

Leaf Morpho-Anatomical Variability in Mulberry (*Morus* spp.) Germplasm

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Leaf anatomical parameters in 330 diverse mulberry germplasm resources (indigenous-182, exotic-108 and polyploids-40) were studied to estimate the variability and to identify potential mulberry varieties for stress tolerance. Estimates of genetic parameters showed high coefficients of variation (GCV and PCV) coupled with high magnitude of heritability and genetic advance for idioblast length and frequency, and upper and lower cuticular thickness. The least coefficient of variation was recorded in respect of leaf thickness and number of chloroplast/stomata. The leaves of exotic accessions were more thick and succulent than indigenous accessions. Significant positive correlations were observed between stomatal size, idioblast length, palisade thickness, spongy thickness, leaf thickness and number of chloroplast/stomata. Nine indigenous and 11 exotic accessions, which showed multiple drought tolerant characters were identified for stress condition.

Key Words: Leaf anatomy, Indigenous and exotic germplasm, Mulberry, Variability,

Mulberry, the principal food plant of silkworm (*Bombyx mori* L.) is cultivated for its leaf in different agroclimatic conditions for commercial production of silk. Major constraints to mulberry cultivation in India are the limited irrigation facilities, depleting ground water resources and prolonged dry spell with high temperature which reduces the crop yield to a greater extent. Thus, an assessment of variability of leaf anatomical parameters is of prime importance to identify the potential genotypes for stress tolerance.

Leaf anatomical features related to drought tolerance have been studied in crop plants (Dobrenz *et al.*, 1969; Oleinikowa and Kozhushoko, 1970; Miskin and Rasmussen, 1970; Ciha and Brun, 1975; Bhat and Andal, 1979 and Turner, 1979). Physiological and leaf anatomical parameters associated with drought tolerance was examined in mulberry by a few workers (Gupta *et al.*, 1988; Susheelamma and Jolly, 1986; Cappellozza *et al.*, 1996 and Dorcus and Vivekanandan, 1997). However, these studies in mulberry involved very few genotypes and were limited to a few parameters. Thus, an extensive study with a large number of mulberry varieties (330) was undertaken to estimate the nature and magnitude of variability of 12 leaf morpho-anatomical parameters related to stress tolerance.

Materials and Methods

In the present study, a total of 330 mulberry accessions (exotic-108, indigenous-182 and polyploid-40) were studied. The diploid exotics (2n=28) represent collections from 21 sericultural countries. The diploid indigenous accessions were collected from 13 Indian states including 47 locals besides 84 selections, 34 open pollinated

hybrids, 2 cross pollinated hybrids, 5 mutants and 10 wild collections. The polyploid accessions (2n=42, 56 and 84) were collected from different sources. The plantations are maintained as tree with 2.4 x 2.4 m spacing and crown height of 1.5 m at the CSGRC, Hosur, Tamil Nadu situated at latitude 12.45° N, longitude 77.51° E and altitude 942 m. Four plants are maintained per accession, out of which three plants were used for leaf sampling and data recording. Fully expanded leaves (5th to 7th position from top) in descending order were collected at 9-10 am from 3 month old shoots and small rectangular pieces were taken out from the central portion of the leaf blade avoiding veins and veinlets (Metcalfe and Chalk, 1979) and preserved in FAA solution (formalin 5 ml, glacial acetic acid 5 ml and 70 % ethanol 90 ml). The stomatal and idioblast studies were conducted by taking a thin layer of Wimbley's quickfix impressions on the abaxial (lower) and adaxial (upper) surfaces of the leaf respectively which were observed under Leica Leitz, DMRB Wetzlar microscope. Stomata and idioblasts were counted per unit area and the frequency per sq. mm was calculated. For observing the cuticular, epidermal, palisade, spongy mesophyll, leaf thickness and idioblast length, thin hand sections were made and observed under microscope after staining with 1 % safranin and mounted in 50 % glycerine. Chloroplast number per stomata was counted using epidermal peelings of the freshly collected leaf samples and stained with 2 % potassium iodide-iodine solution and observed under microscope. In each case nine microscopic fields were observed for data recording. The statistical analyses were carried out using software package developed by INDOSTAT services Ltd, Hyderabad, India.

Results and Discussion

Estimates of genetic parameters (Table 1) show the wide spectrum of variability in the mulberry germplasm under study. Exotic accessions exhibited higher mean and range for palisade, spongy, cuticular, epidermal and leaf thickness and lower values for stomatal size, idioblast length and frequency than indigenous accessions and offer scope as potential genetic material for stress tolerance breeding programmes. Polyploids recorded higher mean values over diploid indigenous and exotic accessions in all the traits except palisade thickness, stomatal and idioblast frequency. The estimates of genotypic and phenotypic coefficients of variation varied very little for all the traits indicating the importance of additive gene effects in the expression of the characters. The indigenous accessions exhibited higher GCV and PCV values in respect of idioblast length and frequency, palisade,

spongy, upper cuticle, lower cuticle and leaf thickness when compared to exotics. Exotic accessions recorded higher values for stomatal size, stomatal frequency, upper epidermal thickness than indigenous accessions. Polyploid accessions recorded higher GCV and PCV values in respect of stomatal size, idioblast length, upper and lower epidermal thickness and leaf thickness. The analysis of genetic parameters further showed that high GCV and PCV values coupled with high magnitude of heritability and genetic advance in respect of idioblast length and frequency followed by upper and lower cuticular thickness which indicates the scope for improvement through selection. The stomatal size and chloroplast number/stomata had less genotypic and phenotypic coefficient of variation with low heritability and genetic advance indicating low priority in selection programme.

Table 1. Estimates of variability and genetic parameters for leaf morpho-anatomical parameters in mulberry

Parameters	Acc.	Mean±SE	Range		GCV	PCV	h ² (%)bs	GA % of mean
			Min.	Max.				
Stomatal size (sq. µm)	Ind	261.59 ± 4.52	142.85	468.40	21.48	26.66	64.92	35.65
	Exo	250.55 ± 6.49	122.12	458.92	25.07	30.31	68.41	42.71
	Poly	316.07 ± 14.63	173.43	542.79	27.39	32.72	70.08	47.24
Stomatal frequency/sq.mm	Ind	673.35 ± 12.40	338.70	1240.96	24.47	25.63	91.14	48.11
	Exo	632.82 ± 17.41	344.87	1333.39	28.40	28.95	96.23	57.39
	Poly	574.09 ± 19.81	329.30	997.89	21.29	22.86	86.69	40.83
Idioblast length (µm)	Ind	10.73 ± 0.62	1.91	64.37	77.28	78.52	96.85	156.66
	Exo	10.67 ± 0.64	2.02	43.20	62.28	63.66	95.71	125.52
	Poly	14.97 ± 3.08	3.07	90.80	129.74	130.40	98.99	265.90
Idioblast frequency/sq.mm	Ind	20.97 ± 0.68	6.66	81.28	43.19	43.95	96.57	87.43
	Exo	17.60 ± 0.58	7.92	38.75	34.11	34.97	95.14	68.53
	Poly	17.45 ± 0.80	9.75	38.46	28.60	29.35	94.97	57.41
Palisade thickness (µm)	Ind	52.29 ± 1.02	18.22	146.14	26.63	26.52	97.92	53.50
	Exo	59.51 ± 1.34	33.33	107.66	24.13	24.32	98.47	49.34
	Poly	58.13 ± 2.14	39.46	102.68	23.32	23.52	98.27	47.62
Spongy thickness (µm)	Ind	61.07 ± 1.07	27.00	104.46	23.63	23.89	97.77	48.12
	Exo	62.14 ± 1.11	37.93	106.13	18.49	18.81	96.59	37.43
	Ploy	64.10 ± 2.13	36.79	103.45	20.96	21.13	98.33	42.81
Upper cuticle thickness (µm)	Ind	5.03 ± 0.14	2.02	10.15	37.09	38.04	95.10	74.52
	Exo	6.56 ± 0.21	2.02	11.81	33.38	34.27	94.88	66.97
	Poly	6.91 ± 0.31	3.55	10.73	28.14	29.37	93.94	56.19
Lower cuticle thickness (µm)	Ind	2.51 ± 0.10	0.81	7.28	54.17	55.20	96.29	109.50
	Exo	3.60 ± 0.14	1.01	7.28	40.56	42.81	89.78	79.18
	Poly	4.02 ± 0.24	1.68	10.15	37.92	39.36	92.79	75.24
Upper epidermal thickness (µm)	Ind	22.36 ± 0.43	6.90	45.98	25.63	27.26	88.36	49.62
	Exo	24.32 ± 0.64	10.23	43.20	27.07	27.88	94.24	54.13
	Poly	29.58 ± 1.41	11.11	49.81	30.06	30.63	96.26	60.75
Lower epidermal thickness (µm)	Ind	8.14 ± 0.16	26.02	19.54	25.43	26.69	90.73	49.89
	Exo	9.10 ± 0.23	3.45	18.77	25.42	26.46	92.30	50.31
	Poly	10.13 ± 0.67	5.17	24.71	41.49	42.07	97.27	84.30
Leaf thickness (µm)	Ind	151.33 ± 1.71	84.03	239.96	15.15	15.36	97.31	30.79
	Exo	165.23 ± 2.26	122.85	263.88	14.16	14.31	97.92	28.86
	Poly	172.86 ± 4.42	117.15	283.52	16.11	16.23	98.53	32.95
Chloroplast number/ stomata	Ind	10.18 ± 0.07	7.22	13.56	8.07	12.87	39.35	10.43
	Exo	10.72 ± 0.13	8.89	13.89	10.71	14.60	53.79	16.18
	Poly	15.15 ± 0.28	14.00	22.00	10.32	13.85	55.56	15.85

Ind- Indigenous, Exo-Exotic, Poly-Polyploid

Correlation studies on leaf anatomical parameters in mulberry are very limited. Hence, in the present study character association was attempted. Genotypic and phenotypic coefficient of correlations (Table 2) was in the same direction and the genotypic correlations were higher than the phenotypic correlations both in indigenous and exotic accessions indicating the inherent association between the characters. Stomatal size and frequency are negatively correlated as reported in barley (Miskin and Rasmussen, 1970) and in mulberry (Yang and Yang, 1995). Stomatal size and idioblast length showed significant positive correlations with palisade, spongy, lower cuticular, epidermal and leaf thickness whereas stomatal and idioblast frequency showed non-significant and negative correlations with these characters. This indicated the role of stomatal size for water retention in the leaves. Similar results in mulberry were also obtained Susheelamma and Jolly (1986). Positive correlations

were also found between palisade and spongy, leaf thickness and chloroplast number/stomata in both indigenous and exotic accessions.

The mulberry accessions are grouped into low, medium and high frequency groups based on observed range of variability for various leaf anatomical parameters (Table 3). The accessions from lower frequency group for stomatal size, stomatal frequency, and idioblast length and idioblast frequency are desirable for stress condition. Various stomatal characters like smaller size with lesser frequency which is responsible for low conductance have been suggested as desirable traits to improve drought resistance as they reduce water loss and lower the probability of dehydration (Dorcus and Vivekanandan, 1997). The mulberry genotypes which have leaves with small stomata and extensive deep root system reduce water loss and show better tolerance in stress condition (Susheelamma *et al.*, 1986).

Table 2. Genotypic (G) and phenotypic (P) coefficient of correlation in indigenous and exotic accessions

Parameters		1	2	3	4	5	6	7	8	9	10	11	12
1. Stomatal size (sq.µm)	G		0.192**	0.204**	-0.121	0.222**	0.231**	-0.028	0.146*	0.286**	0.180*	0.373**	0.285**
	P		-0.121	0.164*	-0.096	0.179*	0.189**	-0.019	0.017	0.214**	0.138*	0.299**	0.173**
2. Stomatal frequency/sq.mm	G	-0.375**		0.142*	0.241**	0.071	-0.079	0.116	0.166*	-0.019	-0.097	-0.009	0.111
	P	-0.307**		0.136	0.225**	0.067	-0.074	0.108	0.155*	-0.020	-0.086	-0.008	-0.069
3. Idioblast length (µm)	G	0.165	-0.061		0.035	0.179*	0.213**	-0.014	0.052	0.152*	0.287**	0.286**	0.102
	P	0.148	-0.061		0.033	0.175*	0.208**	-0.013	0.051	0.145*	0.267**	0.279**	0.068
4. Idioblast frequency/sq.mm	G	0.356**	0.531**	-0.038		0.093	-0.217**	0.011	0.006	0.054	-0.091	-0.075	-0.060
	P	0.289**	0.514**	-0.034		0.089	-0.210**	0.011	0.005	0.051	-0.083	-0.072	-0.030
5. Palisade thickness (µm)	G	0.464**	0.120	0.022	0.275**		0.109	0.041	0.034	-0.007	-0.017	0.661**	0.141*
	P	0.380**	0.116	0.020	0.267**		0.106	0.037	0.034	-0.005	-0.014	0.657**	0.098
6. Spong thickness (µm)	G	0.229*	-0.061	0.064	-0.144	0.181		0.045	0.037	0.087	0.233**	0.736**	0.228**
	P	0.187	-0.064	0.065	-0.138	0.175		0.045	0.035	0.080	0.219**	0.732**	0.154*
7. Upp. cuticle thickness (µm)	G	-0.191	-0.151	-0.043	0.314**	-0.018	0.138		0.604**	0.155*	0.087	0.207**	0.287**
	P	-0.143	-0.143	-0.048	-0.294**	-0.018	0.128		0.578**	0.147*	0.080	0.204**	0.188**
8. Lower cuticle thickness (µm)	G	0.063	0.068	0.188	-0.062	0.064	0.070	0.454**		0.231**	0.131	0.214**	0.268**
	P	0.074	0.064	0.176	-0.064	0.058	0.069	0.412**		0.213**	0.123	0.210**	0.165*
9. Upper epidermal thickness (µm)	G	0.238*	0.007	0.165*	0.034	0.288**	0.093	0.020	0.291**		0.283**	0.355**	0.259**
	P	0.178*	0.006	0.153	0.027	0.279**	0.093	0.019	0.266**		0.251**	0.359**	0.168*
10. Lower epidermal thickness (µm)	G	0.109	-0.086	0.161	-0.135	0.007	-0.039	0.161	0.188	0.144		0.310**	0.097
	P	0.064	-0.077	0.155	-0.132	0.007	-0.40	0.149	0.107	0.136		0.300**	0.053
11. Leaf thickness (µm)	G	0.461**	0.027	0.115	0.061	0.787**	0.642**	0.200*	0.272**	0.538**	0.147		0.344**
	P	0.374**	0.024	0.111	0.057	0.782**	0.641**	0.195*	0.260**	0.537**	0.147		0.230**
12. Chloroplast number/stomata	G	0.307**	-0.010	-0.105	-0.058	0.144	-0.057	0.112	0.076	0.008	0.084	0.086	
	P	0.160	-0.023	-0.075	-0.037	0.103	-0.036	0.081	0.036	0.015	0.068	0.067	

Above diagonal - Indigenous (diploid) and below diagonal - Exotic (diploid) **, * Significant at 1% and 5 % respectively.

Table 3. Frequency distribution of mulberry accessions in respect of leaf morpho anatomical-parameters

Parameters	Acc.	Frequency distribution					
		Low		Medium		High	
		Range	No. of acc.	Range	No. of acc.	Range	No. of acc.
Stomatal size (sq.µm)	Ind	< 200	17	200- 400	159	>400	6
	Exo		18		85		5
	Poly		4		26		10
Stomatal frequency/sq.mm	Ind	<400	4	400- 800	144	>800	34
	Exo		6		83		19
	Poly		2		36		2
Idioblast length (µm)	Ind	< 5	28	5-15	120	>15	34
	Exo		8		84		16
	Poly		7		23		10
Idioblast frequency/sq.mm	Ind	< 15	42	15 - 30	123	>30	17
	Exo		47		56		5
	Poly		15		24		1
Palisade thickness (µm)	Ind	< 50	83	50 - 75	90	>75	9
	Exo		24		71		13
	Poly		12		25		3
Spongy thickness (µm)	Ind	< 50	44	50-75	104	>75	34
	Exo		11		86		11
	Poly		3		28		9
Upper cuticle (µm) thickness	Ind	< 4	52	4-8	119	> 8	11
	Exo		13		66		29
	Poly		3		28		9
Lower cuticle thickness (µm)	Ind	< 2	76	2-4	84	> 4	22
	Exo		16		48		44
	Poly		1		25		14
Upper epidermal thickness (µm)	Ind	< 20	64	20 - 40	115	>40	3
	Exo		29		77		2
	Poly		5		31		4
Lower epidermal thickness (µm)	Ind	< 5	7	5-10	144	>10	31
	Exo		4		67		37
	Poly		0		24		16
Leaf thickness (µm)	Ind	< 150	92	150-175	64	>175	26
	Exo		31		47		30
	Poly		6		17		17
Chloroplast number/stomata	Ind	< 14	182	14-16	0	>16	0
	Exo		108		0		0
	Poly		0		34		6

Ind- Indigenous, Exo-Exotic indicates diploid accessions separately and Poly-Polyploid accessions both for indigenous and exotics

Idioblasts with long projecting spines in the leaves, which contain high quantity of tannins, affects the nutritional quality, palatability and digestibility of leaves by the silkworm (Cappellozza *et al.*, 1996). In the lower frequency group 28 indigenous, 8 exotic and 7 polyploid accessions (Table 3) have smaller idioblast length and 42 indigenous, 47 exotic and 15 polyploid accessions have fewer idioblasts per unit area.

The mesophyll tissue consisting of palisade and spongy parenchyma is the main photosynthetic zone. Thick, smooth, succulent and highly cutinized (glaucous) leaves, which can conserve water most efficiently, are desirable for dry sericultural zones. These two characters contribute most to leaf thickness and are highly heritable with moderate genetic advance. Hence, emphasis should be given for these traits during selection. The leaves of exotic accessions are comparatively thick and succulent

which can be used in crossing with promising indigenous varieties.

Further, chloroplast number per stomatal guard cells among 330 accessions ranged between 8-14 for diploids (2n=28), 14-16 triploids (2n=42) and >16 for tetraploids (2n=56) indicating positive association of chloroplast number with ploidy status which was also reported by Yang and Yang (1995) and Tikader *et al.* (1999) in mulberry. Polyploids are characterised by increased vigour and hardiness and particularly triploids exhibit more drought tolerant characters (Singh and Handique, 1995).

A perusal of data on ten promising varieties (Table 4) revealed that the accessions MI-119, 77, 54, 141 among indigenous and ME- 51, 2, 2, 65 among exotics had lowest stomatal size, stomatal frequency, idioblast length and frequency, respectively. Similarly,

Table 4. Ten promising accessions for leaf morpho anatomical parameters

Parameters	Acc.	Range	Accessions
Stomatal size (sq.µm)	Ind	142.85-180.50	MI-119,137,3,123,33,26,19,90,70,48
	Exo	122.12-174.47	ME-51,20,116,115,49,98,113,114,108,99
	Poly	173.43-245.82	MI-205,184,201,200,ME-84,59,72,92,97,38
Stomatal frequency/sq.mm	Ind	338.70-455.72	MI-77,87,8,161,142,80,116,9,28,78
	Exo	344.87-422.90	ME-2,62,90,91,49,54,89,53,82,51
	Poly	329.30-514.18	MI-212,203,196,204,192,202,200,ME-81,126,72
Idioblast length (µm)	Ind	1.91-3.64	MI-54,98,123,58,85,102,177,57,92,94
	Exo	2.02-5.23	ME-2,65,116,57,62,128,22,47,87,25
	Poly	3.07-6.51	MI-195,199,183,181,173,205,189,187,192,ME-84
Idioblast frequency/sq.mm	Ind	6.66-11.37	MI-141,118,35,30,115,52,139,124,27,86
	Exo	7.92-12.07	ME-65,87,60,93,105,42,49,102,20,82
	Poly	9.75-13.83	MI-184,204,173 ME-84,92,59,38,126,56,97,
Palisade thickness (µm)	Ind	74.93-146.14	MI-67,76,12,53,99,31,168,30,153,10
	Exo	80.08-107.66	ME-110,65,76,9,32,136,43,60,80,90
	Poly	66.83-102.68	MI-191,185,164,212,ME-52,97,59,72,126,81
Spongy thickness (µm)	Ind	82.35-104.46	MI-109,78,141, 135, 22,52,85,66,60,116
	Exo	75.48-106.13	ME-48,64,9,32,110,36,39,66,94,80
	Poly	72.41-103.45	MI-205,200,172,185,180,191,164, ME-72,92,81
Upper cuticle thickness (µm)	Ind	8.05-10.15	MI-147,161,68,76,99,160,102,43,207,177
	Exo	9.86-11.81	ME-96,83,5,17,42,13,131,80,136,36
	Poly	7.86-10.73	MI-186,180,182,212,195,200,202,172,ME-97,72
Lower cuticle thickness (µm)	Ind	5.36-7.28	MI-150,175,117,127,210,171,121,166,207,168
	Exo	6.32-7.28	ME-95,107,64,130,96,42,88,4,23,77
	Poly	4.31-10.15	MI-178,188,182,191,172,203,ME-84,81,38,126
Upper epidermal thickness (µm)	Ind	32.57-45.98	MI-144,72,53,156,73,166,168,13,207,162
	Exo	34.10-43.70	ME-128,117,122,23,85,116,43,3,9,76
	Poly	36.01-49.81	MI-195,234,190,180,198,212,196,201,199,ME-81
Lower epidermal thickness (µm)	Ind	11.71-19.54	MI-52,66,77,83,86,207,159,124,81,162
	Exo	12.15-18.77	ME-11,35,43,60,53,28,20,48,105,131
	Poly	11.11-24.71	MI-212,195,184,234,182,191,204,ME-63,81
Leaf thickness (µm)	Ind	186.20-239.96	MI-22,135,166,99,162,116,76,168,207,67
	Exo	197.51-263.87	ME-77,66,110,85,32,76,9,43,90,80
	Poly	187.36-283.52	MI-172,185,199,180,164,212,191,ME-72,81
Chloroplast number/ stomata	Ind	12.00-13.56	MI-50,155,168,69,162,171,179,211,197,207
	Exo	12.00-13.89	ME-14,64,91,102,77,108,78,85,67,68
	Poly	15.56-22.00	MI-181,201,212,195,203,164,185,234,ME-81,126

Ind- Indigenous, Exo- Exotic, Poly-Polyploid

the indigenous accessions MI-67, 116, 177, 168, 162, 162 and 67 and exotic accessions ME-90, 80, 36, 77, 76, 81, 131 and 80 which had highest palisade, spongy, upper and lower cuticular thickness, upper and lower epidermis and leaf thickness, respectively, are promising for further exploitation. However, the accessions like MI-212, 195, 197, 210, 116, 172, 234, 191, 162 and ME- 81, 80, 43, 9, 65, 90, 116, 49, 62, 63 and 84 which exhibited three or more drought tolerant traits may be useful for evolving drought tolerant genotypes through appropriate breeding programme. The promising triploids like MI-172, 173, 187, 188 and ME-52, 84, which had chloroplast number 14 to 16 are high yielders and possess all the desirable characters may also be used for direct exploitation. Thus, the results indicated that no single variety possesses all the desirable characters for drought tolerance hence, repeated intermating of the promising accessions followed by selection of the

recombinants for pyramiding the drought tolerant traits are suggested.

Mulberry genotypes are heterozygous due to out crossing and hence selections can be practised for desirable variants among the progeny. The extent of variability in leaf anatomical parameters in the mulberry germplasm resources revealed in the present study gives better scope for breeders for selection. Stress tolerance in cross-pollinated crops is generally polygenic (Hurd, 1976). The promising genotypes identified through screening can be utilised to develop varieties through suitable breeding programmes.

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