Uttar Pradesh J. Zool. 32(2) : 217-221, 2012 ISSN 0256-971X

EFFECTS OF PLOIDY ON MORPHOLOGY, LEAF ANATOMY AND PROPAGATION EFFICIENCY IN MULBERRY (*MORUS* SP.)

SUDHIR DAS, SUKHEN ROYCHOWDHURI*, LALTANMAWII*, BIJAN MANDAL, KUNAL SARKAR AND GOPINATH BAUR
KRISHNATH COLLEGE, MURSHIDABAD, INDIA.
CENTRAL SERICULTURAL RESEARCH & TRAINING INSTITUTE, BERHAMPOR-742 101, INDIA*. .

Mulberry (*Morus* sp.) foliage are the source of nutrients for silkworm (*Bombyx mori* L.) rearing. It is basically a diploid plant with 28 chromosomes (2n=28) have differences in ploidy due to its high heterozygous nature. Morphology, leaf anatomical study and propagation efficiency at diploid, triploid and tetraploid of mulberry showed significant differences. Among different ploidy levels, triploid were found better than diploid and tetraploid. Thickness of upper and lower cuticle was significantly lower in triploid (6.21; 4.51 µm) than the tetraploid (10.65; 6.64 µm) and the diploid (6.29; 5.39 µm). Thickness of upper and lower epidermis was higher in triploid (26.73; 18.15 µm) than the tetraploid (24.64; 14.19 µm) and diploid (22.11; 10.56 µm respectively). Palisade and spongy parenchyma cells showed significantly higher values in tetraploid (71.06; 59.18 µm) followed by triploid (43.23; 40.48 µm and diploid (37.84; 35.68 µm respectively). The characters namely tickness of cuticle, epidermis, palisade and spongy parenchyma cells and the size of stomata of mulberry leaf are influenced with the levels of ploidy, where thickness of cuticle, palisade and spongy parenchyma cells may be considered as indicators for identification of ploidy, as these parameters showed positive with the increase of ploidy levels. In propagation study, number of roots /plant, root area (cm), root length (cm), root hair zone (cm), root volume (cm3/litre), fresh wt. (g) and dry wt. (g) of root showed significantly higher in triploid than the tetraploid but was *at par* with the diploid indicate the superiority of triploid variety. Moreover, cuttings obtained from the base of the mulberry shoots ensure higher survival.

Key words : Anatomy, leaf, morphology, *Morus* sp., ploidy, stomata, silkworm breeding.

INTRODUCTION

Mulberry (*Morus* sp.) is a perennial tree of economic importance mainly grown for its leaves to rear silkworm (*Bombyx mori* L.) for sericulture industry. It being basically a diploid plant with 28 chromosomes in its somatic cells (2n=28), due to heterozygous, cross pollinated nature and frequent mixing of pollens in mulberry, a greater number of ploids is created in the nature with highest number of chromosomes of 308 (Das & Prasad, 1971). However, mulberry has the advantages of propagating vegetatively through stem or shoots clones and restores the inherent qualities perpetuating through the clones without deterioration of qualities. On sericulture point of view, diploids and triploids mulberry are superior in quality and productivity than the higher levels of ploidy. Information on morphology, anatomy and propagation efficiency of mulberry varieties at different ploidy levels are meager, this being an important pre-requisite for selection of suitable parents in breeding programme, the study therefore, was conducted on morphology, leaf anatomy and adaptability of mulberry varieties at diploid, triploid and tetraploid levels so that the information on quality of mulberry at different ploidy shall be used for identification and preliminary selection and further exploitation before their cytological confirmation.

MATERIALS AND METHODS

Mulberry varieties one each at three levels of ploidy namely *Morus indica* of diploid, S-1635 of triploid and *Morus laevigata* of tetraploid were studied. Data were recorded on morphological characters of the plants, leaf anatomical characters and propagation efficiency. For morphological study, three plants of each ploidy level were observed and data recorded. For leaf anatomy study, leaves were cross sectioned, mounted with 5% glycerin solution for leaf thickness and observed under the compound microscope (Olympus, 600x). For stomata study leaf samples 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 and studied with Litz dia-plane phase contrast microscope (1000 x). For propagation study, fifty cuttings of each variety were planted in the nursery beds in three replications. Uniform cuttings at two categories *i.e.* hard wood (base and middle) and soft wood (tip) of a shoot were taken for the propagation and root proliferation study. Data recorded on survival on 15th day of cuttings plantation and continued upto 60th day. For root proliferation study, weight of root was taken with electronic balance. Root length and root area was measured with the help of scale, number of roots and number of leaves was counted, root volume was measured with the help of one litre measuring cylinder. Data were statistically analysed.

RESULTS AND DISCUSSION

Plant morphology

Morphological characters showed variation with the levels of ploidy. Branching was erect, colour of young shoots varied from green to dark green, mature shoot green to brown; phyllotaxy 1/3 and 2/5; leaf shape entire to trilobed; leaf colour green to dark green and leaf surface smooth, rough and leaf apex was acute and acuminate. Leaf margin was serrate to dentate and leaf base cordate to ovate (Table I).

Leaf anatomy study : Thickness of cuticle, epidermis, parenchyma cells and size of stomata were significantly varied with the differences of ploidy level (Table II).

Cuticle thickness : Thickness of upper and lower cuticle was significantly lower in triploid (6.21; 4.51 µm) than tetraploid (10.65; 6.64 µm) but was at par with the diploid (6.29; 5.39 µm).

Epidermis : Thickness of upper and lower epidermis was significantly higher in triploid (26.73; 18.15 µm) than the tetraploid (24.64; 14.19 µm) diploid (22.11; 10.56 µm).

Parenchyma cells : Thickness of palisade and spongy parenchyma cells showed significantly higher in tetraploid (71.06; 59.18 µm) followed by triploid (43.23; 40.48 µm and diploid (37.84; 35.68 µm, respectively).

Study of stomata : Among the ploids, frequency of stomata per unit area was significantly higher in diploid *M. indica* (118.8) followed by triploid S-1635 (97.7) and tetraploid *M. laevigata* (59.4), while, length, breadth and size of stomata were significantly higher in tetraploid (97.35 µm, 59.95 µm and 440 µm, respectively) than the triploid (80.3 µm, 52.41 µm and 265.48 µm) and diploid (67.65 µm, 35.22 µm and 217.25 µm). Number of chloroplasts count in the guard cell of stomata showed that in tetraploid, chloroplasts in the guard cells were higher and bigger in size. An average of 7-

Table I : Morphological characters of mulberry at different ploidy levels.

Parameters	*M. indica*	S-1635	*Morus laevigata*
Level of ploidy	**Diploid (2n = 28)**	**Triploid (2n= 3x=42)**	**Tetraploid (2n= 4x=56)**
Nature of plant	Erect	Erect	Erect
Branching pattern	Erect	Erect	Erect
Young shoot colour	Dark green	Dark green	Green
Mature shoot colour	Dark brown	Brown	Brown
Phyllotaxy	1/3	2/5	1/8
Trichome density/cm^2	6.26	7.8	8.9
Stipule nature	Free lateral	Free lateral	Free lateral
Stipule duration (Days)	Cauducous (7-8)	Cauducous (8-10)	Cauducous 10-12)
Leaf lobation	Entire	Entire	Entire, sometimes three
No. of lobes/ leaf	0, entire	0, entire	0, trilobed
Leaf colour	Dark green	Dark green	Green
Leaf texture	Smooth, glossy	Rough	Rough and hairy
Leaf apex	Acuminate, acute	Acuminate	Acute
Leaf margin	Serrated	Serrated	Dentate
Leaf base	Ovate/ truncate	Cordate	Cordate
Leaf shape	Ovate	Ovate	Ovate
Leaf length (cm)	16.3	19.2	16.2
Leaf width (cm)	10.1	14.9	13.3
Petiole length (cm)	2.6	2.5	1.8
Petiole diameter (mm)	3.2	4.1	4.2
Leaf size (cm)	168	278.4	209
Lenticel size (mm)	0.5-1	2	1
Lenticels density (No. / cm^2)	7	5	12

Table II : Leaf anatomy and stomatal features..

Variety	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	
Morus indica	6.29	5.39	22.11	10.56	37.84	35.68	113.3 *	67.65	35.22	217.25
S-1635	6.21	4.51	26.73 **	18.15 **	43.23 **	40.48 **	97.7 **	80.3 **	52.41 **	365.46 **
Morus laevigata	10.65 **	6.64 **	24.64 **	14.19 **	71.06 **	59.18 **	59.40	97.55 **	58.95 **	440.0 **
CD at 5%	1.054	0.881	1.365	1.004	2.865	3.034	10.52	5.145	5.247	53.977

Table III : Root propagation efficiency of mulberry varieties at different ploidy level.

Ploidy	Cuttings sprouted / survivability (%)				Shoot length (cm)		
	15th day	30th day	45th day	60th day	30th day	45th day	60th day
Position of cuttings							
Base	84.44	72.78*	68.33**	67.78**	11.76	20.41	23.39
Middle	83.89	85.00*	55.56	55.56	9.82	16.51	20.22
Tip	76.11	51.67	41.11	40.00	7.53	12.38	17.06
Level of ploidy							
Diploid	89.44**	80.56	73.89**	73.89**	10.01	18.52	21.07
Triploid	98.33**	82.22*	77.22**	76.67**	10.06	18.80	23.83
Tetraploid	56.67	56.67	43.89	42.78	9.04	11.98	15.77
CD at 5%	**10.05**	**18.67***	**21.50****	**22.29****	**NS**	**NS**	**NS**

Table IV : Root proliferation of mulberry varieties at different ploidy level.

Level of ploidy	No. of roots/ plant	Root area (cm)	Root length (cm)	Root hair zone (cm)	Root volume (cm^3/litre)	Root weight	
						Fresh wt. (g)	Dry wt. (g)
Diploid	10.28**	5.39	12.42	1.88	4.32	0.24	0.18*
Triploid	12.67**	6.92**	14.08	2.27**	5.32**	0.28	0.18*
Tetra-ploid	8.06	4.72	11.78	1.73	3.86	0.23	0.08
CDat 5%	**2.005**	**1.215**	**NS**	**0.166**	**0.643**	**NS**	**0.072**

8 chloroplasts was observed in each guard cell. However, in diploid the number was lessbut was *at par* with the triploid. 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. Leaf anatomical features being associated with many physiological functions influence the quality of leaf, resistance of water vapour and exchange of carbon dioxide and other gases directly related to stomatal frequency and size (Lea *et al.*, 1977).

In mulberry quality of leaf influences the palatability by the silkworms. Higher thickness and more coarseness of leaves in tetraploids than the triploids and diploids indicate poor quality and palatability of leaves to silkworms. The bigger size and lesser number of stomata in leaf recorded in the tetraploid followed by triploid and diploid supports the observations of Yang & Yang (1995). Chloroplasts in stomatal guard cells at different ploidy showed an increase in number with the increase of ploidy supports the observation that heredity of chloroplast number bears a close relationship with the chromosome numbers and it is indirectly associated with the ploidy (Tikader & Rao, 2001). Thickness of cuticle, epidermis, palisade and spongy parenchyma cells and the size of stomata are influenced with the levels of ploidy, while, thickness of cuticle, palisade and spongy parenchyma layers showed positive correlation with the numbers chromosomes increased (Laltanmawii & Roy Chowdhuri, 2010). Differences in stomatal characteristics and leaf thickness observed among ploidy levels, leaf characters and stomatal count may be used reliably to screen mulberry germplasm and also large plant populations developed through breeding programmes (Mishra, 1997) and rapid indirect methods to identify ploidy level (Beck *et al.*, 2002).

Propagation and root proliferation

Data recorded on number of cuttings sprouted on fifteenth day after plantation of cuttings and lengthy of shoot at fifteen days interval upto 60th day revealed that survival of cuttings on 15th, 30th, 45th and 60th days for the cuttings prepared from the base of the shoots were 84.4%, 72.7%, 68.3% and 67.8%, respectively followed by cuttings of middle portion of the shoot 83.8%, 85%, 55.5% and 55.5% respectively, while, survival cuttings of tip portion of the shoots was 76.1%, 51.6%, 41.1% and 40%, respectively. Considering the level of ploidy, triploid (76.7%) showed higher survival followed by diploid (73.9%) and tetraploid (42.8%). Number of cuttings though initially found sprouted, but decreased upto 45th day and was *at par* on 60th day of cuttings plantation. Growth performance was recorded highest for the cuttings prepared from base of the shoots followed by middle cuttings. However, length of saplings was lowest in the cuttings of tip of the shoots and tetraploid (Table III). Analysis of data revealed that number of roots /plant, root area (cm), root length (cm), root hair zone (cm), root volume (cm3/litre), fresh wt. (g) and dry wt. (g) of root were significantly higher in triploid than the tetraploid but was *at par* with the diploid (Table IV). Saplings with higher growth and rooting behaviours in triploids by exhibiting superiority between the diploid and tetraploid support that triploid possess higher rate of growth and development (Yang & Yang, 1995).

ACKNOWLEDGEMENT

The authors are thankful to the University of Kalyani, Kalyani, Nadia, West Bengal and the Director, CSR&TI, Berhampore, West Bengal for providing necessary supports for this study, are duly acknowledged.

REFERENCES

BECK, S.L., DUNLOP, R.W. & FLOSSY, A. 2002. Stomatal length and frequency as a measure of ploidy level in black wattle, *Acacia mearnsii* (de Wild). *Botanical J Linnean Society*. **141**, 177-181.

DAS, B.C & PRASAD, D.N. 1974. Evaluation of tetraploid and triploid mulberry varieties through chemical analysis and feeding experiment. *Ind. J. Seric.* **13**, 17-22.

LEA, H.Z., DUNN, G.M. & KOCH, D.W. 1977. Stomatal diffusion resistance in three ploidy levels of smooth bromegrass. *Crop Science*. **17**, 91-93.

MISHRA, M.K. (1997) Stomatal characteristics at different ploidy levels in *Coffea* L. *Annals of Botany*, **80**, 689-692.

LALTANMAWII AND ROY CHOWDHURI, S. 2010. Effects of chromosomal variations on morphology and leaf anatomical behaviours in mulberry (*Morus* sp.). *J. Crop and Weed.* **6**(2): 35-39.

TIKADER, A. & RAO A.A. 2001. Morpho- anatomical and pollen studies in mulberry germplasm. *Sericologia.* **41**(1), 69-76.

VIJAYAN, K., SAHU, P.K., CHAKRABORTI S.P. & ROY, B.N. 1999. Evaluation of tetraploids for triploid breeding in mulberry. *Sericologia.* **59**(4), 515-522.

YANG J.H. & YANG, X.H. 1995. Relationship between stomatal chloroplast number and ploidy levels in mulberry (*Morus* spp.). *Sericologia.* **35**, 289-292.