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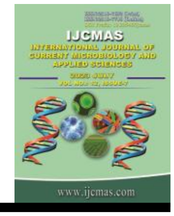

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Genetic Inter-Relationship and Principal Component Analysis for Determination of the Selection Criteria in Mulberry Genotypes

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ABSTRACT

The identification of superior clones in mulberry breeding programs requires the knowledge on genetics of economic traits and efficient tools for selection. A field experiment was carried to evaluate two pseudo F_2 populations of CSRS-1 × V1 and Kajli OPH × V1 for leaf yield and quality attributes during *Kharif* 2018. The objective of this study was to determine the interrelationship among different traits and to identify superior clones by principal component analysis. One way analysis of variance revealed that the progenies of both populations are highly significant for all quantitative traits studied indicating opportunity for genetic selection. Higher mean, standardized range and genotypic coefficient of variances was observed for fresh leaf weight, leaf area, primary shoots, total shoots length and leaf yield per plant. High to moderate heritability in broad sense and genetic advance in percent of mean was recorded for all the characters except for chlorophyll content index, specific leaf area, leaf moisture content and leaves per meter shoot. Phenotypic correlation coefficients and path analysis revealed that selection based on higher fresh leaf weight, primary shoots and total shoots length would be effective for increasing the leaf yield of mulberry. Platykurtic and positively skewed distribution noticed in these traits indicated the involvement of a several minor genes and dominance based complementary gene interaction suggests that rapid genetic gain under mild selection. PCA identified four principal components (PC) and explained 72 to 77% of total variation present in both the population. PC1, PC2, and PC3 were related to leaf productivity, quality and leaf size, respectively. PCA based index identified superior clones CV2, CV109, KV40, KV103 and KV169 may be further evaluated to develop improved mulberry varieties for subtropical sericulture.

Introduction

Sericulture also known as "Industry of the Poor" is an agro-based industry and the end product of which is silk, "the queen of fabrics". India is the second largest producer of raw silk (34903 MT) after China and also one of the biggest consumers. Five commercially traded varieties of natural silks are produced in India and mulberry silk alone contributes 74% of total silk output (Anonymous, 2022). To meet the demand of silk goods for growing population and to achieve self sufficiency in the country, the present silk production levels need to be increased by 2000 tons every year. In mulberry sericulture, more than 60% of the total cost of cocoon production goes towards mulberry leaf production alone. Hence, productivity of the host plant needs to be enhanced through heterosis breeding or other innovative breeding approaches for sustainability and profitability of sericulture.

Mulberry is a fast growing, deciduous woody perennial tree species of Moraceae family (Vijayan, 2010; Yuan and Zhao, 2017). It is widely cultivated across different agro-climatic conditions for feeding silkworm (*Bombyx mori*) and production the silk. Enhancement of silk production to a great extent depends on increased leaf productivity of mulberry (Sarkar *et al.,* 1987). The mulberry leaf productivity is determined by edaphic factors, climatic conditions, management and the genetic potential of the variety. The genetic resources enable the creation of cultivars that are higher productivity and more resilient to biotic or abiotic stress (Tanksley and McCouch, 1997). Genetic variability is the fundamental requirement for initiation of any crop improvement programme and effectiveness of selection will be depends on the extent of variability in the breeding materials. Mulberry leaf yield is a complex trait and is contributed by a number of component traits that are highly influenced by environment and management practices. Correlation and path analysis draws a clear image of inter-relationships and relative contribution of independent variables, which enables a plant breeder to plan an efficient breeding program (Dewey and Lu, 1959 and Bhat, 1973). Genetic variability and correlation studies for various morphological traits and leaf yield in mulberry has been reported by several authors (Sarkar *et al.,* 1987; Bari *et al.,* 1989; Susheelamma *et al.,* 1998; Goel *et al.,* 1998; Vijayan *et al.,* 1998; Masilamani *et al.,* 2000; Tikader and Roy, 2001; Tikader *et al.,* 2004; Tikader and Dandin, 2005; Rahman *et al.,* 2006; Doss *et al.,* 2006; Banerjee *et al.,* 2007; Mallikarjunnappa *et al.,* 2008; Vijayan *et al.,* 2010; Doss *et al.,* 2011; Doss *et al.,* 2012; Biradar *et al.,* 2015; Suresh *et al.,* 2017; Saini *et al.,* 2018; Chanotra *et al.,* 2019 and Magadum and Singh, 2021).

Selecting superior genotypes is a complex process because the economically important yield traits are quantitative by nature and are associated with each other. Multivariate techniques are required to identify and prioritize the important traits for effective selection. Principal component analysis (PCA) is an exploratory tool that transforms a number of correlated variables into a smaller number of uncorrelated variables called principal components (Ringer, 2008). This technique assist in identification of traits that help in distinguishing selected genotypes based on similarities in one or more traits and classify the genotypes into separate groups (Ariyo, 1987 and Nair *et al.,* 1998). Principal component analysis studies have been performed to identify and cluster the genotypes in mulberry (Banerjee *et al.,* 2016; Wang *et al.,*2017; Suresh, *et al.,* 2018; Chanotra *et al.,* 2019; Zhenjiang *et al.,* 2021; Toolir and Mirjalili, 2023; Sun *et al.,* 2023). PCA based selection index can be computed using principal components where in data was normalized and weights were assigned based on the PCs having Eigen value greater than one. Based on the average of important PC scores, the ranks were assigned to each genotype for identification of superior genotypes. The present study was conducted to estimate the genetic interrelationships among yield attributes in two pseudo F_2 population and to identify superior genotypes in mulberry.

Materials and Methods

The present study was carried out at Central Sericulture Research and Training Institute, Berhampore located at an altitude of 19 m above mean sea level with humid sub-tropical climate [34° 0' 28" North, 71° 34' 24" East]. The female parents CSRS-1 and Kajli OP had moderate leaf yield potential and adapted to subtropical region, while male parent is a popular tropical variety V1. Ten year old established plantation of two pseudo F_2

seedling population *viz.*, CSRS-1 × V-1 (155 No.) and Kajli OP × V-1 (173 No.) along with parents and check varieties (S-1& S-1635) in paired row system [(2'×3') ×5'] was utilized. The recommended package of practices for irrigated mulberry cultivation was followed throughout the crop period. The field experiment was conducted during the *Kharif* 2018 season to evaluate segregating populations for leaf yield and quality attributes.

Observations such as fresh leaf weight, fresh leaf area, chlorophyll content index, specific leaf area, leaf moisture content, leaves per meter shoot, leaf fall at harvest, length of longest shoot, primary shoots per plant, total shoots length and leaf yield per plant were recorded after 75 days after pruning. Chlorophyll content index (CCI) was recorded using Chlorophyll Content Meter (CCM-200, USA) on 7th leaf from the top of main axis of longest shoot in each individual plant. Leaf area was recorded using leaf area meter (Li-COR Model-3100). Specific leaf area was determined from the ratio of leaf area to the dry weight of leaves from top, middle and bottom positions of individual plant. The remaining growth traits were recorded on individual progenies at the time of harvest.

The mean data of the above mentioned eleven traits were statistically analyzed using SPSS Professional Statistics version 10.1(SPSS Inc., Chicago, III) and online statistical analysis tool OPSTAT (Sheoran *et al.*, 1998). One way analysis of variance (ANOVA) was done by the method suggested by Panse and Sukhatme (1985). Critical difference (C.D.) was calculated wherever the ‘F' test was found significant and data was presented with the level of significance at 5 per cent. Genetic variability was assessed using first-degree statistics mean, standardized range and second degree statistics, phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) (Burton and De vane, 1953). Genetic parameters such as broad sense heritability (Lush, 1945) and genetic advance as per cent mean (GAM) were also estimated (Johnson *et al.*, 1955).
Skewness, and kurtosis are the third and fourth degree statistics used to understand nature of genetic control and relative number of genes controlling the traits, respectively (Snedecor and Cochran, 1994). Skewness is a measurement of symmetry of the population and is often used to indicate the nature of gene action for any trait (Fisher, 1932). Positive skewness corresponds to complementary gene action, whereas negative skewness corresponds to duplicate epistasis (Pooni *et al.*, 1977). Kurtosis is a measure of peakedness or tailness of a data and measuring whether it is right tailed or left tailed to the normal distribution. Kurtosis is manifested in three levels *viz.*, leptokurtic (positive kurtosis) signifying that the traits are controlled by fewer genes, platykurtic (negative kurtosis) signifying that the traits are controlled by a larger number of genes and mesokurtic (normal distribution at 0) (Kapur, 1981).

The coefficients of correlation (Johnson *et al.*, 1955) and the path coefficient analysis (Dewey and Lu, 1959) were worked out to understand the interrelationship among the different traits. Significance of correlation coefficient was tested by comparing table ‘r' value with the obtained value. The path coefficient is a standardized partial regression coefficient and correlation coefficient of any character was split into direct and indirect effects adopting the standard formula. The principal component analysis was followed in the extraction of the components (Harman, 1976). The PCA based selection index for each genotype is computed by averaging the important PC scores. Based on the index values, the ranks were assigned to each progenies in both populations for selection of superior genotypes in mulberry.

Results and Discussion

The one way analysis of variance revealed significant mean sum of squares for all the eleven characters among progenies in both the population, indicating existence of a good amount of genetic variability. Mean performance of parents and their pseudo F_2 populations CSRS-1 × V-1 and KOP × V-1 along with two checks for all the traits studied is presented in Table 1. The parents recorded higher mean values for most of the characters studied than check variety S-1. The leaf yield per plant varied from 0.154 to 1.728 kg and 0.122 to 1.724 kg in CSRS-1 × V-1 and Kajli OP× V-1, respectively. Higher standardized range was recorded for fresh leaf weight, leaf area, chlorophyll content index, primary shoots per plant, total shoot length, leaf to shoot ratio and leaf yield per plant in both population indicate the presence of enormous amount of genetic variability. Genetic variability is a key for the success of any plant breeding program and assessment of the variability present for different yield components provides an opportunity for effective selection of genotypes. Genetic parameters such as genotypic (GCV) and phenotypic coefficients of variation (PCV), heritability in broad sense and genetic advance in percentage of mean are presented in Table 1.

The magnitude of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits studied. Higher magnitude (> 20%) of PCV along with GCV was recorded for leaf yield per plant (48-66%), total shoot length (37-47 %), primary shoots per plant (33-45%), fresh leaf weight (25-32%) and fresh leaf area (22-26%) in both the population. However, leaf fall at harvest (17-28%), chlorophyll content index (14-23%), length of longest shoot (13-15%), leaves per meter shoot (10-13%) specific leaf area (8-11%) and leaf moisture content (1.73-2.11%) recorded moderate and lower estimate of PCV and GCV. The presence of higher PCV and GCV for most of the characters in both the population revealed that their phenotypic expression would be a good indication of genetic potential and considerable improvement could be achieved through single plant selection. The existence of high variability for several leaf yield traits in mulberry was also reported by Goel *et al.*, (1998); Tikader *et al.*, (2004); Banerjee *et al.*, (2007); Mallikarjunappa *et al.*, (2008); Doss *et al.*, (2012); Biradar *et al.*, (2015); Suresh *et al.*, (2017); Saini *et al.*,(2018); Chanotra *et al.*, (2019) and Magadum and Singh, (2021). The quality characters such as leaf moisture content, chlorophyll index, leaf thickness(SLA) exhibited moderate phenotypic (PCV) and genotypic coefficient of variation (GCV) are likely to allow reasonable scope of improvement through selection.

Heritability plays a significant role as it serves as an extrapolative guide to realize the breeding value. The heritability (bs) ranged from 38.10 to 87.80% in CSRS1 × V1 and 40.93 to 98.01% in Kajli OP × V1 population. The higher heritability was recorded for longest shoot height (98.04 %), primary shoots per plant (90.01 %), leaf area (84.28 %), leaves per meter shoot (83.30%), leaf moisture content (83.18%), leaf yield per plan(73.32%), fresh leaf weight (67.83%) and total shoots length (65.03%) in Kajli OP × V1. Moreover, CSRS-1×V1 also recorded higher heritability for total shoots length (87.80%), leaf moisture content (86.70%), leaf area (86.56%), leaves per meter shoot (74.96%), longest shoot height (73.59%), fresh leaf weight (70.50%), primary shoots per plant (65.15 %) and leaf yield per plant (64.87%) indicated high rate of trait transmissibility and improvement can be made by simple selection. The rest of the traits recorded moderate to low in heritability values. High heritability estimates indicated that the variation observed was mainly under genetic control and such characters are considered to be dependable from the breeding point of view.

The estimation of heritability along with genetic advance is more applicable than the heritability value alone (Johnson *et al.*, 1955). Genetic advance as percentage of mean (GAM) ranged from 3.26% to 82.83% for leaf moisture content and primary shoots per plant, respectively. Leaf yield per plant exhibited highest value of genetic advance as percentage of mean (76-79%) followed by primary shoots per plant (55-83%), total shoots length (62-81%), fresh leaf weight (43-46%), fresh leaf area (41-46%), length of longest shoot (22-28%) and leaf fall at harvest (22%) in both the population. High heritability coupled with high Genetic advance as percentage of mean (GAM) was observed for all the traits except chlorophyll content, leaf moisture content, specific leaf area, and leaves per meter shoot indicating that most likely the heritability is due to additive gene effects and thus the improvement by selection would be highly effective.

Moderate heritability and low to moderate genetic advance was noticed in chlorophyll content index, specific leaf area, leaf moisture content and leaves per meter shoot indicating that traits might be governed by non-additive gene action and also the negative influence of environment in the expression of the traits. These traits can be improved through further crossing and selection. High heritability coupled with high genetic advance for several leaf yield traits in mulberry was also report by Goel *et al.,* (1998); Das and Krishnaswamy (1969); Tikader (1997); Tikader *et al.,* (2004); Doss *et al.,* (2006); Banerjee *et al.,* (2007); Mallikarjunappa *et al.,* (2008); Keshava Murthy *et al.,* (2010); Biradar *et al.,* (2015); Suresh *et al.,* (2017); Saini *et al.,* (2018); Chanotra *et al.,* (2019) and Magadum and Singh (2021).

Frequency distribution for eleven quantitative or metric characters in two pseudo F_2 populations was studied using skewness and kurtosis (Fig 1). The number of genes controlling the traits and the nature of gene action are the important criteria for the selection of individuals in segregating population. The skewness and kurtosis coefficients of all the traits studied in both the population is furnished in Table 1.

Significant and positive skewness was observed for traits leaves per meter shoot (0.178), chlorophyll content index (0.280), primary shoots per plant (0.376), total shoots length (0.455), leaf fall at harvest (0.568), fresh leaf area (0.629), fresh leaf weight (0.643), leaf moisture content (1.248) and leaf yield per plant (1.457) in CSRS-1 × V-1 population, while in Kajli OP × V-1 population it was recorded for leaves per meter shoot (0.123), leaf fall at harvest (0.330), specific leaf area (0.386), fresh leaf weight (0.517), unit leaf area (0.558), chlorophyll content index (0.569), length of longest shoot (0.917) and leaf yield per plant (1.097). The study of skewness explains degree of departure from the normal distribution and provides information about nature of gene action that controls a trait. The positively skewed distribution indicated dominance based complementary gene interaction having decreasing effect in the inheritance and suggests intensive selection of superior segregants will be effective. The traits specific leaf area (-0.102) and leaf moisture content (-0.166) in segregating population C-1 and C-2 respectively, displaying dominant based duplicate epistasis with increasing effect as indicated by significant negative skewness and suggests that these traits necessitates the more proportion of segregants for evaluation.

Kurtosis gives details about flatness or peakedness of a distribution and it estimates the number of genes controlling a trait. Lower kurtosis values indicate that data has light tails or lack of outliers. Positive kurtosis indicates a relatively peaked distribution. Negative kurtosis indicates a relatively flat distribution. The traits fresh leaf weight, unit leaf area, chlorophyll content index, specific leaf area, leaves per meter shoot, leaf fall at harvest, length of longest shoot, no of primary shoots, total shoots length and leaf yield per plant appeared to controlled by many segregating genes as indicated by platykurtic distribution. The trait leaf moisture content appeared to be controlled by few segregating genes as indicated by the leptokurtic (5.197 and 3.182) distribution in both the population. The platykurtic and positively skewed distribution for most of the characters indicated suggests intensive selection of superior segregants in both populations for mulberry improvement. This is our first report on frequency distribution analysis of leaf yield traits in mulberry by skewness and Kurtosis.

The phenotypic correlation coefficients among eleven leaf yield components in two segregating population of mulberry is furnished in Table 2. In the present investigation, leaf yield per plant is positively and significantly correlated with fresh leaf weight, leaf area, length of longest shoot, primary shoots and total shoots length per plant indicates the simultaneous improvement of these characters through selection.

Table.1 Estimates of genetic parameters for leaf yield and its attributes in two pseudo F_2 population of mulberry

Traits	Cross	FLW	FLA	CCI	SLA	LMC	LLS	NPS	TSL	LMS	LFH	LYP
CSRS-1	P1	3.744	186.45	14.5	269.46	72.45	208	9.48	1198	21.5	14.56	643
Kajli OPH	P2	3.014	154.56	14.83	185.74	78.55	166	9.07	1085	19.58	19.34	570
V-1	P3	4.153	175.55	17.16	147.41	76.85	213	14.75	1475	24.06	7.06	1023
S-1	Check1	2.740	186.45	14.50	269.46	72.45	168	9.48	1454	21.50	7.28	543
S-1635	Check2	4.208	254.77	18.47	246.80	80.83	185	16.00	2340	23.00	24.30	1169
Pseudo F_2 Mean	C1	2.75	154.04	14.02	304.68	81.38	156	12.35	1873	22.52	30.77	518
	C2	2.71	154.25	12.51	293.18	80.36	153	10.82	1634	23.62	34.06	535
Standardised range	C1	1.60	1.34	1.12	0.59	0.16	0.80	1.70	1.81	0.65	1.76	3.04
	C2	1.59	1.20	1.24	0.62	0.15	0.68	2.13	2.11	0.65	1.40	3.22
PCV (%)	C1	31.54	25.60	21.24	11.18	2.11	14.80	41.15	47.35	11.09	28.17	59.47
	C2	30.68	23.64	22.75	11.43	1.90	13.98	44.67	45.94	12.94	26.20	66.10
GCV (%)	C1	26.48	23.82	14.42	8.04	1.97	12.69	33.22	44.37	9.60	17.39	47.90
	C2	25.26	21.70	14.55	8.09	1.73	13.84	42.38	37.04	11.81	16.89	56.60
h^2_{bs}(%)	C1	70.50	86.56	46.09	51.73	86.70	73.59	65.15	87.80	74.96	38.10	64.87
	C2	67.83	84.28	40.93	50.14	83.18	98.04	90.01	65.03	83.30	41.57	73.32
GAM (%)	C1	45.80	45.65	20.17	11.91	3.77	22.43	55.23	80.64	17.12	22.11	79.48
	C2	42.86	41.05	19.18	11.81	3.26	28.24	82.83	61.54	22.20	22.43	75.85
Skewness	C1	0.643**	0.629**	0.280**	-0.102**	1.248**	-0.003	0.376**	0.455**	0.178**	0.568**	1.457**
	C2	0.517**	0.558**	0.569**	0.386**	-0.166**	0.917**	-0.003	0.529**	0.123**	0.330**	1.097**
Kurtosis	C1	0.224**	0.220**	-0.091	-0.331**	5.197**	0.227**	-0.563**	-0.683**	0.395**	1.383**	2.290**
	C2	0.352**	0.152**	0.167**	0.103**	3.182**	0.495**	0.047*	-0.450**	-0.238**	-0.114**	1.108**

Note: C1**:** CSRS1 × V1**;** C2: Kajli OP × V1, FLW: Fresh leaf weight (g), FLA: Fresh leaf area (cm^2), CCI: chlorophyll content index, SLA: specific leaf area ($cm^2\ g^{-1}$), LMC: Leaf moisture content (%), LLS: length of longest shoot(cm), NPS: No of primary shoots per plant(No),TSL: Total shoots length per plant(cm). LMS: leaves per meter shoot length (No), LFH: Leaf fall at harvest (%) and LYP: leaf yield per plant (g)

Table.2 Phenotypic correlation coefficients and Path Coefficients (Direct Effects) of growth traits with leaf yield in Mulberry

Traits	Cross	FLA	CCI	SLA	LMC	LFH	LMS	NPS	LLS	TSL	LYP	Direct Effects
Fresh Leaf Weight [FLW]	C 1	0.872**	0.231**	-0.549**	-0.138	-0.091	-0.203**	0.281**	0.409**	0.336**	0.330**	0.050
	C 2	0.843**	0.215**	-0.310**	0.107	-0.020	-0.172*	0.293**	0.355**	0.340**	0.315**	-0.036
Fresh leaf Area [FLA]	C 1	1	0.157*	-0.326**	-0.138	-0.103	-0.215**	0.277**	0.380**	0.325**	0.339**	0.044
	C 2		0.094	-0.0480	0.044	0.065	-0.167*	0.248**	0.306**	0.290**	0.307**	0.142
Chlorophyll content Index [CCI]	C 1		1	-0.490**	-0.407**	-0.068	-0.011	0.060	0.081	0.045	-0.032	-0.109
	C 2			-0.266**	-0.044	-0.100	0.021	0.007	0.096	0.042	-0.043	-0.094
Specific leaf area [SLA]	C 1			1	0.570**	0.209**	-0.020	-0.187*	-0.279**	-0.207**	-0.189*	0.034
	C 2				0.412**	0.232**	-0.026	-0.307**	-0.144	-0.271**	-0.306**	-0.060
Leaf moisture content [LMC]	C 1				1	0.231**	-0.039	-0.124	-0.141	-0.103	-0.125	-0.016
	C 2					0.251**	-0.060	-0.220**	-0.036	-0.180*	-0.297**	-0.129
Leaf fall at harvest [LFH]	C 1					1	0.165*	-0.040	-0.334**	-0.091	-0.342**	-0.249
	C 2						-0.222**	-0.116	-0.122	-0.123	-0.153*	-0.044
Leaves /meter shoot [LMS]	C 1						1	-0.201**	-0.292**	-0.258**	-0.245**	0.003
	C 2							0.004	-0.323**	-0.115	-0.297**	-0.047
No of primary shoots /plant[NPS]	C 1							1	0.542**	0.942**	0.748**	0.505
	C 2								0.384**	0.941**	0.747**	0.695
Length of longest shoot [LMS]	C 1								1	0.683**	0.651**	0.190
	C 2									0.626**	0.455**	0.199
Total shoots length /plant [TSL]	C 1									1	0.771**	0.122
	C 2										0.747**	0.107

Table.3 Eigen values, Cumulative proportion and PC values in pseudo F_2 population

Traits	CSRS-1 × V-1				Kajli OP × V-1			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Fresh leaf weight	0.326	-0.401	0.364	0.207	0.337	0.242	0.521	0.003
Fresh leaf area	0.288	-0.449	0.237	0.283	0.318	0.168	0.552	-0.044
Chlorophyll content	0.081	-0.010	0.579	-0.242	0.126	0.468	-0.162	0.158
Specific leaf area	-0.242	-0.285	-0.414	0.232	-0.260	-0.485	0.105	-0.087
Leaf moisture content	-0.143	-0.470	-0.125	0.275	-0.164	-0.432	0.413	-0.029
Leaves per meter shoot	-0.105	0.356	0.158	0.738	-0.163	-0.076	0.318	0.730
Leaf fall at harvest	-0.106	-0.370	-0.269	-0.164	-0.174	0.156	-0.184	0.473
Length of longest shoot	0.349	-0.136	-0.130	-0.283	0.381	-0.276	-0.169	0.344
No of primary shoots	0.432	0.164	-0.245	0.176	0.387	-0.136	-0.078	-0.147
Total shoots length	0.463	0.085	-0.261	0.046	0.409	-0.284	-0.134	0.259
Leaf yield per plant	0.425	0.138	-0.216	0.065	0.395	-0.259	-0.169	-0.033
Eigen values	3.716	1.818	1.402	0.995	4.130	1.999	1.303	1.096
Proportion	33.8	16.5	12.7	9.0	37.5	18.2	11.8	10.0
Cumulative Proportion	33.8	50.3	63.1	72.1	37.5	55.7	67.6	77.5

Fig.1 Frequency distribution curves in pseudo F_2 population of CSRS-1×V1 and Kajli OP×V1

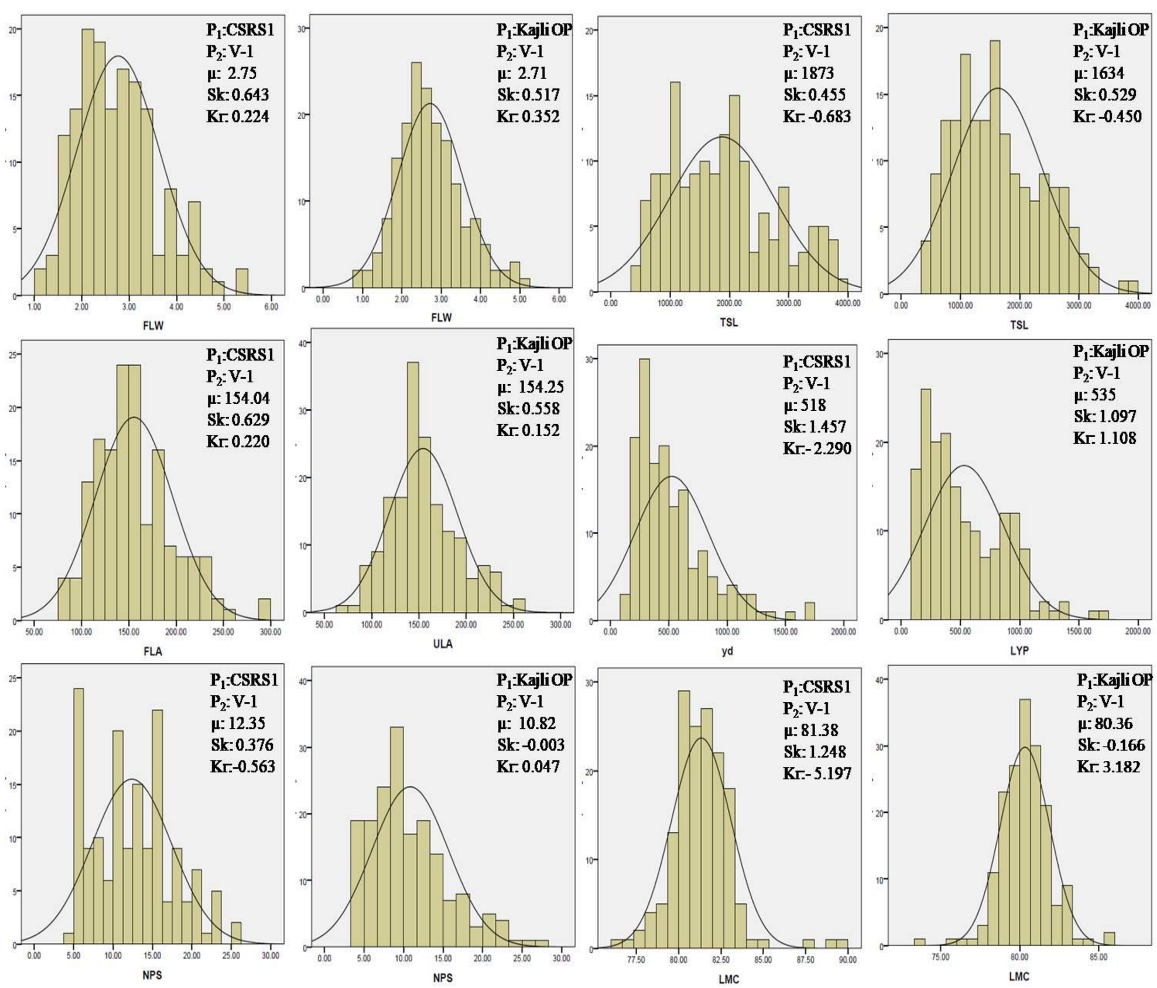

Table.4 Superior genotypes for leaf yield based on principle component index

Genotypes	PC1	PC2	PC3	PC4	FLW	FLA	NPS	TSL	LMC	LYP	% gain
CV-2	2.350	0.401	-0.876	0.253	3.873*	267.53*	25*	2705*	79.58	1.728*	48
CV-109	2.473	0.054	0.640	-1.238	5.467*	289.30*	21*	3510*	78.70	1.526*	31
KV-40	3.211	-0.932	0.550	1.262	4.540*	250.79*	27*	3754*	80.15	1.724*	47
KV-103	2.238	0.104	-1.136	0.599	3.410	195.67	22*	3256*	77.75	1.664*	42
KV-169	1.999	-1.845	-0.149	-0.589	3.320	168.52	24*	2920*	82.69*	1.443*	23
S-1635	*1.227*	*0.623*	*2.035*	*0.049*	*3.208*	*206.80*	*16*	*2340*	*80.83*	*1.169*	
CD5%					0.221	14.56	3	197	0.39	0.109	

Significant correlation of characters suggested that there is much scope for direct and indirect selection for further improvement. Specific leaf area, leaf moisture content, leaves per meter shoot and leaf fall at harvest recorded significant negative correlation with leaf yield per plant.

Chlorophyll content index showed non-significant negative association with leaf yield per plant. Several leaf yield component traits also recorded significant correlation among each others. Fresh leaf weight recorded significant positive association with leaf area, chlorophyll content index, shoots growth traits and also significant negative correlation with specific leaf area and leaves per meter shoot.

Leaves per meter shoot recorded significant negative correlation with fresh leaf weight, leaf size, length of longest shoot and leaf yield per plant. The weight and size of leaves was observed to inversely relate to leaves per meter shoot or plant. Specific leaf area had significant negative association with chlorophyll content index, and shoots growth traits. While, leaf moisture content and leaf fall at harvest had significant negative correlation in both the populations. The growth related traits like shoots per plant, longer shoot length and total shoots length per plant exhibited significant positive association among themselves. The leaf yield association with other quantitative traits in mulberry was also reported by Sarkar *et al.*, (1987); Bari *et al.*, (1989); Vijayan *et al.*, (1997b and 1998); Tikader and Roy (1999 and 2001); Tikader and Dandin (2005); Rahman *et al.*, (2006); Banerjee *et al.*, (2007); Mallikarjunnappa *et al.*, (2008); Doss *et al.*, (2012); Biradar *et al.*, (2015) and Suresh *et al.*, (2017); Saini *et al.*, (2018); Sathyanarayana and Sangannavar (2020); Magadum and Singh (2021) and Ravikumara and Ramesh (2022). Since, mulberry crop yield is directly and multiply determined by yield-component traits, the significant association will provide scope for mulberry improvement through indirect selection for development of superior cultivars for sericulture industry.

Path coefficient analysis has been widely employed in plant breeding to understand the nature of interactions between leaf yield and its contributing factors and to identify those factors that have a major impact on yield for prospective use as selection criteria (Mohammadi *et al.*, 2003). The phenotypic correlation coefficients of different quantitative traits were subjected to path coefficient analysis for estimating direct effects of component traits on leaf yield per plant. The direct effects of various traits on leaf yield per plant were presented in Table 2.

Path coefficient analysis revealed that, fresh leaf area, longer shoot length, primary shoots per plant, total shoots length had higher magnitude of direct effect on leaf yield in both the segregating population. Leaf moisture content recorded significantly negative association and direct effect with leaf yield per plant with low in magnitude (-0.129). The leaf fall at harvest showed negative direct effect on leaf yield per plant (-0.249). High positive direct effects for some of these characters were reported by Banerjee *et al.*, (2007); Doss *et al.*, (2012); Suresh *et al.*, (2017); Saini *et al.*, (2018);

Sathyanarayana and Sangannavar (2020); Magadum and Singh (2021) and Ravikumara and Ramesh (2022). Considering overall, fresh leaf area, longer shoot length, primary shoots per plant and total shoots length may be the most valuable traits for the selection programme to evolve high yielding mulberry variety.

Principal Component analysis was used to identify the important traits which contribute maximum variability for effective selection and also to rank genotypes on the basis of PC scores. PCA is appropriate tool for developing a smaller number of principal components that will account for most of the variance in the observed variables. In present investigation, principal component analysis explained the genetic variation for eleven leaf yield traits among the progenies in a segregating population under study.

The Eigen values, proportion of total variance and the component loading of different characters for the important principal components are presented in Table 3. Principal components with more than one Eigen values and higher than 4% variation were considered as main PC (Brejda *et al.,* 2000). Out of eleven PCs, only four principal components (PCs) exhibited more than 1.00 Eigen value and accounted about 72 to 75% variability among the traits studied (Table 2). Similar results for yield and biochemical traits were reported earlier in mulberry by Chang *et al.*, (2014); Eva María *et al.*, (2017); Suresh *et al.*, (2018); Zhenjiang *et al.*, (2021); Toolir and Mirjalili (2023). Eigen values measure the importance and contribution of each component to total variance, while each value indicates the degree of contribution of each original variable associated with each main component. The first principal component (PC1) in both the populations recorded the highest variation (34 to 37%) was strongly associated with primary shoots per plant (0.387, 0.432), longer shoot length (0.349, 0.381), total shoots length (0.409, 0.463) leaf yield per plant (0.395, 0.425). fresh leaf weight (0.326, 0.337) and fresh leaf area (0.288, 0.318) (Table 3). While, the other characters studied were negatively contributed. This component was regarded as a leaf and shoot growth component since it included several growth traits which mainly determine the leaf yield potential. The characters coming together in different principal components describe the variability to that remains together and these characters must be taken into consideration during the breeding program (Chakravorty *et al.,* 2013).

The PC2 accounted for about 16 to 18 per cent of total variance and it reflected positive loading of leaves per meter shoot (0.356) in C1 and chlorophyll content index (0.468) in C2, while other traits were noticed to be negatively loaded. The third PC3 was characterized noticeably by high loading of fresh leaf weight (0.364), chlorophyll content index (0.579) in C1 and fresh leaf weight (0.521), fresh leaf area (0.552), leaf moisture content (0.413) and leaves per meter shoot (0.318) in C2. This factor accounted for 12% of the variation and regarded as a leaf quality component.

The fourth principal component accounted for 9 to 10% of the variation and positively associated with leaves per meter shoot (0.730) in both populations. Through outcome of the PCA we could identify the characters responsible for genotypic variation within the group. PCA helped in identify the characters which have great impact in phenotype of different genotypes in segregating population of mulberry which is most important in the selection procedure of breeding programme. PCA has been used by various researchers like Chang *et al.*, (2014); Eva María *et al.*, (2017); Suresh *et al.*, (2018); Zhenjiang *et al.*, (2021) and Toolir and Mirjalili (2023) for characterization of different mulberry clones.

Selecting superior genotypes is a complex process because the economically important yield traits are quantitative by nature and interrelated. Principal component analysis transforms a number of correlated variables into a smaller number of uncorrelated variables called principal components. It also assists in identification of important traits for effective selection. Selection index can be computed using principal components having Eigen value greater than one. Based on the average of important PC scores, the ranks were assigned to all the progenies of two pseudo F_2 population for identification of superior genotypes.

Based on average score of PCs, several superior genotypes were identified and five having more than 20 percent higher leaf yield over S1635 were selected. The genotypes CV2 and CV109 from CSRS1×V1 and KV40, KV103 and KV169 from Kajli OP × V-1 recorded 1.44 to 1.72 kg leaf yield per plant under paired row cultivation (Table 4). These potential genotypes may further be evaluated for economical characters of silkworm and at different environments for identification of stable mulberry variety for subtropical sericulture.

Knowledge about the genetic variability of segregating population is essential for the crop improvement through selection a superior genotypes. In conclusion the high variability and heritability was recorded for traits fresh leaf weight, fresh leaf area, primary shoots per plant and total shoot length indicated high rate of trait transmissibility. Further, strong correlation and direct effects of these traits on leaf yield provide scope for prospective use as selection criteria. Platykurtic and positively skewed distribution of these characters indicated several segregating genes with dominance based complementary gene interaction and suggests intensive selection of superior segregants in both populations for mulberry improvement.

The results also indicate that leaf yield can be improved by direct and indirect selection. Principle component analysis revealed that these four traits mostly contributed to the variation in different principal components and mainly determined the leaf yield potential of mulberry genotypes. Hence, selection of the genotypes with the highest leaf yield along with higher fresh leaf weight, fresh leaf area, primary shoots per plant and total shoot length are recommended as selection strategy for genetic improvement of mulberry.

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