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Genetic studies in mulberry seedlings by diallel analysis

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Abstract
Genus *Morus* includes more than 20 species, some of which are commercially important mulberries with different utilities. Even though these species are propagated by rooting of cuttings in practice, mulberry seeds are of importance for breeding studies and rootstock seedling propagation. For that reason, this study was conducted to know the seed performance for germination and emergence traits of four mulberry varieties *viz,* V1, G4, S36, MR2 using 4× 4 complete diallel mating design. According to the results both non – additive and additive gene actions are important in the inheritance of these traits. Progenitors MR2 and V1 were found to be the best combiners for most of the traits studied.

Keywords: Germination, emergence, diallel, combining ability, additive and non-additive gene action

1. Introduction
Sericulture is an art of cultivation of mulberry and rearing of silkworms to produce silk. Mulberry foliage is the only food for the silkworm (*Bombyx mori*) which is being grown under varied climatic conditions. Mulberry is a fast-growing deciduous perennial plant with deep – root system belonging to the family Moraceae. Plants are generally dioecious, hence cross pollination takes place. Inflorescence is catkin with pendent or drooping peduncle bearing unisexual flowers. The pollination is aided by wind and insects. Fruit is a sorosis and the colour of the fruit is mainly violet black. Most of the species of the genus *Mours* and cultivated varieties are diploid with 28 chromosomes.
Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area has a direct bearing on cocoon harvest. One of the reason for the difference in productivity is due to varied climatic conditions and various problems at different zones. Hence development of improved mulberry varieties with high yield, quality and tolerant to abiotic and biotic stress is necessary.
Though, mulberry is propagated by rooting of cuttings, seeds are important for propagation of rootstocks to be grafted and budded cultivars, especially poor rooting cultivars and for growing hybrid plants in breeding programme (Westwood 1995, Hartmann *et al.*, 1997 [11, 5].) Germination rate and time are two main items that are important for using the seeds for propagation which were influenced by a number of factors both internally and externally (Bewley and Black 1994) [2]. Hence, the present study was designed to assess the germination, emergence traits and the genes involved in their inheritance.

2. Materials and Methods
2.1 Location of the experiment
This study was conducted at the Department of Sericulture, Forest College and Research Institute, Mettupalayam, which comes under western zone of Tamil Nadu. The experimental site is rested at 11.20˚ N latitude, 76.56˚ N longitude at an altitude of 320m above MSL with an annual mean rainfall of 922.0mm. The soil of this zone are Red loamy soils.

2.2 Genetic Materials
Four parental mulberry genotypes *viz.* V1, G4, S36 and MR2 were selected based on desirable characters. The present study examined these four commercial popular varieties in Tamil Nadu, and their progenies. These genetic materials had phenotypic variations for morphological characters and yielding capacity (Table-1). V1 variety is high yielding, G4 variety has high yielding with high rooting ability, MR2 variety is resistant to powdery

mildew. S36 variety is suitable for chawki rearing as the leaves are soft and succulent, tolerant to leaf spot and powdery mildew.

2.3 Development of F1 hybrids and survivability assessment

A 4 × 4 full diallel cross was carried out to generate 16 F1 progenies. The progenies were evaluated in a completely randomized design (CRD) with three replications during the year 2021 - 2022.

2.4 Data collection

As a part of the study, the parameters of germination percent (GP), emergence percent (EP), mean emergence time (MET), seedling fresh weight (SFW), seedling dry weight (SDW) and seedling vigour index (SVI) were evaluated.

Germination percentage (GP %)

The term germination refers to the seeds of protrusion of a root or shoot from the seed coat (Hadas and Russo 1974) [6]. Germination tests were conducted in petri plates using filter paper moistened with water (Fig 1). All the seedlings with the radicle at least 2mm in length were considered as germinated. The seed germination was recorded for 30 days and germination percentages were calculated.

Table 1: Codes, varieties, genetic origin, sex expression, potential yield and salient traits

Code for parents	Variety	Genetic origin	Sex expression	Potential yield (Mt/ha/yr)	Salient traits
P1	V1	Intervarietal hybridization	Mostly male, female	70	High yielding variety, resistant to leaf spot
P2	G4	Interspecific hybridization	Male, female	65	High yielding variety
P3	S36	Mutation	Male, female	38 – 45	Tolerant to leaf spot and mildew
P4	MR2	Phenotypic selection	Male, female, Bisexual	30-35	Resistant to powdery mildew

Emergence percentage (EP %)

Emergence is the visible penetration of the shoot above the soil surface (Benech Arnold *et al.*, 1991). Seedling emergence was recorded after the hypocotyls had risen above the surface of the growing media (Fig 2). In this experiment, the seeds were sown in seedling trays with 20 cavities each and dimensions of 35×35×25 cm, filled with mixture of fine soil, vermiculite and Farm Yard Manure (1:1:1) (Vijayan *et al*., 2010) [9]**.** These seedling trays with seeds were placed under room conditions (minimum 21˚C and maximum 27˚C temperatures). The observations were recorded for 40 days.

Mean Emergence Time (MET - days)

MET is an accurate measure of the time taken for a lot to germinate, but does not correlate this well with the time spread or uniformity of germination. It focusses instead on the day when most germination events occurred (M.A. Kader, 2005) [8]. The mean emergence time (MET) was calculated by the following formula.

$$MET = \frac{\sum(tn)}{\sum n}$$

For the evaluation of seedling performance, seedling fresh weight, seedling dry weight and seedling vigour index were calculated.

Seedling fresh weight (SFW)

It represents the weight of seedlings when they had 2-4 true leaves.

Seedling dry weight (SDW)

After taking the fresh weight seedlings were dried in a hot air oven at 65˚ C until they reached constant weight.

Seedling vigour index (SVI)

Calculated with the help of the following formula

$$SVI = \frac{\text{Emergence percent}}{\text{Seedling dry weight}}$$

As a part of the statistical analysis the percentage of germination and emergence percent were transformed by the angular transformation before subjects to analysis.

2.5 Data Analysis

All the data obtained were subjected to Diallel analysis using R software to determine Analysis of variance for combining ability, genetic components and combining ability effects by following Model 1 and Method 1 of Griffing approach. A fixed model (Model 1) was used because parents used were not randomly selected from a segregating population. Parents were selected on the basis of their prior performance. The following statistical models were used for combining ability analysis:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + b_k + e_{ijk}$

Where Y_{ijk} is the observed trait value from each experimental unit

μ = the overall means

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

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

s_{ij} = the SCA effect of the ij^{th} cross

r_{ij} = the reciprocal effect of the ij^{th} cross

b_k = the replication effect and

e_{ijk} = the random residual effect (experimental error).

As a part of the statistical analysis, the percentage values, germination, emergence percentage values were transformed by the angular transformation before variance analysis.

3. Results

3.1 Mean performance of the sixteen F1 progenies for germination and emergence traits

The variations in germination and emergence traits among the 16 F1 progenies were assessed (Table 2). Germination events are also important indicators of seed vigour and stress

resistance (Kader and Jutzi 2002) [7]. Highest germination percent (GP) of 84.26 per cent was recorded in T6 and T9 followed by 81.86% in progenies T1, T5, T10 and T7 (80.025%)) which were on par with each other, whereas T15 showed the least GP (70.63%) which was significantly different from the F1's showing highest germination percent. Similarly, emergence percent (EP) was observed highest in cross T9 (84.26%) which significantly differed with all other crosses. The lowest EP was recorded in cross T5 (62.027%) which was on par with T10 (81.86), T14 (74.65) and T7 (80.025). Cross T12 (13.1 days) recorded highest mean emergence time (MET) which differed significantly with all other crosses, and the lowest was recorded in T3 (3.0 days) and T10 (3.0 days) which were on par with T14 (3.9 days).
Seedling fresh weight (SFW) was highest in T1 (0.99g) followed by T7 (0.98g) and T2 (0.95g), lowest SFW was recorded in T12 (0.100g). F1 hybrids of cross T3 (0.036g) showed highest seedling dry weight (SDW) followed by T6 (0.034g) and T4 (0.033g) which recorded no significant difference among them. Cross T3 (2.729) recorded the elevated seedling vigour index (SVI) and the least was recoded in T14 (1.459) each differed significantly.

Table 2: Mean performance of the 16 F1 hybrids for germination and emergence traits

Crosses	GP (%)	EP (%)	MET (Days)	SFW (g)	SDW (g)	SVI
T1	81.86^a	70.630^c	4.2^g	0.99^a	0.029^b	2.0482^b
T2	77.079^b	67.21^c	7.4^e	0.95^a	0.026^b	1.747^c
T3	71.565^c	75.821^b	3.0^h	0.108^c	0.036^a	2.729^a
T4	78.463^b	77.079^b	5.0^g	0.103^c	0.033^a	2.543^a
T5	81.869^a	62.027^d	9.0^d	0.89^b	0.020^d	1.240^e
T6	84.260^a	78.463^b	5.0^g	0.106^c	0.034^a	2.667^a
T7	80.025^a	66.421^d	6.5^f	0.98^a	0.027^b	1.793^c
T8	75.821^b	69.732^c	10.1^c	0.101^c	0.031^b	2.161^b
T9	84.260^a	84.260^a	8.5^d	0.86^b	0.018^d	1.516^d
T10	81.869^a	62.725^d	3.0^h	0.94^a	0.025^c	1.568^d
T11	78.463^b	68.027^c	5.0^g	0.89^b	0.020^d	1.360^e
T12	75.821^b	73.570^b	13.1^a	0.100^c	0.029^b	2.133^b
T13	73.570^c	78.463^b	7.2^e	0.87^b	0.019^d	1.490^d
T14	74.658^b	63.434^d	3.9^h	0.93^a	0.023^c	1.459^d
T15	70.630^c	67.213^c	4.5^g	0.104^c	0.033^b	2.218^b
T16	73.570^c	74.658^b	12^b	0.94^a	0.024^c	1.791^c
S Ed	1.1612	2.2599	0.319	0.0318	0.0013	0.0757
Cd (0.05)	3.285	4.60	0.6394	0.0647	0.026	0.1542
F value	**	**	**	**	**	**

GP - Germination percent; EP – Emergence percent; MET – Mean emergence time; SFW – Seedling fresh weight; SDW – Seedling dry weight; SVI – Seedling vigour index.
Values were expressed in mean with three replications (n=3).
****** - Significant difference
T1 (V1×V1), T2 (V1× G4), T3 (V1× S36), T4 (V1 ×MR2), T5 (G4× V1), T6 (G4 × G4), T7 (G4 ×S36), T8 (G4 ×MR2), T9 (S36 ×V1), T10 (S36 ×G4), T11(S36 ×S36), T12 (S36× MR2), T13 (MR2 × V1), T14 (MR2× G4), T15 (MR2× S36), T16 (MR2 × MR2) Means followed by different small superscript in a column were statistically different at $p \leq 0.05$

Fig 1: Germination of seeds

Fig 2: Emergence of seedlings

3.2 Analysis of Variance

A combined analysis of variance was performed on the data of germination and emergence traits to estimate the amount of variability for these characters among the parents, their F1 direct and F1 reciprocals. Analysis of variance for diallel cross of mulberry is presented in Table 3. The results showed highly significant ($p < 0.001$) difference for almost all the measured traits. Analysis of variance of combining ability indicated that effects of general combining ability (GCA) were found to be highly significant ($p < 0.001$) the traits GP, EP, MET and SFW, whereas SDW is significant ($p < 0.05$) and SVI is very significant ($p < 0.01$). Specific combining ability (SCA) were highly significant for all the traits. The mean squares for the reciprocals also showed high significant differences for all the traits.

Table 3: Analysis of variance for combining ability in mulberry emergence

Source	DF	GP (%)	EP (%)	MET (Days)	SFW (g)	SDW (g)	SVI
GCA	3	47.852***	34.004***	11.0969***	0.0588***	0.0000*	0.018**
SCA	6	5.939***	72.346***	5.0763***	0.2403***	0.0000***	0.262***
Reciprocal	6	19.749***	16.145***	13.5247***	0.1539***	0.0001***	0.282***
ERR	30	0.331	2.44	0.0479	0.0005	0.00000	0.0029

*, **, ***Significantly different from zero at the 5%, 1%, and 0.1% probability levels, respectively. GP – Germination percent; EP – Emergence percent; MET – Mean emergence time; SFW – Seedling fresh weight; SDW – Seedling dry weight; SVI – Seedling vigour index. Values were expressed in mean with three replications (n=3).

3.3 General combining ability effects (GCA)

The effects of GCA for all the parents are presented in the Table 4. Progenitor G4 recorded the highest significant positive GCA effect of 2.2587 for the GP. On the other hand, MR2 genotype with negligible GP, had significant negative GCA effect of – 3.3662. Negative GCA's for GP indicates unsuitability of specific progenitors as combiners when targeting high germination percent in the progeny.

However, G4 showed highly significant negative GCA of – 2.67 for EP, while V1 recorded highest positive significant GCA of 2.03 for EP. Only progenitor MR2 (1.7626) had positive and significant GCA for MET and SVI (0.0443). Significant and positive GCA of 0.1038 was recorded only in V1 for SFW, while G4 recorded highest positive and significant GCA effect of 0.0007 for SDW, which are presented in Table 4.

Table 4: General combining ability (GCA) effects of parents used in a 4 × 4 diallel analysis of 6 traits

Progenitor	GP (%)	EP (%)	MET (Days)	SFW (g)	SDW (g)	SVI
P1- V1	1.223**	2.03**	-0.6500**	0.1038**	-0.0004 ns	0.0162 ns
P2 – G4	2.2587**	-2.67**	-0.4750**	0.0090 ns	0.0007**	0.0089 ns
P3 – S36	-0.1163 ns	-0.48 ns	-0.6376**	-0.0074 ns	-0.0007**	-0.0694**
P4 – MR2	-3.3662 **	1.1173**	1.7626**	-0.1055**	0.0004 ns	0.0443**
S Ed	0.2875	0.7821	0.1094	0.0111	0.0005	0.0272

*, **, *** significantly different from zero at the 5%, 1%, and 0.1% probability levels, non- significant respectively. ns – non significant, GP – Germination percent; EP – Emergence percent; MET – Mean emergence time; SFW – Seedling fresh weight; SDW – Seedling dry weight; SVI – Seedling vigour index. Values were expressed in mean with three replications (n=3).

3.4 Specific combining ability effects (SCA)

SCA effects for all the crosses are given in the Table 5. For GP three combinations showed significant and positive SCA effect among which T13- MR2 × V1 and T15 – MR2 × S36 recorded the higher of 2.500 SCA. Overall, there was high variation in SCA effects for the EP, where nine crosses showed significance, among which five combinations recorded positive SCA effect. T3 – V1× S36 had the most positive and significant SCA effect of 7.2506.

For MET, there were six crosses with positive significant SCA effects, with T15- MR2 × S36 as the best specific combination. Meanwhile, SFW had only two crosses T7- G4 × S36 and T2 -V1 × G4 with significant positive SCA effect of 0.19087. On the other hand, for SDW and SVI, highest significant and positive SCA was recorded in T9 – S36 × V1, 0.00883 and 0.6065 respectively presented in Table 5.

Table 5: Specific combining ability (SCA) effects for 4 × 4 diallel analysis of 6 traits

Crosses	GP (%)	EP (%)	MET (Days)	SFW (g)	SDW (g)	SVI
T2	-1.7238**	-5.9712**	2.6124**	0.19087**	-0.00385**	-0.4353**
T3	-0.8486**	7.2506**	0.3250**	-0.2285**	0.00122**	0.2715**
T4	0.4012 ns	3.3880**	-1.7249**	-0.1283**	-0.00047 ns	0.0522 ns
T5	-1.9996**	2.5928**	-0.8000**	0.0300 ns	0.003166**	0.2535**
T7	1.1161**	-3.5112**	-0.8501**	0.3417**	-0.00072 ns	-0.1630**
T8	-1.6336**	-3.0940**	-0.9997**	-0.0043 ns	-0.00093 ns	-0.1473**
T9	-6.4998**	-4.2196**	-2.7500**	-0.3761**	0.00883**	0.6065**
T10	-0.4998 ns	1.8481 ns	1.7498**	0.0200 ns	0.00133 ns	0.1126**
T12	-1.2587**	-1.4838 ns	0.9624**	-0.4016**	0.004479**	0.2964**
T13	2.5001**	-0.6921 ns	-1.0998**	-0.3836**	0.00716**	0.5265**
T14	0.5001 ns	3.1485**	3.1000**	-0.4145**	0.00383**	0.3516**
T15	2.5000**	3.1781**	4.3000**	-0.0018 ns	-0.00183**	-0.0425 ns
SEd	0.6428	1.7488	0.2447	0.0248	0.0010	0.609
Var GCA	5.9401	3.9449	1.3811	0.0073	0.0010	0.0020
Var SCA	5.6103	69.8997	5.0284	0.2398	0.070	0.2600
GCA/SCA Var	1.0588	0.0564	0.2747	0.0304	0.0133	0.0077

*, **, *** significantly different from zero at the 5%, 1%, and 0.1% probability levels, respectively. ns- non significant; GP – Germination percent; EP – Emergence percent; MET – Mean emergence time; SFW – Seedling fresh weight; SDW – Seedling dry weight; SVI – Seedling vigour index. Values were expressed in mean with three replications (n = 3). T1 (V1×V1), T2 (V1× G4), T3 (V1× S36), T4 (V1 ×MR2), T5 (G4× V1), T6 (G4 × G4), T7 (G4 ×S36), T8 (G4 ×MR2), T9 (S36 ×V1), T10 (S36 ×G4), T11(S36 ×S36), T12 (S36× MR2), T13 (MR2 × V1), T14 (MR2× G4), T15 (MR2× S36), T16 (MR2 × MR2)

4. Discussion

Analysis of variance for the following traits *viz.* GP, EP, MET, SFW, SDW and SVI were recorded and reported in four *Morus* sp. by Kazim *et al.*, (2018). They recorded GP from 9.3 to 66.5%, EP ranged between 67 to 80%, MET were 14 to 16.3 days, SFW recorded as 132.0 to 248.3 mg, SDW as 18.3 to 28 mg and SVI was reported as 0.726 to 2.362.7. There is an increase in germination rate and reduction in mean germination time due to the dormancy breaking effect of GA_3 treatment for seeds. In order to evaluate plant vigour characteristics of seedlings, both fresh and dry weights of the seedlings and seedling vigour indices were recorded which showed more or less similarity to Kazim *et al.*, (2018) studies. Griffing's analysis of variance revealed significant GCA and SCA effects among the diallel crosses with both additive and non - additive gene effects being important for the inheritance of the germination and seedling traits studied in mulberry. The significant positive GCA effects (Table 4) for GP, EP, MET,SFW,SDW and SVI were observed for G4, V1, MR2, V1,G4 and MR2 respectively, indicates that these parental lines could be useful for contributing favourable alleles for improving these traits. Significant GCA effect is a manifestation of additive and additive ×additive gene interactions, which represents a heritable portion of genetic variation which is transmittable from the parent to its progeny, which was also reported in cowpea (Owusu *et al.*, 2020).

The crosses MR2 × V1 and MR2 × S36 showed positive significant SCA effect for GP, when MR2 showed negative significant GCA and S36 showed non-significant GCA, In the cross MR2 × V1, V1 with positive GCA showed the presence of additive gene action and also indicated that one parent of the worst combination could make the best combination if the other parent was selected properly. On the other hand, combination MR2 × S36 with high positive SCA had progenitors that did not show significant GCA effect in desirable direction. Same result was recorded by Vijayan *et al.*, 1997 [10] for high leaf yielding combination in mulberry. This high effect of SCA manifested by low × low cross may be due to dominance × dominance type of allelic gene interaction producing over dominance thus being non – fixable. High SCA confined the non-additive gene action. Along with above crosses, the variances of GCA is more than SCA variance and their ratio is more than unity, concluded that both additive and non-additive gene actions were involved in the inheritance of the germination trait.

As per the trait EP the variance of GCA is less than the variance of SCA and GCA/SCA variance ratio is less than one showed the dominance gene action. The parents involved in the crosses showing high positive SCA for MET had high negative GCA effects. Significant negative GCA suggests that these parents consist of favourable dominant or partial dominant alleles, which could contribute for earliness, which means seeds might germinate earlier than usual resulting in lowest MET. Likewise same significant negative GCA effects were also found in cowpea that can be used to develop early maturing cowpea lines (Owusu *et al.*, 2020).

For SFW the GCA variance is lesser than SCA variance and the ratio of GCA/SCA variance is less than unity which shows predominance of non–additive gene effect. The progenitors involved in the crosses with high SCA effect for SFW showed non-significant GCA might be due to the epistatic gene effect. The same pattern was observed for SDW also. The Variance of SCA is greater than GCA and GCA/SCA variance ratio less than unity for SVI shows dominance gene action (Non – additive effect).

5. Conclusion

Selection of compatible parents with desirable GCA and crosses with superior SCA effects has a crucial role in all successful breeding programmes. In the present study variations were observed in all the four progenitors and 16 F1 progenies of mulberry. Both F1 hybrids and parents had significant amount of GCA and SCA respectively. Due to the variations, screening of better mulberry genotype is quite easy. The crosses of MR2 × V1 for GP, V1 × MR2 for EP, MR2 × S36 for MET, V1 × G4 for SFW, G4 × V1 for SDW and S36 × MR2 for SVI were found to be the best crosses and parents MR2 and V1 found to be the best combiners for most of the traits. Hence, these parents and F1 progenies may be further used in breeding programmes when the above traits have to be taken into consideration.

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