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Effects of chromosomal variations on morphology and leaf anatomical behaviours in mulberry (*Morus* sp.)

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ABSTRACT

Mulberry (Morus sp.) used as feed to silkworm (Bombyx mori L.) is basically a diploid plant with 28 chromosomes (2n=28). The cross pollinated and heterozygous nature of mulberry created wide variations in number of chromosomes which resulted in phenotypic differences in the progenies. Study on mulberry genotypes showed differences on morphological and leaf anatomical characters with the differences of basic sets of chromosomes number. Triploids (2n=3x=42) were superior to the diploids and tetraploids. Upper cuticle (r = 0.77 µm), palisade (r = 0.71 µm) and spongy parenchyma (r = 0.74 µm) layers of leaf showed positive correlation with the increased number of chromosomes. The bigger size and lesser number of stomata in leaves were recorded in tetraploids followed by triploids and diploids. Leaf thickness and number of stomata were indicative of ploidy and can be used as preliminary identifying characteristics for screening of mulberry germplasm for breeding.

Key words: Leaf anatomy mulberry, morphology and ploidy.

Mulberry (*Morus* sp.) is basically a diploid (2n=28) plant. Due to heterozygous and cross pollinated nature, large populations of mulberry were found in the nature with the differences in their morphological features and levels of basic number of chromosomes. These progenies were at triploids, tetraploids, hexaploids, octaploidy and even decosoploid levels having 2n=22x=308 chromosomes. The phenotypic and genotypic behaviours of these plant populations were different. Mulberry being the chief food plant of silkworm *Bombyx mori* L., its leaves were found to be not palatable to silkworms when number of chromosomes were higher in the plants. Information on parents to be used for crossing being important for drawing selective breeding plan, the study was conducted in order to have database on the morphological and leaf anatomical behaviours of mulberry at differential levels of chromosomes number for utilizing the suitable germplasm with desirable quality in breeding programmes.

MATERIALS AND METHODS

Six mulberry genotypes two each at three levels of chromosomes number such as, S-1 and V-1 (2n=28 chromosomes, diploid), S-1635 and C-1730 (2n=3x=42, triploid) and T-1 and T-23 (2n=3x=56, tetraploid) were studied during 2009-10 at Central Sericultural Research & Training Institute, Berhampore, West Bengal. Data were recorded on plant morphology and microscopic observations made on leaf anatomical characters. Leaves were cross sectioned for leaf anatomy study and mounted with 5% glycerin solution for leaf thickness; for stomata study leaf sample were decolourized fixing in 1:3 acetic-ethanol for 12 hours and in 95% ethanol for overnight followed by gradual transferring through alcohol grades in descending order and stained with 2% iodine-potassium iodide solution. Cystolith and trichomes were observed by staining the decolorized leaf segments with 1% methylene-blue for one minute.

RESULTS AND DISCUSSION

Plant morphology

Data recorded on morphological characters showed variations among the genotypes at different levels of chromosomes number (Table 1). Branching nature was erect, young shoot colour varied from green to dark green, mature shoot colour green to brown, phyllotaxy ranged between 1/3 and 2/5, leaf shape entire to trilobed, leaf colour green to dark green with smooth, rough surface with acute and acuminate apex. Leaf margin was serrate to dentate with cordate to ovate leaf bases.

Variations in morphological characters of the genotypes exhibited the heterozygous nature of mulberry. Rough and hairy leaf surface, dentate leaf margin and higher thickness of leaf lamina were recorded in tetraploid as compared to triploid and diploid genotypes which corroborates the earlier findings that morphological characters being taxonomic and heritable in nature are used for classification of genotypes and species.

Leaf anatomy study

Analysis of variance (ANOVA) of data of leaf anatomy characters (Table 2) revealed that thickness of upper cuticle was significantly higher in tetraploids (10.52 µm) followed by triploids (6.28 µm) and diploids (6.27 µm), however, between diploids and triploids, upper cuticular thickness was *at par* within the genotypes except tetraploid where, significant variation exists between T-1 (10.94 µm and T-23

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(10.09 μm). Thickness of lower cuticle was significantly lower in triploid (4.01 μm) but was *at par* in diploid (6.24 μm) and tetraploid (5.21 μm). Upper epidermis was thicker in tetraploids (22.57 μm) than the diploids (21.04 μm) and triploids (22.05 μm). Thickness of upper epidermis was higher in S-1635 (25.74 μm) and least in C-1730 (18.37 μm) while lower epidermis in diploids (14.05 μm) followed by tetraploids (13.11 μm) and triploids (12.81 μm). However, thickness of upper and lower epidermis in all the test genotypes was *at par*.

A significant variation in palisade parenchyma was observed within the ploidy. Measurement of palisade parenchyma showed significantly higher values in tetraploids (70.40 μm) but was *at par* in diploids (43.17 μm) and triploids (42.35 μm). Among the genotypes, T-23 (73.70 μm) of tetraploids showed significantly higher values followed by T-1 (67.1 μm) of tetraploid, V-1 (47.85 μm) of diploid and S-1635 (44.55 μm) of triploids. Thickness of spongy parenchyma varied significantly with the levels of ploidy and significantly higher values were recorded in tetraploids (58.02 μm) and triploids (39.98 μm) than the diploids (35.47 μm). However, in diploids and triploids, spongy parenchyma thicknesss was *at par*. Although genotypes in diploid and triploid were *at par*, tetraploids, T-23 (64.35 μm) and T-1 (51.70 μm) showed significantly higher values. Mulberry genotypes with thicker leaves observed in higher ploidy are not suitable for silkworm feeding due to coarseness, hairiness and less succulence of leaf as was found in tetraploids with the morphological characters such as, coarseness and dentations in leaf margin (Vijayan *et al.*, 1999).

Number of stomata per sq. mm was significantly higher in diploids (149.05) than the triploids (78.1) and tetraploids (49.5). Among the genotypes, frequency of stomata was higher in V-1 (179.3) followed by S-1 (118.8), S-1635 (99.0), C-1730 (57.0), T-1 (53.9) and least in T-23 (45.1). Stomata length, breadth and size were found significantly higher in tetraploids (83.87 μm, 53.90 μm and 427.13 μm respectively) than triploids (71.22 μm, 42.46 μm and 280.41 μm) and diploids (57.37 μm, 34.59 μm and 215.65 μm). Among the diploids and triploids, the values were significantly higher in triploids than in diploids. Chloroplasts counted in the guard cells of stomata showed that in tetraploids, number of chloroplasts in the guard cells were higher and bigger in size. An average of 7-8 chloroplasts was observed in each guard cell. However, the number was less in diploid but was *at par* with the triploids. Number of chloroplast was higher in T-1 and T-23 followed by S-1635, C-1730 and S-1 genotypes. The mean chloroplast number per guard cell was 5.6±1.6, 6.2 ±1.2 and 8.5 ±1.4 in the diploid, triploid and tetraploid, respectively. Cystolith and trichomes were varied in size with the ploidy. The trichomes on the upper surface of the leaves were pointed at the tip in all the genotypes. In tetraploids, trichome density was higher than the triploids and diploids.

Leaf anatomy and presence of trichomes on leaf indicate the quality of leaf. Higher thickness and more coarseness of leaves was found in tetraploids than the triploids which is responsible for poor quality and palatability of mulberry to silkworm. The bigger size and lesser number of stomata in leaf were recorded from tetraploids followed by triploids and diploids (Yang and Yang, 1995) (Fig. 1-3). Chloroplasts in stomatal guard cells at different ploidy showed an increase in number with the increase of ploidy supports the observation that it is directly associated with the ploidy (Tikader and Rao, 2001). Variations of cuticle, palisade and spongy parenchyma cells were influenced by the levels of ploidy. Significant differences in all the parameters of stomata were observed in diploid and triploid but no significant difference observed in tetraploid.

A highly positive correlation was obtained between upper cuticle (r = 0.77 μm), palisade parenchyma (r = 0.71 μm) and spongy parenchyma (r = 0.74 μm) and the levels of ploidy (Table 3) while the upper epidermis though have positive correlation but was *at par*. Number of stomata, stomata length and breadth and size showed significant negative correlation with the ploidy. Upper epidermis and lower cuticle were not influenced by the level of ploidy.

Leaf anatomical features are associated with many physiological functions which in turn influence the quality of leaf. Resistance of water vapour and exchange of Carbon dioxide and other gases are directly related to stomatal frequency and size (Lea *et al.* 1977). In mulberry quality of leaf influences the palatability to the silkworm. Differences in stomatal characteristics, its frequency and thickness of leaf alongwith anatomical features recorded at differential levels of chromosomes number in the genotypes were indicative of ploidy and corroborates the observations of earlier workers (Chaudhari and Barrow, 1975; Sikdar *et al.*, 1986; Yang and Yang, 1995 and Tikader and Rao, 2001) and can be used as preliminary and rapid indirect methods to identify ploidy level of mulberry parents for breeding programmes (Mishra, 1997 and Beck *et al.*, 2002).

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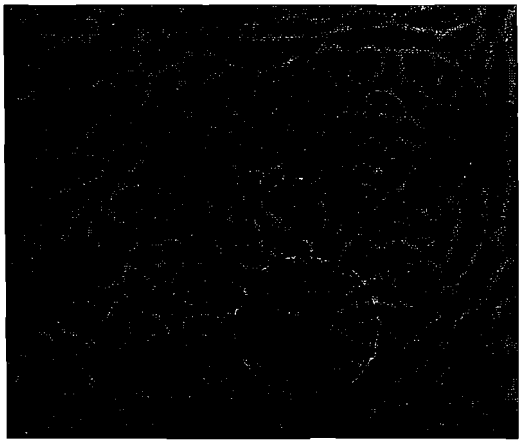

Fig. 1: Stomata in diploid mulberry

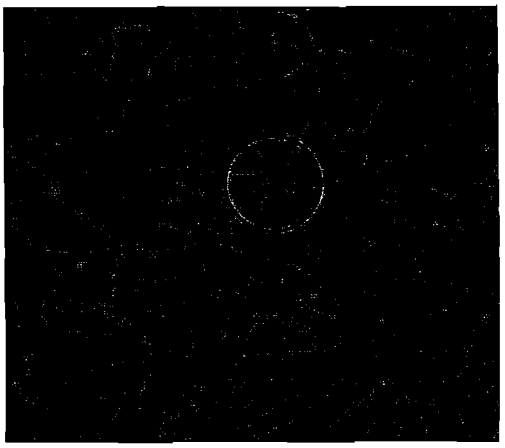

Fig. 2: Stomata in triploid mulberry

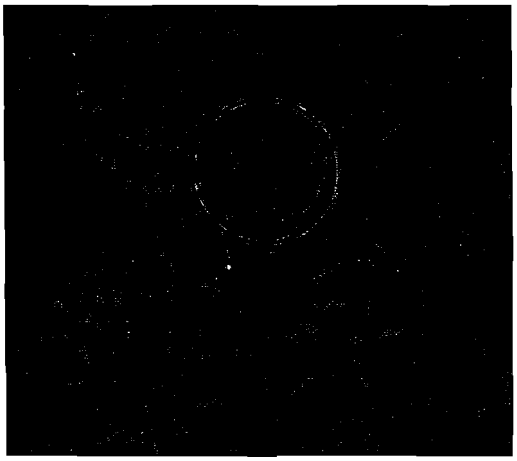

Fig. 3: Stomata in tetraploid mulberry

REFERENCES

Beck, S. L., Dunlop, R. W. and Flossy, A. 2002. Stomatal length and frequency as a measure of ploidy level in black wattle, *Acacia mearnsii* (de Wild). *Bot. J. Linnean Soc.,* **141**: 177-81.

Chaudhari, H. K. and Barrow, J. R. 1975. Identification of cotton haploid by stomatal chloroplast technique. *Crop Sci*, **15**: 760-63.

Lea, H. Z., Dunn, G. M. and Koch, D.W. 1977. Stomatal diffusion resistance in three ploidy levels of smooth bromegrass. *Crop Sci*, **17**: 91-93.

Mishra, M. K. 1997. Stomatal characteristics at different ploidy levels in *Coffea* L. *Annals of Bot.* **80**: 689-92.

Sikdar, A. K., Dwivedi N. K., Dandin S. B., Kumar R., Giridhar. 1986. Stomatal chloroplasts count technique as a tool to ascertain different ploidy levels in mulberry. *Indian J. Seric.*, **25**: 88-90.

Tikader, A. and Rao, A. A. 2001. Morpho- anatomical and pollen studies in mulberry germplasm. *Sericologia* , **41**: 69-76.

Vijayan, K., Sahu, P. K., Chakraborti, S. P. and Roy, B. N. 1999. Evaluation of tetraploids for triploid breeding in mulberry. *Sericologia,* **59**: 515-22

Yang, J. H. and Yang, X. H. 1995. Relationship between stomatal chloroplast number and ploidy levels in mulberry (*Morus* spp.). *Sericologia,* **35**: 289-92.

Table 1: Morphological characters at different ploidy levels

Parameters	Diploid		Triploid		Tetraploid	
	S-1	V-1	S-1635	C-1730	T-1	T-23
Level of ploidy	Diploid (2n = 28)	Diploid (2n = 28)	Triploid (2n= 3x=42)	Triploid (2n= 3x=42)	Tetraploid (2n= 4x=56)	Tetraploid (2n= 4x=56)
Nature of plant	Erect	Erect	Erect	Erect	Erect	Erect
Branching pattern	Erect	Erect	Erect	Erect	Erect	Erect
Young shoot colour	Dark green	Green	Dark green	Dark green	Green	Green
Mature shoot colour	Dark brown	Reddish brown	Brown	Reddish brown	Brown	Green
Phyllotaxy	1/3	1/8	2/5	1/3	1/8	1/3,1/4
Trichome density/cm^2	6.21	5.6	7.4	6.9	8.9	9.2
Stipule nature	Free lateral	Free lateral	Free lateral	Free lateral	Free lateral	Free lateral
Stipule duration (days)	Cauducous (7-8)	Cauducous (7-8)	Cauducous (7-8)	Cauducous (7-8)	Cauducous (7-8)	Cauducous (7-8)
Leaf lobation	Entire	Entire	Entire	Entire	Entire, sometimes three	Three
No. of lobes/ leaf	0, entire	0, entire	0, entire	0, entire	0, trilobed	3, deep lobe
Leaf colour	Dark green	Dark green	Dark green	Dark green	Green	Dark green
Leaf texture	Smooth, glossy	Smooth, glossy	Rough	Rough, Glossy	Rough and hairy	Rough and hairy
Leaf apex	Acuminate, acute	Acute	Acuminate	Acute	Acute	Acuminate
Leaf margin	Serrate	Serrate	Serrate	Serrate	Dentate	Dentate
Leaf base	Ovate/ truncate	Cordate	Cordate	Cordate	Cordate	Cordate
Leaf shape	Ovate	Ovate	Ovate	Ovate	Ovate	Ovate, deep lobed
Leaf length (cm)	16	19.5	19	20.5	16	30
Leaf width (cm)	10	10	14.5	14	13	21
Petiole length (cm)	2.5	3.5	2	3.5	1.6	3.5
Petiole diameter (mm)	3	3	4	4	4	6
Leaf size (cm)	160	195	275.5	287	208	630
Lenticel size (mm)	0.5-1	2	2	1-2	1	1.5
Lenticels density (No. / cm^2)	7	5	5	10	12	14

Table 2: Leaf anatomy and stomatal features

Genotypes	Cuticle thickness (µm)		Epidermis thickness (µm)		Parenchyma cells (µm)		No. of stomata /mm^2	Size of stomata (µm)		Size of stomata (µm)
	Upper	Lower	Upper	Lower	Palisade	Spongy		Length	Breadth	
Diploid										
S-1	6.17	5.170	21.98	11.44	38.50	37.40	118.80*	71.500	35.200	230.670
V-1	6.37	7.315	20.11	16.66	47.85	33.50	179.30*	63.250	33.990	200.640
Mean	***6.27***	***6.243***	***21.04***	***14.05***	***43.17***	***35.47***	**149.05***	**67.375**	**34.595**	**215.655**
Triploid										
S-1635	5.99	3.960	25.74	18.81	44.55	41.47	99.00*	77.000	46.200	330.000
C-1730	6.56	4.070	18.37	6.82	40.15	38.50	57.00*	65.450*	38.720*	230.835
Mean	***6.28***	***4.015***	***22.05***	***12.81***	***42.35***	***39.98****	***78.10****	***71.225****	***42.460****	***280.418****
Tetraploid										
T-1	10.94*	5.885	22.93	11.27	67.10*	51.70*	53.90	75.900	57.750	402.050
T-23	10.09*	5.940	22.22	14.96	73.70*	64.35*	45.10	91.850	53.845	452.210
Mean	***10.52***	***5.913***	***22.57***	***13.11***	***70.40****	***58.02****	***49.50***	***83.875****	***55.798****	***427.130****
LSD(0.05)	**0.659**	**1.212**	**NS**	**NS**	**5.135**	**4.394**	**10.53**	**6.662**	**6.126**	**60.566**

Table 3. Correlation coefficients among leaf anatomical features

	Upper cuticle	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Lower epidermis	Lower cuticle	No. of stomata mm^{-2}	Stomata length	Stomata breadth	Stomata size
Ploidy level	0.771**	0.152	0.715**	0.743**	-0.086	-0.063	-0.820**	0.493**	0.670**	0.659**
Upper cuticle		-0.017	0.765**	0.634**	-0.093	0.205	-0.537**	0.341**	0.489**	0.477**
Upper epidermis			0.097	0.186	0.358**	-0.080	-0.047	0.279*	0.337**	0.355**
Palisade parenchyma				0.665**	0.116	0.230	-0.410**	0.413**	0.471**	0.498**
Spongy parenchyma					0.063	0.123	-0.576**	0.524**	0.561**	0.603**
Lower epidermis						0.189	0.349**	0.143	0.062	0.136
Lower cuticle							0.199	-0.089	-0.055	-0.046
No. of stomata mm^{-2}								-0.394**	-0.510**	-0.507**
Stomata length									0.572**	0.830**
Stomata breadth										0.921**

** $P<0.01$, * $P<0.05$