of pollination
✓ Specifics of the cross (name of the Female parent × Male parent)

#### 2.3.2.4 Collection of fruits

The fruits (sorosis) started to ripen after 19–23 days of pollination. The green turned to a reddish-black hue. The ripened sorosis from each of the chosen mother plants was carefully collected, and each cross's seeds were taken out separately and without blending.

#### 2.3.2.5 Seed extraction

The mature or ripened fruits were gathered and allowed to soak in water for a whole day in order to soften them. After the berries were mashed and their water removed, the seeds became visible. After adding water to the mashed berries, the sunken and floating seeds were identified. Using a flotation test, viable (yellowish brown) and non-viable seeds were separated [16]. Fruits were stored in a cold room if the seeds could not be extracted right away after harvest. After being treated with 1000 ppm GA3 for the entire night, the seeds were sown in seedling trays [17].

## 2.4 Design of Experiment

The seeds were planted in poly bags. A mixture of fine soil, vermiculite, and farm yard manure (1:1:1) was used to fill poly bags. Watering and all other intercultural procedures were carried out on a regular basis. There were three replications kept, each with five plants. A completely randomized design (CRD) was employed in the evaluation of the F1 progenies (16).

## 2.5 Data collection

Observations pertaining to mulberry growth and survivability were recorded on the 45, 60, 75 and 90 days after sowing.

### 2.5.1 Number of leaves per plant

Number of leaves on the seedlings per plant were counted manually.

### 2.5.2 Internodal distance (cm)

The distance between two plant nodes was measured in centimetres with the help of scale and expressed in cm.

### 2.5.3 Single leaf area (cm$^2$)

In plant growth analysis and photosynthesis, leaf area is very essential. It was estimated using the factor technique and given in cm$^2$.

Single leaf area = L×B×0.69

Where,

L = Length
B = Breadth

### 2.5.4 Chlorophyll estimation (mg/g)

The chlorophyll content in leaves was assessed by the method of Arnon [18], chlorophyll was extracted with 80% acetone and the absorbance was read at 663 nm and 645 nm in spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated [19].

## 2.6 Statistical Analysis

The data recorded for F$_1$ progenies were subjected to the following statistical analysis.

### 2.6.1 ANOVA

To estimate the variance among the crosses Completely randomized design was used.

### 2.6.2 Combining ability analysis

The methodology outlined by Griffing [12] was used to quantify the variance related to parents' general combining ability (GCA) and F1 progenies' specific combining ability (SCA). TNAU stat software was used to determine the GCA and SCA values.

## 3. RESULTS AND DISCUSSION

### 3.1 Mean Performance of the Sixteen F1 Progenies

For shoot diameter the crosses T4 (2.01 cm), T5 (1.97 cm), and T7 (1.90 cm) were on par with each other. Among the crosses T4 showed best performance and the cross T1 (0.54cm) had the least performance (Table 1).

Number of leaves per plant showed no much difference among all the crosses. The highest internodal distance was recorded in cross T2 (4.85 cm) which differed significantly from all other crosses, and the poorest internodal distance was recorded in T13 (0.50 cm). The single leaf area was highest in the cross T11 (9.715cm$^2$), which recorded a significant variation. While T15 (0.828 cm$^2$) recorded the least single leaf area which was on par with T10 (0.883 cm$^2$) and T14 (0.966 cm$^2$).

The cross T12 (2.08 mg/g) recorded highest chlorophyll-a content, while the crosses T13 (1.03 mg/g), T10 (2.44 mg/g) recorded the highest for chlorophyll-b and total chlorophyll content respectively. T5 (1.63 mg/g) for chlorophyll-a, T11 (0.74 mg/g) for chlorophyll-b, and T8 (1.88 mg/g) for total chlorophyll recorded the least. The results revealed the presence of significant differences among the progenies and progenitors. Similar results were reported by Bhuvana [20] and Ghosh [21]. All the 16 progenies exhibited wide variations for all the studied characters, except for number of leaves per plant. This character showed no variation, which indicated that the seedlings does not show any larger variation for number of leaves per plant until 90 days form sowing.

### 3.2 Anova for Combining Ability

A combined analysis of variance was performed on the data for growth traits to estimate the amount of variability for these characters among the parents, their F$_1$ direct and F$_1$ reciprocals. Analysis of variance for diallel cross of mulberry

**Table 1. Mean performance of the 16 $F_1$ crosses for growth traits**

| Treatments | Crosses | Shoot diameter(mm) | Number of leaves/plant | Internodal distance (cm) | Singleleaf area ($cm^2$) | Chlorophyll-a (mg/g) | Chlorophyll-b (mg/g) | Total Chlorophyll (mg/g) |
|---|---|---|---|---|---|---|---|---|
| T1 | V1×V1 | 0.54$^f$ | 7.00 | 0.70$^f$ | 5.630$^d$ | 1.85$^{de}$ | 0.76$^{de}$ | 2.12$^{bc}$ |
| T2 | V1×G4 | 1.83$^b$ | 6.00 | 4.85$^a$ | 4.705$^{ef}$ | 2.01$^{abcd}$ | 0.81$^{bcde}$ | 2.40$^a$ |
| T3 | V1×S36 | 1.24$^e$ | 8.00 | 0.70$^f$ | 4.485$^f$ | 1.99$^{abcd}$ | 0.75$^{de}$ | 1.92$^d$ |
| T4 | V1×MR2 | 2.01$^a$ | 8.00 | 0.65$^{fg}$ | 5.030$^e$ | 2.05$^{ab}$ | 0.79$^{cde}$ | 2.18$^b$ |
| T5 | G4 × V1 | 1.97$^a$ | 8.00 | 0.55$^{gh}$ | 3.049$^g$ | 1.63$^g$ | 1.01$^a$ | 2.00$^{cd}$ |
| T6 | G4 ×G4 | 1.79$^{abc}$ | 7.00 | 1.05$^d$ | 3.015$^g$ | 1.97$^{abcd}$ | 0.85$^{bc}$ | 2.10$^{bc}$ |
| T7 | G4 × S36 | 1.90$^b$ | 6.00 | 0.65$^{fg}$ | 7.728$^b$ | 2.04$^{ab}$ | 0.99$^a$ | 1.90$^d$ |
| T8 | G4× MR2 | 1.36$^{de}$ | 7.00 | 0.90$^e$ | 5.554$^d$ | 2.10$^a$ | 1.00$^a$ | 1.88$^d$ |
| T9 | S36 × V1 | 1.45$^d$ | 8.00 | 1.60$^c$ | 1.035$^{ef}$ | 1.70$^{fg}$ | 0.77$^{de}$ | 2.14$^{bc}$ |
| T10 | S36 × G4 | 1.77$^{bc}$ | 9.00 | 0.90$^e$ | 0.883$^i$ | 1.69$^{fg}$ | 0.82$^{bcd}$ | 2.44$^a$ |
| T11 | S36 ×S36 | 1.54$^{cd}$ | 7.00 | 0.60$^{fgh}$ | 9.715$^a$ | 1.74$^{efg}$ | 0.74$^e$ | 1.97$^{cd}$ |
| T12 | S63×MR2 | 1.62$^c$ | 7.00 | 0.95$^{de}$ | 6.099$^c$ | 2.08$^a$ | 0.79$^{cde}$ | 2.18$^b$ |
| T13 | MR2× V1 | 1.84$^b$ | 7.00 | 0.50$^h$ | 4.830$^{ef}$ | 1.88$^{cde}$ | 1.03$^a$ | 1.99$^{cd}$ |
| T14 | MR2× G4 | 1.75$^{bc}$ | 8.00 | 1.05$^d$ | 0.966$^i$ | 1.90$^{bcde}$ | 0.87$^b$ | 2.01$^{bcd}$ |
| T15 | MR2×S36 | 1.66$^{bc}$ | 8.00 | 0.60$^{fgh}$ | 0.828$^{gi}$ | 1.75$^{fg}$ | 0.98$^a$ | 1.90$^d$ |
| T16 | MR2×S36 | 1.44$^d$ | 7.00 | 2.80$^b$ | 2.484$^{gh}$ | 1.94$^{abcd}$ | 1.00$^a$ | 1.98$^{cd}$ |
| **Sed** | | 0.07 | 0.81 | 0.05 | 0.180 | 0.08 | 0.03 | 0.08 |
| **CD (0.05)** | | 0.14 | 1.66 | 0.10 | 0.368 | 0.16 | 0.07 | 0.17 |
| **F value** | | * | Ns | * | * | * | * | * |

* Significant at 5%

**Table 2. Analysis of Variance for Combining Ability**

| Source | Df | Shoot diameter(mm) | Number of leaves/plant | Internodal distance (cm) | Single leafarea (cm$^2$) | Chlorophyll-a (mg/g) | Chlorophyll-b (mg/g) | Total Chlorophyll (mg/g) |
|--------|----|----|----|----|----|----|----|----|
| GCA | 3 | 0.161** | 0.028 | 0.491*** | 7.231*** | 0.020** | 0.022*** | 0.017** |
| SCA | 6 | 0.224*** | 1.458 | 1.305*** | 3.170*** | 0.004 | 0.004*** | 0.010* |
| RCA | 6 | 0.022*** | 0.250 | 1.627*** | 8.269*** | 0.044 *** | 0.015*** | 0.053*** |
| ERR | 30 | 0.003 | 1.693 | 0.0015 | 0.0175 | 0.0032 | 0.0006 | 0.0036 |

*** 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)*

is presented in Table 2. The variance for combining ability indicated that general combining ability (GCA) effects were very highly significant ($p < 0.001$) for the traits internodal distance (0.491), single leaf area (7.231), and chlorophyll – b (0.022). Whereas GCA for shoot diameter, chlorophyll - a and total chlorophyll were highly significant (p < 0.01).

The SCA effect for total chlorophyll (0.010) was significant at $p < 0.05$ and chlorophyll - a was non- significant (Table 2). The mean sum of squares for the reciprocals also showed highly significant differences for all the traits. Number of leaves per plant was non- significant for GCA and SCA where as it was little significant for reciprocal combining ability (RCA) at $p < 0.1$. The findings have revealed that there is a chance to analyse combining ability for recognizing the good combiners and pledging hybrids for growth and survivability traits. Results were similarly reported by Chakrabarty [22], except for number of leaves which also showed significant variation. He stated that the variations between the genotypes are a must for all breeding programmes.

## 3.3 Combining Ability Analysis

### 3.3.1 Estimation of general combining ability effects of parents

The estimates of GCA effects of parents for the characters studied were given in Table 3. It is more preferable to choose parents based on the effectiveness of their off-springs than to choose them based on per se performance Fasahat [23]. A low GCA with either positive or negative values suggests that there is not much of a difference between the overall mean of the crosses and the mean of one parent when crossing with the other. A high GCA number, on the other hand, suggested that the parental mean and the overall mean differed. This gives information on the concentration of primarily additive genes as well as compelling evidence of favourable gene flow from parents to offspring at a high level. Elevated GCA additionally suggested increased heredity with reduced environmental impact. The results could be less gene interaction and increased selection success rates.

**Growth traits:** Shoot diameter G4 (0.163) exhibited positive and significant GCA and V1 (-0.179) exhibited significantly negative GCA (Table 3) which was not in accordance with Bhuvana [20] studies, where V1 had highest

positive GCA for shoot diameter. No significant difference was exhibited by all the parents for number of leaves per plant (Table 3).

G4 exhibited highly positive and significant value of 0.184 cm for internodal distance and high negative significant GCA effect was exhibited by S36 (-0.366 cm). The negative significant GCA show that S36 parent had less internodal distance. Internodal distance and number of leaves per plant are correlated negatively with each other [24]. The short internodal distance would increase the number of leaves per unit length of the shoot. GCA for internodal distance is higher for parent G4(0.184). However, G4 showed the least non-significant GCA for the quantity of leaves per plant, which was consistent with the findings of the previous study. Parents V1 and MR2 can be the best combiners for short internodal distance.

GCA estimates for chlorophyll content are given in Table 3. The highest GCA effect for chlorophyll-a was recorded by the parent MR2 (0.060) and significantly negative GCA effect was exhibited by S36 (-0.054). MR2 recorded highest and significant positive GCA effect (0.060) for chlorophyll-b, while highest negative and significant effect was recorded by V1 (-0.037). V1 (0.039) recorded highest total chlorophyll content and negative significance value was shown by MR2 (-0.057).

The most important quantitative attribute is leaf area and the largest individual leaf area was strongly correlated with shoot biomass and leaf production. It serves as a predictor variable for figuring out how much chlorophyll content is present in the leaves. GCA values ranged from -0.818 (MR2) to 1.158 (S36) for single leaf area. Among the four parents S36 showed highly significant and positive GCA effect (1.58), whereas, S36 showed significant negative GCA for chlorophyll-a (-0.054) and chlorophyll-b (0.050). This result was in controversy with the report given by Satoh [25] where LA $_{max}$ was recorded with high concentration of chlorophyll pigments. The parent S36 can be a superior combiner for the trait followed by V1.

A genotype with higher chlorophyll content can produce higher number of leaves due to high photosynthetic rate [26]. According to the above statement, V1 (0.039) had high significant positive GCA for total chlorophyll (Table 3). So V1 can be the superior combiner for leaf quality and quantity traits.

**Table 3. Estimation of General Combining Ability effects for Parents**

| Progenitor | Shoot diameter(mm) | Number of leaves/plant | Internodal distance (cm) | Single leaf area (cm$^2$) | Chlorophyll-a (mg/g) | Chlorophyll-b (mg/g) | Total Chlorophyll (mg/g) |
|---|---|---|---|---|---|---|---|
| **P1- (V1)** | -0.179** | 0.042 | 0.091** | 0.397 ** | -0.025 | -0.037** | 0.039* |
| **P2- (G4)** | 0.163** | 0.000 | 0.184** | -0.737** | 0.019 | 0.027** | 0.034 |
| **P3-(S36)** | -0.017 | 0.042 | -0.366** | 1.158** | -0.054 ** | -0.050** | -0.017 |
| **P4-(MR2)** | 0.033* | -0.083 | 0.091** | -0.818** | 0.060 ** | 0.060** | -0.057** |
| **Sed** | 0.025 | 0.656 | 0.019 | 0.066 | 0.028 | 0.012 | 0.031 |

*\*\*\* 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 Specific Combining Ability effects for F$_1$ progenies**

| Crosses | Shoot diameter(mm) | Number of leaves/plant | Internodal distance (cm) | Single leafarea (cm$^2$) | Chlorophyll-a (mg/g) | Chlorophyll-b (mg/g) | Total Chlorophyll (mg/g) |
|---|---|---|---|---|---|---|---|
| V1 × G4 | 0.309 ** | 0.042 | 1.234 ** | -0.143 | -0.068 * | 0.047** | 0.057 |
| V1 × S36 | -0.066 * | -0.333 | 0.234 ** | -1.321** | 0.029 | -0.025 | -0.662 |
| V1 × MR2 | 0.464 ** | -0.708 | -0.797 ** | 0.988** | 0.035 | 0.015 | 0.033 |
| G4 × V1 | -0.070 | -0.667 | 2.149 ** | 0.828 ** | 0.189 ** | -0.099 ** | 0.200** |
| G4 × S36 | 0.082 ** | 0.875 | -0.234 ** | -0.477** | 0.005 | 0.055 ** | 0.083 * |
| G4 × MR2 | -0.248 ** | 0.333 | -0.491 ** | 0.492** | 0.026 | -0.025 | -0.102 ** |
| S36 × V1 | -0.105 ** | 0.000 | -0.450 ** | -0.110 | 0.145 ** | -0.010 | -0.110 * |
| S36 × G4 | 0.065 | -0.500 | -0.125 ** | 3.422 ** | 0.175** | 0.085 ** | -0.269 ** |
| S63 × MR2 | 0.017 | 0.792 | -0.141 ** | -1.238** | 0.014 | 0.002 | 0.044 |
| MR2 × V1 | 0.085 * | 0.167 | 0.075 * | 0.099 | 0.085 * | -0.120** | 0.095 * |
| MR2 × G4 | -0.195 ** | 0.167 | -0.075 * | 2.332 ** | 0.100 * | 0.065 ** | -0.065 |
| MR2 × S36 | -0.020 | 0.000 | 0.175** | 2.636** | 0.165 ** | -0.095** | 0.140 ** |
| Sed | 0.057 | 0.148 | 0.043 | 0.148 | 0.063 | 0.0279 | 0.071 |

*\*\* significant at 1% level (p< 0.01); \*significant at 5% level (p< 0.05)*

### 3.3.2 Specific combining ability effects of hybrids

SCA refers to the performance of crossings that varies from the average general combining ability of two parental lines [27]. If the cross shows a greater deviation from the general mean, it has higher SCA and is good for the breeding program's further improvement. According to the report by Thorat [28], good pairings are found using the SCA estimate, which results in the generation of useful hybrids. Inference about the gene action have been made based on observations of the performance of various cross patterns.

**Growth traits:** SCA effect was high and positively significant in cross T4 (0.464) followed by T2 (0.309) for the shoot diameter (Table 4) for shoot diameter. Number of leaves per plant showed no significant effect among the 16 crosses. Internodal distance for cross T13 recorded least significant positive SCA (0.075) and T5 (2.149) recorded highest positive SCA for internodal distance.

A genotype with higher chlorophyll content can produce higher number of leaves due to high photosynthetic rate [26]. According to the above statement, V1 (0.039) had high significant positive GCA for total chlorophyll (Table 3). So V1 can be the superior combiner for leaf quality and quantity traits.

Estimates of SCA for single leaf area are given in Table 4. Highest positive and significant value for single leaf area was exhibited by the cross T10 (3.422) and highest negative significant SCA effect was recorded by T7 (-0.477). Among the crosses T5 (0.189) exhibited highest positive and significant SCA effect for chlorophyll – a, while T2 exhibited highly negative and significant SCA (-0.068). The crosses T10 (0.085) recorded highest positive and significant SCA followed by T14 (0.065) for chlorophyll-b. SCA value for total chlorophyll content for T5 (0.200) was highly significant and positive followed by T15 (0.140) and highly significant negative SCA (-0.102) was recorded in the cross T8 (Table 4).

More number of leaves can be produced by the genotype with shorter internodal distance Results depicted that MR2 × G4 (-0.075) had poorest SCA effect for internodal distance and it had highest SCA (0.167) for number of leaves per plant, that showed no variation among all the other crosses for leaves per plant (Table 4). Mulberry is mostly used for its foliage. So single leaf area became a crucial trait in mulberry. Mostly all the crosses showed a significant variation for single leaf area and this variation in genotypes was due to leaf shape, lobation and genetic nature. Among the progenies S36 × G4 (3.422) exhibited highest SCA for single leaf area and significant SCA for both chlorophyll-a (0.175) and chlorophyll- b (0.085). This result was in accordance with Satoh [25] who reported that plants with maximum leaf area had high concentration of chlorophyll pigments.

## 3.4 Combining Ability Variances

The GCA and SCA variances for the studied traits given in Table 5.

Estimates for GCA and SCA variance were (-0.013) and (-0.099), respectively and their ratio was 0.132. The SCA variance was greater than GCA, which shows that number of leaves was governed by non- additive genes. Occurrence of high SCA variance was noticed for internodal distance (1.303) over GCA variance (0.061). The ratio of GCA variance to SCA variance was 0.047 which was less than one. The observed variance of SCA and GCA for leaf area were 3.152 and 0.902, respectively. The ratio of variance was less than unity (0.286).

GCA variance was higher than SCA variance (0.0020 > 0.0009) and their ratio was 2.286, Which shows dominant gene action was involved in controlling the trait chlorophyll – a (Table 5). GCA to SCA variance ratio for chlorophyll-b was 0.874, which was nearly close to one and the magnitudes of GCA and SCA variance were equal (0.003and 0.003). For total chlorophyll, the GCA variation was less than the SCA variance (0.002 < 0.008), and the ratio of GCA to SCA variance was 0.26. Except for trait chlorophyll – a, all the other traits recorded SCA variance greater than GCA variance and GCA to SCA variance ratio was less than unity (Table 5). This depicts many of the traits were governed by non-additive gene action.

Estimates of GCA for parents rendered that parents V1, S36 and MR2 are good combiner for studied traits. V1 shows high significance for single leaf area and significance for total chlorophyll content. Parent G4 showed high significance for shoot diameter, parent S36 significant negative for internodal distance and high significance for single leaf area. MR2 is significant for shoot diameter, highly significant for chlorophyll a, b. These genotypes could be further utilized for the mulberry hybrid development.

**Table 5. Ratio of variances**

| S.No | Traits | $\sigma^2$ GCA | $\sigma^2$SCA | $\sigma^2$RCA | $\sigma^2$GCA/$\sigma^2$SCA |
|------|--------|--------|--------|--------|--------------|
| 1 | Shoot diameter (mm) | 0.020 | 0.222 | 0.007 | 0.089 |
| 2 | Number of leaves /plant | -0.013 | -0.099 | 0.176 | 0.132 |
| 3 | Internodal distance (cm) | 0.061 | 1.303 | 0.813 | 0.047 |
| 4 | Single leaf area (cm$^2$) | 0.902 | 3.152 | 4.125 | 0.286 |
| 5 | Chlorophyll-a (mg/g) | 0.002 | 0.0009 | 0.020 | 2.286 |
| 6 | Chlorophyll-b (mg/) | 0.003 | 0.003 | 0.007 | 0.874 |
| 7 | Total Chlorophyll (mg/g) | 0.002 | 0.007 | 0.017 | 0.266 |

Estimates of SCA depicted that crosses V1 × G4 (shoot diameter and chlorophyll - b) and it's reciprocal cross G4 × V1 (chlorophyll – a, total chlorophyll) showed high significant SCA effects. V1 × MR2 for shoot diameter and lowest positive SCA for internodal distance. All these crosses with highest SCA for particular trait had a parent V1 with best GCA effect. When breeding for specific trait is required the above combinations for those particular traits can be utilized in breeding programme.

## 4. CONCLUSION

The analysis revealed notable genetic variability among the genotypes, with most traits showing a dominance of non-additive gene effects. This indicates that improving these traits will likely be more successful in advanced generations. The crosses V1 × G4 and its reciprocal G4 × V1 demonstrated the best performance for survivability traits, with MR2 × V1 also showing strong results. Both V1 and MR2 were identified as excellent combiners. Therefore, these specific crosses are recommended for genetic enhancement to identify transgressive segregants and to develop pure lines for cultivar release.

## 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.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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