RESEARCH ARTICLE

GENETIC STUDIES OF LINE X TESTER ANALYSIS IN MULBERRY (*MORUS* SPP.)

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Abstract: Sericulture is the agricultural activity that traditionally consists in the cultivation of mulberry trees (*Morus* spp.) to yield leaves that are used for feeding silkworms (*Bombyx mori* L.), reared for silk production. In order to improve the leaf quality of mulberry leaf, general combining ability of the parents and specific combining ability of hybrids were estimated for F_1 hybrids of mulberry for survivability and growth traits. Combining ability studies were done using line x tester mating design involving eight genotypes of mulberry. Combining ability analysis of variance revealed that the hybrids were highly significant for all characters because of the preponderance of non-additive genes. Among the parents, MI-0543, MI-0615, MI-0685 and V-1 were the best general combiners for most of the traits in the study. Two best combinations viz. MI-0543 x V-1 and MI-0685 x V-1 had high significant variance coupled with high SCA and can be utilized for further mulberry crop improvement programme.

Keywords: Mulberry leaf, Line x Tester, GCA, SCA, Silkworm

INTRODUCTION

Sericulture is the art of cultivation of mulberry (*Morus* Spp.) and rearing of silkworms (*Bombyx mori*) to produce silk. Mulberry foliage is the only food for the silkworm 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. Male catkins are loosely arranged and lengthier than female catkins. After shedding the pollen, catkins dried and fall off. Female catkins are compactly arranged and after fertilization the fleshy bases of the perianth altered become sorosis. (Vijayan *et al*., 2011). Identifying the differences in sex expression in mulberry is an enigmatic process. Sex of particular species in mulberry is not static, it varies from season to season based on the environmental fluctuations, cultural practices and soil factors. Sex may change from male to female and *vice versa* due to pruning (Tikader *et al*., 1995).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 because of the monophagous nature of silkworm. One of the reasons 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. Mulberry exhibits high plasticity and acclimatize itself to various climatic conditions. Environmental conditions in India are most favourable for mulberry growth and development throughout the year.

New hybrids with novel desirable characters developed through various breeding methods might be boosting the Sericultural economy. In India, mulberry crop improvement programme was initiated during 1960's. So far 1292 accessions of different *Morus* sp collections are available at Central Sericultural Germplasm Research Institute, hosur. In mulberry breeding, meticulous screening and selection of promising F_1 hybrids is a lengthy and cumbersome process due to high degree of heterogeneity. Present study was carried out to estimate the combining ability effects for growth and root traits and the survival rate was analyzed for F_1 mulberry hybrids.

MATERIALS AND METHODS

Selection of compatible parents and suitable procedure are the two main pre-requisite for all breeding programs. A sound procedure for estimating combining ability is line x tester analysis and it was introduced by Kempthrone (1957). This mating

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design is an extension of top cross method, in which several testers are used to evaluating the lines precisely. Selection is not only based on phenotypic performance but also on their intrinsic genetic value such as genetic variability, combining ability and heterosis. A total of fifteen crosses produced by crossing five lines with three testers were used for this study, which were procured from Central Sericulture Germplasm Resources Centre, Hosur.

In biometrical genetics, two types of combining abilities are estimated i.e. General Combining Ability (GCA) and Specific Combining Ability (SCA). Average performance of parental lines in its cross are known as general combining ability and specific combining ability refers to the performance of certain crosses deviates from the expected value based on the average performance of the parental lines involved (Sprague and Tatum, 1942). The observations of F_1 progenies pertaining to survivability traits were recorded on 60^{th}, 75^{th} and 90^{th} day after sowing. Morphological traits of the progenies were observed and apart from that some of the quantitative traits were estimated such as shoot diameter, growth rate, leaf chlorophyll contents, number of roots per seedling and fresh root weight.

Qualitative traits

Leaf shape

Leaf shape of the F_1 progenies were recorded based on visual observation and categorized as cordate or ovate or serrate (Gray and Gray, 1987).

Phyllotaxy

Arrangement of leaves around the stem is known as phyllotaxy. Divergence in phyllotaxy as seen in mulberry genotypes, it may be opposite or alternate (Krishna *et al*., 2019). Phyllotaxy was recorded based on visual observation and categorized as mentioned above.

Quantitative traits

Shoot diameter (mm)

Shoot diameter was measured by using the digital verniar caliper. It was placed just below the cotyledon scar ensuring that the caliper was perpendicular to the stem during measurement. Readings were recorded at three different places *viz*. base, middle and top portion and then average thickness was calculated. It is an indicator of seedling quality (Haase, 2008). It expressed in mm.

Growth rate (cm/day)

Changes in offspring's height was recorded at the time interval between 60^{th} to 75^{th} day as well as 75^{th} to 90^{th} day after sowing. Growth rate was calculated using the following formula and expressed in cm/day.

$$\text{Growth rate} = \frac{w2-w1}{\gamma}$$

Where,

W_1- first measurement;

W_2- second measurement;

T- time interval between W_1 and W_2.

Chlorophyll content (mg/g)

Chlorophyll content was measured using the leaves of 90^{th} day old seedlings by method. One gram of leaf samples were homogenized with 80 per cent acetone, supernatant solution was taken after centrifugation at 5000rpm for 5 minutes. Then the absorbance readings were recorded at 663nm and 645nm in spectrophotometer. Chlorophyll content was calculated as per the following formulae by using spectrophotometer readings and expressed in mg.

$$\text{Chlorophyll a} = 12.7\,(OD663) - 2.69\,(OD645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 12.7\,(OD663) - 2.69\,(OD645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(OD645) - 4.68\,(OD663) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = 20.2\,(OD\,645) + 8.02\,(OD663) \times \frac{V}{1000 \times W}$$

Where,

OD = Optical Density at specific wavelength

V = Volume of chlorophyll extract

W = Weight of leaf sample taken.

Number of roots/plant

Roots of the individual seedlings were counted on 90th day. Seedlings were uprooted, remove the adhering soil by washing the roots and number of roots counted manually.

Fresh root weight (g)

Ninety days old seedlings were separated and the whole root was cut off from the seedling then weighed using electronic weighing balance, weight expressed in grams.

TNAUSTAT software used to evaluate GCA and SCA values. This package was developed by Manivannan (2014). Significant variance was found among the genotypes for all the studied traits.

RESULTS

Analysis of variance for combining ability values and contribution to total variances were represented in the Table 1. Significant variance was found among the genotypes for all the studied traits. It revealed that the estimation of combining ability is possible due to the presence of substantial genetic variability among the genotypes. In most of the evaluated traits, contribution of lines was higher compared to testers whereas, contribution of line x tester interaction was higher for most of the traits. The results pertaining to general combining ability effects for the five quantitative traits were presented in the Table 2.

Shoot diameter (mm)

Among the lines, estimates of GCA ranged from MI-0718 (-1.51) to MI-0543 (0.59) and for testers V-1 had highly positive significant GCA (0.64) while G4 had highly significant and negative GCA effect (-0.96) for shoot diameter.

Growth rate (cm/day)

For growth rate at 60 – 75 days, GCA estimates ranged from MI-0718 (-0.06) to MI-0615 and MI-0543 (0.03) among lines and for testers, from G4 (-0.02) to V-1 (0.02). Out of eight parental genotypes MI-0543 and MI-0615 had highly positive significant GCA effect and the value of MI-0543 was on par with MI-0615 (0.03).

MI-0543 recorded highest positive significant GCA value (0.06) for growth rate at 75-90 days. Estimates of GCA for lines, ranged from MI-0718 (-0.14) to MI-0543 (0.06) and G4 (-0.04) to V-1 (0.05) for testers.

Chlorophyll-a (mg/g)

GCA estimates ranged from MI-0685 (-0.18) to MI-0543 (0.18) among lines. Out of three testers V-1 had highly significant positive GCA (0.09) for Chlorophyll-content.

Chlorophyll-b (mg/g)

Among the lines, MI-0651 recorded highly significant positive GCA (0.06) followed by MI-0543 (0.05). Other three lines *viz*., MI-0685, MI-0718 and MI-0615 showed highly significant and negative GCA effect (-0.04, -0.04 and 0.03 respectively). Among the testers, V-1 had highest positive GCA (0.13).

Total chlorophyll content (mg/g)

Estimates of GCA for lines, ranged between MI-0685 (-0.43) to MI-0543 (0.45) for total chlorophyll contents. Similarly, for testers GCA ranged from G4 (-0.08) to V-1(0.13). Out of eight parental genotypes, MI-0543 had highly significant positive GCA effect (0.45) followed by MI-0651 (0.28) for total chlorophyll content.

Number of roots per plant

Out of five lines MI-0615 exhibited highest and significant GCA effect (1.20) followed by MI-0543 and MI-0685 (0.59 and 0.57 respectively) for number of roots per plant. Among testers, V-1 had highly significant positive GCA (0.48) whereas, G4 recorded highly significant and negative GCA effect (-0.64).

Fresh root weight (g)

GCA estimates ranged from MI-0718 (-0.82) to MI-0685 (0.68) among lines and for testers values lies between G4 (-0.28) to V-1 (0.36) for fresh root weight. MI-0685and MI-0543 showed highly significant positive GCA effects (0.68 and 0.54 respectively).

Specific combining ability effects

Results of SCA effects are presented in the Table 3. SCA estimates varied from MI-0718 x V-1 (-1.66) to MI-0615 x G4 (1.38) for shoot diameter. MI-0615 x G4 and MI-0651 x V-1 had highly significant positive SCA effect (1.38 and 1.11 respectively) for shoot diameter. With respect to growth rate at 60-75 days interval, MI-0543 x V-1 showed highest positive significant SCA (0.11) followed by MI-0615 x G4 and MI-0718 x G4 (0.08). Hence, both crosses were on par with each other. For 75-90 days interval, MI-0685 x MI-0663 and MI-0718 x G4 showed highly positive significant SCA value (0.23 and 0.21 respectively). Conversely, MI-0718 x MI-0663 had highly significant negative SCA effect (-0.32).

MI-0615 x MI-0663 showed highly significant positive SCA effect (0.42) for Chlorophyll-a content followed by MI-0685 x MI-0663 and MI-0651 x G4 (0.31 and 0.24 respectively). For chlorophyll-b, MI-0718 x V-1 was found to be significant with highest positive SCA effect (0.09) followed by MI-0685 x MI-0663 (0.08). MI-0718 x V-1, MI-0651 x G4, MI-0685 x MI-0663 recorded highly significant positive SCA values for total chlorophyll content (0.43, 0.34 and 0.27 respectively).

Number of roots per plant was highly significant with positive SCA (2.98) in MI-0543 x V-1. Whereas, MI-0615 x G4 had highly significant positive SCA effect for fresh root weight (4.25) followed by MI-0685 x V-1 (3.28) and MI-0543 x V-1 (3.08). and the observations of morphological traits were given in table 4.

DISCUSSION

Arrangement of the leaves on the shoot is called as phyllotaxy. Alternate type of leaf arrangement was

observed in all the fifteen crosses. In most of the mulberry genotypes leaves are alternatively arranged with the stem. Observation of the present study was in conformity with the findings of Dandin and Jolly (1986) and Pooja (2016). Lobation of the leaves is a genetic trait which influences the leaf yield via single leaf area. Out of fifteen only two crosses viz. MI-0543 x MI-0663 and MI-0651 x MI-0663 exhibited lobed leaves. Shape of the leaves varied among the genotypes due to the genetic nature of the particular genotype. Narrow variation was observed for this trait, among the F_1 progenies. Most of the crosses were found to have cordate-ovate and cordate leaves. Ovate shaped leaves were observed in two crosses named as MI-0651 x G4 and MI-0685 x G4.

Shoot diameter is directly proportional to shoot height whereas, both shoot diameter and shoot height were negatively correlated with shoot biomass as reported by Cao *et al*. (2019). Among the parents, V-1 (0.64) and MI-0543 (0.59) had highly positive and significant GCA for shoot diameter. In the current study, GCA effect for growth rate was greater in 75-90 days interval compared to 60-75 days interval. It denotes that, maximum variation in the growth of the seedlings occurred between 75-90 days and the result was similar to the findings of Pooja *et al*. (2016).

High concentration of chlorophyll pigments has more photosynthetic efficiency as reported by Satoh *et al*., (1977). In the present study, total chlorophyll pigments were higher in MI-0543 (0.45) but it showed negative GCA effect for single leaf area which was contrary with the earlier report. Waktole and Wosene (2016) suggested that the genotype with high chlorophyll contents leads to produce better leaf yield due to high photosynthetic rate. Similarly, in the present study, MI-0543 is the best parent for leaf quality and quantity traits of mulberry.

Fresh weight of the roots and their number were recorded for an individual seedling. Among the genotypes, MI-0615 obtained with more number of roots per plant (1.20) simultaneously low fresh root weight (0.26) due to thin roots. For fresh root weight, MI-0685 (0.68) and MI-0543 (0.54) showed highest significant GCA value and also exhibited positive GCA effect for number of roots per plant (0.57 and 0.59 respectively). Out of three testers, V-1 had good result for both number of roots (0.48) and fresh root weight (0.36).

Results showed that MI-0685 x MI-0663 was significant for growth rate (0.23) as well as chlorophyll-a (0.31) which was on par with the results of the earlier work done by researchers. Results of the present study showed that highest SCA effect was observed in MI-0615 x G4 (4.25) and MI-0543 x V-1 (2.98) for fresh root weight and for number of roots per plant respectively.

Table 1. ANOVA for combining ability of mulberry

Source of variation	Df	G.R (60-75 D)	G.R (75-90 D)	Shoot dia (mm)	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	No. of roots/plant	Fresh root weight (g)
Replication	2	0.0013	0.0035	0.1743	0.0018	0.0043	0.0471	0.4866	0.0101
Crosses	14	0.0144	0.0809	6.5713	0.2255	0.0151	0.4984	15.0554	15.4813
Lines	4	0.0109	0.0606	6.8777	0.1670	0.0212	1.2097	11.6875	4.3133
Testers	2	0.0045	0.0301	10.8355	0.0868	0.0044	0.1861	5.0346	1.6378
L x T	8	0.0187	0.1037	5.3521	0.2895	0.0147	0.2208	19.2445	24.5261
Error	28	0.0005	0.0027	0.3641	0.0103	0.0010	0.0197	0.3667	0.2760
SED		0.0173	0.0421	0.4927	0.0828	0.0263	0.1146	0.4944	0.4289
CD (5%)		0.0355	0.0862	0.0100	0.1697	0.0539	0.2349	1.0135	0.8793
CD (1%)		0.0478	0.1161	1.3598	0.2285	0.0725	0.3163	1.3646	1.1839
Contribution of lines		21.53	21.41	29.90	21.16	40.08	69.35	22.18	7.96
Contribution of testers		4.42	5.31	23.56	5.50	4.13	5.33	4.78	1.51
Contribution of interactions		74.05	73.28	46.54	73.35	55.79	25.31	73.04	90.53

Table 2. General combining ability effects of parents in mulberry

Parents / Traits	G.R 60-75 D	G.R 75-90 D	Shoot dia (mm)	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	No.of roots/plant	Fresh root weight (g)
MI-0543	0.03**	0.06**	0.59**	0.18**	0.05**	0.45**	0.59**	0.54**
MI-0615	0.03**	0.04*	0.54*	-0.07	-0.03*	-0.02	1.20**	0.26
MI-0651	-0.01	-0.01	0.02	0.08*	0.06**	0.28**	-0.79**	-0.65**
MI-0685	0.01	0.05**	0.36	-0.18**	-0.04**	-0.43**	0.57**	0.68**

MI-0718	-0.06**	-0.14**	-1.51**	-0.02	-0.04**	-0.27**	-1.57**	-0.82**
SED	0.0100	0.0243	0.2845	0.0478	0.0152	0.0662	0.2854	0.2477
CD (5%)	0.0205	0.0498	0.5831	0.0980	0.0311	0.1356	0.5852	0.5077
CD (1%)	0.0276	0.0670	0.7851	0.1319	0.0419	0.1826	0.7878	0.6835
V-1	0.02**	0.05**	0.64**	0.09**	0.02*	0.13**	0.48**	0.36*
G4	-0.02**	-0.04**	-0.96**	-0.02	-0.02	-0.08*	-0.64**	-0.28*
MI-0663	-0.00	-0.00	0.32	-0.06*	-0.00	-0.05	0.16	-0.08
SED	0.0078	0.0188	0.2203	0.0370	0.0118	0.0512	0.2211	0.1918
CD (5%)	0.0159	0.0386	0.4517	0.0759	0.0241	0.1051	0.4533	0.3933
CD (1%)	0.0214	0.0519	0.6081	0.1022	0.0324	0.1414	0.6103	0.5295

Table 3. Specific combining ability effects of hybrids in mulberry

S. No	Crosses	G.R 60-75 D	G.R 75-90 D	Shoot dia (cm)	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	No.of roots/plant	Fresh root weight (g)
1	MI 0543 x V-1	0.11**	0.06*	0.80*	0.12*	0.04	0.04	2.98**	3.08**
2	MI 0615 x V-1	-0.08**	-0.12**	-1.05**	-0.21**	-0.08**	-0.06	-2.93**	-2.48**
3	MI 0651 x V-1	-0.02	0.01	1.11**	0.08	-0.00	-0.24**	-0.32	0.47
4	MI 0685 x V-1	0.03*	-0.07*	0.81*	-0.12*	-0.04*	-0.17*	2.42**	3.28**
5	MI 0718 x V-1	-0.04**	0.12**	-1.66**	0.13*	0.09**	0.43**	0.18	0.85**
6	MI 0543 x G4	-0.07**	-0.06	-0.28	-0.05	0.03	0.03	-1.76**	-2.43**
7	MI 0615 x G4	0.08**	0.07*	1.38**	-0.21*	0.04	-0.04	2.22**	4.25**
8	MI 0651 x G4	-0.00	-0.05	-0.28	0.24**	0.02	0.34**	-0.59	0.93**
9	MI 0685 x G4	-0.08**	-0.16**	-1.65**	-0.19**	-0.04*	-0.10	-2.28**	-1.37**
10	MI 0718 x G4	0.08**	0.21**	0.83*	0.21**	-0.05*	-0.22**	0.09	-1.38**
11	MI 0543 x MI0663	-0.04**	-0.00	-0.52	-0.07	-0.07**	-0.07	-1..22**	-0.65*
12	MI 0615 x MI0663	-0.00	0.06	-0.33	0.42**	0.04*	0.10	0.71	-1.77**
13	MI 0651 x MI0663	0.03*	0.03	-0.82*	-0.32**	-0.02	-0.10	0.92*	-1.40**
14	MI 0685 x MI0663	0.05**	0.23**	0.84*	0.31**	0.08**	0.27**	2.19**	-1.91**
15	MI 0718 x MI0663	-0.04**	-0.32**	0.83*	-0.34**	-0.04	-0.20*	-2.59**	0.53
	SED	0.0173	0.0421	0.4927	0.0828	0.0263	0.1146	0.4944	0.4289
	CD (5%)	0.0355	0.0862	1.0100	0.1697	0.0539	0.2349	1.0135	0.8793
	CD (1%)	0.0478	0.1161	1.3598	0.2285	0.0725	0.3163	1.3646	1.1839
*significant at 5% level (P < 0.05) ; **significant at 1% level (P < 0.01)									

Table 4. Observations on leaf shape and phyllotaxy in mulberry

S. No	Crosses	Leaf shape	Lobation	Phyllotaxy
1	MI 0543 x V-1	Cordate-ovate	Unlobed	Alternate
2	MI 0615 x V-1	Cordate-ovate	Unlobed	Alternate
3	MI 0651 x V-1	Cordate-ovate	Unlobed	Alternate
4	MI 0685 x V-1	Cordate-ovate	Unlobed	Alternate
5	MI 0718 x V-1	Cordate	Unlobed	Alternate
6	MI 0543 x G4	Cordate-ovate	Unlobed	Alternate
7	MI 0615 x G4	Cordate-ovate	Unlobed	Alternate
8	MI 0651 x G4	Ovate	Unlobed	Alternate
9	MI 0685 x G4	Ovate	Unlobed	Alternate
10	MI 0718 x G4	Cordate-ovate	Unlobed	Alternate
11	MI 0543 x MI 0663	Cordate	Hierarchically lobed	Alternate
12	MI 0615 x MI 0663	Cordate	Unlobed	Alternate
13	MI 0651 x MI 0663	Cordate	One-lobed	Alternate
14	MI 0685 x MI 0663	Cordate	Unlobed	Alternate
15	MI 0718 x MI 0663	Cordate-ovate	Unlobed	Alternate

CONCLUSION

Three promising lines and one tester were found to be superior with respect to growth traits. The identified lines *viz*., MI-0543, MI-0615, MI-0685 and tester V-1 can be further used for mulberry crop improvement programme.

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