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EVALUATION OF TETRAPLOIDS FOR TRIPLOID BREEDING IN MULBERRY

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ABSTRACT

Thirty tetraploid mulberry genotypes were evaluated to select desirable donors for breeding purpose. Morphological, anatomical and leaf yield characteristics were studied for two years. Subsequent ranking analysis on the basis of leaf yield, leaf thickness, spongy layer thickness, cystolith and stomatal frequencies, leaf moisture and moisture retention capacities of harvested leaves revealed that tetraploids T36, T21, T13, T19 and T4 were superior for most of the traits. These genotypes can be used for directional breeding programmes to evolve high yielding triploids.

Key words : Evaluation, leaf yield, mulberry, tetraploid, tiploid breeding

Triploid mulberry varieties are known to be superior to other ploidy levels on rooting [1], leaf yield [2, 3], chemical components [4] and silkworm rearing performance [4, 5]. Triploids are, generally, developed through hybridization between tetraploids and diploids. Most of the natural tetraploids were found to be unsuitable for breeding due to higher leaf coarseness, lower rate of plant growth, poor rooting and inferior feeding qualities present in the resultant triploids. Hence, tetraploids with desirable characters were induced [6, 7]. However, most of the induced tetraploids were found to be distinctly different from the parental diploids in many of the desirable traits which necessitated a thorough assessment of the tetraploids before being used for breeding. There are a few reports on assessment of tetraploids [6, 7] which remained to comparative studies between the induced tetraploids and their putative diploids. The present study was, thus, undertaken to evaluate the tetraploid mulberry germplasm maintained at Central Sericultural Research and Training Institute, Berhampore, West Bengal, so as to utilize them more effectively for the development of superior triploids for tropical climatic conditions.

MATERIALS AND METHODS

Thirty tetraploid mulberry genotypes comprising 28 induced and 2 natural viz., *Morus laevigata* Wall and *M. nigra* L., being maintained in the polyploid germplasm under 150 × 150 cm spacing in high bush form, were utilized for the study. The induced tetraploids were developed from hybrids of crosses between *M. indica* var. X and *M. alba* var. S1, *M. alba* var. china white and *M. indica* var. S799. Cultural practices as recommended were followed [8]. Morphological data on days to sprout, number of branches sprouted, length of the longest shoot and internodal distance were recorded on 60th day after pruning. Leaf harvest was recorded 4 times during the year for two years. Leaf moisture content and its retention in harvested leaves were calculated [9]. Rooting ability of the variety was assessed by planting 150 stem cuttings of each variety in randomized block design under 15 × 15 cm spacing and the survival percentage was recorded after 90 days of planting.

Stomatal frequency and size were studied by decolourising leaf samples after fixing in 1:3 aceticethanol for 12 hrs and in 95% ethanol overnight followed by gradual transferring through alcohol grades in descending order. For staining of stomata, 2% iodine-potassium iodide solution was used. Cystolith was observed by staining with 1% methylene blue for 1 min. Leaf anatomy was studied from hand sections. Broad sense heritability of different characters was estimated [10].

Ranking analysis [11] based on leaf yield and 6 other leaf quality parameters viz., pallisade and spongy layer thickness, leaf blade thickness, stomatal and cystolith frequencies and moisture content of the leaf, was done with the help of the selection index programme developed by Barretto *et al.* [12]. Emphasis was given to each and every parameter according to their importance following the guidelines given in the above analysis.

RESULTS AND DISCUSSION

The analysis of variance for morphological and leaf anatomical characters revealed significant variations among genotypes (Table 1 and 2). Days required for sprouting after pruning varied from 5.00 to 11.33. Varieties with early sprouting capacity are considered to be suitable for regions where shoot feeding followed by ground pruning is in vogue. The broad sense heritability (0.92) showed that it was a highly heritable character with less environmental influence.

Primary branches/plant varied among the varieties. Its positive correlation coefficient (0.654) with leaf yield revealed the importance of this character in selection

Table 1. Morphological characters and leaf yield of 30 tetraploids mulberry varieties

Rank	Variety	Days to sprout	No. of primary branches	Length of longest shoot (cm)	Internodal length (cm)	Leaf twig ratio	Leaf yield (kg/plt /yr)	Rooting ability (%)
1	T36	7.67	96.33	107.33	3.26	0.50	3.85	26.67
2	T21	7.00	101.33	127.67	3.15	0.54	5.70	67.78
2	T13	9.33	75.00	114.33	2.84	0.50	5.64	66.67
4	T19	8.00	39.33	118.67	3.18	0.46	1.72	65.56
5	T14	10.00	42.33	138.00	2.91	0.53	2.41	66.67
6	*M.laevigata*	11.33	29.33	119.67	6.56	0.58	1.85	0.00
7	T16	8.00	80.67	150.33	2.95	0.50	2.31	36.67
8	T23	7.67	68.67	107.67	2.71	0.48	4.68	20.00
9	T34	8.67	90.00	74.67	4.03	0.54	1.86	30.00
10	T3	5.00	31.33	101.33	3.45	0.52	1.26	77.78
11	T20	10.00	20.67	90.67	3.18	0.50	0.51	56.67
12	T7	7.00	81.67	149.33	3.80	0.52	3.83	25.56
13	T24	8.00	51.00	61.67	4.62	0.57	0.64	48.89
14	T30	7.00	90.00	92.33	2.98	0.54	2.57	34.44
15	T33	8.00	149.33	90.33	3.55	0.58	2.25	17.78
16	T17	8.00	117.33	132.33	2.92	0.56	5.33	48.89
17	T31	8.00	26.33	79.00	3.37	0.53	0.89	25.56
18	T15	8.00	49.33	112.33	3.29	0.56	1.38	54.49
19	*M. nigra*	8.00	110.67	126.67	2.38	0.52	3.86	74.44
20	T2	8.00	16.33	103.33	4.65	0.55	0.64	74.44
21	T18	8.00	58.33	106.33	2.88	0.61	1.52	24.44
22	T22	8.00	45.67	105.67	3.75	0.57	0.93	31.11
23	T37	8.00	70.00	87.67	3.21	0.53	1.41	74.44
24	T27	8.00	96.33	109.33	3.25	0.41	4.31	48.89
25	T1	8.00	16.33	103.33	4.57	0.45	0.53	57.78
26	T11	8.00	47.67	116.67	3.03	0.58	2.75	27.78
27	T12	8.00	56.67	134.33	2.75	0.58	2.47	45.56
28	T8	10.00	81.67	119.00	3.21	0.52	1.13	33.33
29	T35	7.00	69.00	35.00	3.93	0.52	0.46	14.44
30	T5	8.00	48.67	120.30	3.90	0.53	1.21	48.89
Mean		8.12	63.51	107.89	3.46	0.53	2.33	44.19
SEm±		0.13	3.74	2.86	0.09	0.01	0.18	2.39
PCV(%)		14.75	56.74	25.33	24.85	9.99	75.22	52.16
Heritability		0.81	0.76	0.74	0.77	0.55	0.76	0.73

Table 2. Leaf anatomical characters of 30 tetraploid mulberry varieties

Rank	Variety	Upper epidemis (µm)	Plisade layer (µm)	Spongy layer (µm)	Lower epidermis (µm)	Total leaf thickness (µm)	Stomatal frequency/ mm^2	Stomatal size (μm^2)	Cystolith frequency/ cm^2	Moisture (%)	Moisture retention capacity (%)
1	T36	26.60	48.19	67.86	11.57	154.22	386.51	421.41	7.22	75.28	80.30
2	T21	32.39	37.78	57.06	13.11	140.34	409.45	394.90	14.46	75.68	90.07
3	T13	35.86	47.81	62.85	11.95	158.46	745.41	326.57	10.93	75.19	88.40
4	T19	37.78	37.40	67.09	11.57	153.84	482.90	431.82	15.18	76.31	90.08
5	T14	32.77	40.10	69.01	13.11	154.99	776.90	257.58	23.38	76.03	90.42
6	*M.laevigata*	22.75	50.51	92.15	14.27	179.67	356.96	646.91	25.74	73.75	92.21
7	T16	28.53	39.71	63.23	15.42	146.90	535.43	350.58	24.21	76.01	87.46
8	T23	21.98	34.70	62.07	11.95	130.70	776.90	280.59	24.72	75.86	86.29
9	T34	24.68	45.11	64.77	12.34	146.90	360.53	553.19	8.83	75.07	81.73
10	T3	35.86	45.50	72.87	11.95	166.17	598.43	418.34	25.85	75.42	85.86
11	T20	26.99	54.75	58.75	11.18	151.52	304.46	592.37	9.13	78.05	87.04
12	T7	36.24	37.01	71.33	15.81	160.39	461.94	430.50	24.10	72.62	80.52
13	T24	31.62	35.09	69.01	12.34	148.05	362.49	528.49	15.49	75.93	78.58
14	T30	18.89	45.50	58.94	10.80	134.17	503.44	301.89	13.08	75.00	79.48
15	T33	26.22	37.88	60.92	13.88	138.81	293.60	463.60	10.00	74.23	76.91
16	T17	20.82	35.09	52.05	9.64	117.59	482.94	332.36	12.63	76.02	86.68
17	T31	28.92	41.25	58.60	11.95	140.73	389.76	469.17	9.62	75.37	80.48
18	T15	42.03	47.42	72.87	16.96	179.28	503.84	503.49	15.90	72.22	86.36
19	*M. nigra*	24.29	37.78	52.82	11.95	126.87	545.67	288.52	18.59	74.52	86.26
20	T2	33.93	49.35	47.42	16.58	147.25	524.93	514.02	14.36	76.76	78.53
21	T18	27.27	35.86	51.66	11.18	126.08	598.43	538.00	17.49	74.87	85.07
22	T22	33.16	34.70	68.24	16.19	152.29	419.95	507.28	14.67	71.39	83.53
23	T37	18.89	38.56	52.05	9.64	119.14	295.57	372.86	10.00	74.07	76.99
24	T27	19.28	30.17	41.25	10.41	101.02	526.75	350.51	22.87	75.71	86.32
25	T1	33.54	47.81	47.81	16.6	146.13	493.44	414.63	25.44	74.63	84.09
26	T11	27.37	60.92	62.46	14.66	165.40	598.43	334.02	38.77	71.57	88.01
27	T12	16.96	31.62	60.92	11.57	121.06	477.91	313.41	39.28	73.84	89.29
28	T8	32.77	30.46	41.26	12.34	116.82	524.93	588.90	22.67	75.04	83.49
29	T35	18.89	40.48	45.88	11.95	117.21	383.27	435.38	10.86	74.44	68.09
30	T5	27.37	36.63	56.68	8.10	128.78	755.91	273.72	19.79	70.52	80.39
Mean		28.16	41.16	60.33	12.71	142.36	503.51	421.12	19.10	74.71	83.98
SE m ±		0.70	0.79	1.14	0.26	2.03	16.24	14.67	0.88	0.25	0.62
PCV(%)		22.96	17.54	17.71	17.06	13.35	28.64	24.54	42.32	1.48	5.72
Heritability		0.92	0.90	0.95	0.77	0.94	0.85	0.8	0.90	0.21	0.646

Table 3. Phenotypic (above diagonal) and genotypic correlations (below diagonal) among 17 parameters in tetraploid mulberry genotypes

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	**1.00**	–0.172	0.433**	0.235*	0.052	–0.173	0.252*	0.154	–0.142	0.170	–0.061	0.325**	0.157	0.237*	0.311**	0.410**	–0.025
2	–0.191	**1.000**	0.085	–0.284**	0.005	0.038	–0.122	0.654**	–0.154	–0.343**	–0.277**	–0.105	–0.210*	–0.312**	–0.160	–0.115	–0.308**
3	0.495	0.086	**1.000**	–0.278**	–0.052	–0.060	0.580**	0.416**	0.144	0.200	–0.114	0.166	0.148	0.136	0.383**	0.509**	–0.332**
4	0.341	–0.383	–0.252	**1.000**	0.125	–0.059	–0.142	–0.341**	–0.293**	0.070	0.180	0.369**	0.232*	0.330**	–0.338**	–0.062	0.530**
5	0.092	0.035	–0.083	0.193	**1.000**	–0.235*	–0.106	–0.170	–0.225*	–0.014	0.086	0.239*	0.020	0.166	–0.079	–0.006	0.213*
6	–0.411	–0.071	–0.239	–0.352	–0.564	**1.000**	0.152	0.098	0.188	–0.037	–0.015	–0.149	–0.150	–0.121	–0.078	–0.181	0.085
7	0.358	–0.186	0.790	–0.128	–0.054	0.082	**1.000**	0.347**	0.184	0.191	0.092	0.325**	0.114	0.298**	0.347**	0.477**	–0.076
8	0.155	0.644	0.538	–0.463	–0.207	0.124	0.423	**1.000**	0.014	–0.127	–0.145	0.000	–0.187	–0.124	0.268**	0.254*	–0.462**
9	–0.162	–0.133	0.220	–0.385	–0.321	0.444	0.266	0.104	**1.000**	0.297**	–0.004	–0.193	–0.061	–0.015	0.189	–0.008	–0.288**
10	0.196	–0.417	0.248	0.056	–0.092	–0.094	0.256	–0.167	0.369	**1.000**	0.175	0.272**	0.497**	0.629**	0.075	–0.073	0.217*
11	–0.092	–0.337	–0.156	0.270	0.128	0.010	0.094	–0.180	0.023	0.195	**1.000**	0.308**	0.318**	0.663**	–0.147	–0.009	0.167
12	0.378	–129	0.208	0.440	0.352	–0.321	0.395	0.009	–0.241	0.292	0.323	**1.000**	0.263*	0.808**	–0.041	0.082	0.184
13	0.215	–0.276	0.190	0.362	0.150	–0.239	0.085	–0.214	–0.124	0.587	0.332	0.286	**1.000**	0.569**	–0.101	0.139	0.278**
14	0.269	–0.374	0.163	0.410	0.232	–0.236	0.355	–0.145	–0.015	0.645	0.666	0.817	0.600	**1.000**	–0.067	0.035	0.278**
15	0.365	–0.197	0.548	–0.439	–0.120	–0.224	0.468	0.302	0.249	0.085	–0.148	–0.054	–0.149	–0.075	**1.000**	0.598**	–0.553**
16	0.458	–0.141	0.616	–0.090	0.076	–0.378	0.571	0.288	0.014	–0.079	–0.007	0.105	0.101	0.042	0.680	**1.000**	–0.374**
17	–0.005	–0.399	–0.370	0.618	0.344	0.165	–0.141	–0.569	–0.331	0.280	0.205	0.205	0.342	0.327	–0.663	–0.417	**1.000**

*, ** indicate significance at 5%and 1% level.

1: Days to Sprout, 2 : Number of primary Branches, 3: Length of longest shoot, 4: Internodal length, 5: Leaf twig ratio, 6: Leaf Moisture content, 7: Leaf moisture retention capacity, 8: Leaf Yield, 9: Rooting ability, 10: Thickness of upper epidermis, 11 Thickness of palisade layer, 12: Thickness of spongy layer, 13: Thickness of lower epidermis 14: Total leaf thickness, 15: Stomatal frequency, 16: Cystolith frequency, 17: Stomatal size programme (Table 3). Similar results were reported in diploid mulberry genotypes (13, 14). The heritability estimated (0.76) indicated that environmental conditions have some influence on this character. Hence, parental genotypes considered for breeding for a particular ecozone have to be tested under the same set of environmental conditions. Plant height is another important morphological character determining the leaf yield capacity of a variety as was clear from the positive significant correlation (0.416). Though tetraploids were known to be slow growers [2, 6], the average shoot length of the tetraploids varied from 35.00 to 150.33 cm providing much scope for selection. Heritability of this parameter indicated its susceptibility to environmental vagaries. Internodal distance also showed distinct and significant variations among the varieties. Since internodal distance has negative correlation with leaf yield, higher internodal distance should be considered undesirable in mulberry.

The leaf yield/plant/year varied from 0.46 to 5.70 kg. Since leaf yield is a complex quantitative character contributed by many characters [13], and is influenced by many environmental factors, importance has to be given to the number of primary branches, plant height, shorter internodal distance along with leaf yield, while selecting tetraploid mulberry genotypes for its inclusion in breeding programmes.

Leaf anatomical characters have greater impact on the feeding qualities of the leaf than the yield. Characters like cystolith frequency, hairiness, moisture content are reported to be very important parameters deciding the palatability of the leaf [15, 16]. Leaf thickness along with spongy-palisade thickness is reported to be typical of each varieties [16]. The heritability values for these traits were 0.94, 0.90 and 0.95 respectively, which confirmed the stability of these characters. The leaf thickness in the tetraploids varied form 101.02 to 179.25 μm. Similarly, the thickness of palisade and spongy layers also showed significant variation among the genotypes. The correlation coefficients between leaf yield and leaf anatomical characters were insignificant for most of the characters, meaning due importance should be given not only to leaf yield and yield parameters, but also to the anatomical parameters. The stomatal frequency in tetraploid varied from 293.60 to 776.90/mm^2. Das *et al.* [6] reported that the stomatal frequency in tetraploid was less than that in diploid and triploid. The negative correlation between leaf thickness and stomatal frequency supported the earlier findings [17] that stomatal frequency was inversely correlated with leaf thickness. Cystolith, the calcium carbonate deposit, was reported to be very typical of varieties and has a very detrimental effect on the palatability [16]. The cystolith frequency also varied significantly among varieties (7.22-39.28/cm^2), indicating the need for selection among genotypes. Leaf moisture content and its retention in harvested leaf also showed significant variation among the genotypes, which were directly influenced by the number and size of stomata in leaf [18].

Tetraploids developed from the same parental combinations varied significantly among themselves and also with other varieties. Similarly, the two natural tetraploids differed distinctly from the induced ones. The induced tetraploids were found much better than the natural tetraploids for most of the parameters studied, indicating the necessity of inducing tetraploids from desirable diploids and characterizing them before being utilized for breeding programmes. Since the leaf characters palatability and quality of evolved triploid leaves greatly depend upon those of tetraploid parents, tetraploids with smooth, succulent leaves with less cystolith frequency are to be selected for evolving triploid mulberry varieties. In this present study genotypes T36, T21, T13, T19 and T4 were found superior than other genotypes for most of the characters. Hence, these can be exploited for developing superior triploids in mulberry.

REFERENCES

1. A. Tikader, K. Vijayan, B. N. Roy and T. Pavan Kumar. 1996. Studies on propagation efficiency of mulberry (*Morus* spp.) at ploidy level, Sericologia., **36**: 345-349.
2. N. K. Dwivedi. 1994. Indicated autotriploid in mulberry. Adv. Plant. Sci., **7**: 35-40.
3. A. K. Sikdar and M. S. Jolly. 1995. Induced polyploidy in mulberry (*Morus* spp.) II. Production of triploids and their yield evaluation. Bull. Seric. Res., **6**: 39-46.
4. B. C. Das and D. N. Prasad. 1974. Evaluation of tetraploid and triploid mulberry varieties through chemical analysis and feeding experiment. Indian J. Seric., **13**: 17-22.
5. A. Sarkar. 1993. Effect of feeding different races of silkworm (*Bombyx mori* L.) leaves varying in ploidy level. Sericologia., **33**: 25-34.
6. B. C. Das, D. N. Prasad and A. K. Sikdar. 1970. Colchicine induced tetraploids of mulberry. Caryologia., **23**: 283-293.
7. N. K. Dwivedi, A. K. Sikdar, S. B. Dandin, C. R. Sastry and M. S. Jolly. 1986. Indiced tetraploidy in mulberry. I: Morphological, anatomical and cytological investigations in cultivar RFS 135. Cytologia., **51**: 393-401.
8. D. Ray, L. N. Mondal, A. K. Pain and S. K. Mondal. 1973. Effect of NPK and Farm Yard Manure on the yield and nutritive values of mulberry leaf. Indian J. Seric., **12**: 7-12.
9. K. Vijayan, A. Tikader, K. K. Das, B. N. Roy and T. Pavan Kumar. 1996. Genotypic influence on leaf moisture content and moisture retention capacity in mulberry (*Morus* spp.). Bull. Seric. Res., **7**: 95-98.
10. R. K. Singh and B. D. Chaudhary. 1979. Biometrical methods in quantitative genetic analysis. Kalyani publishers, New Delhi, pp. 52-54.
11. E. Schwarzbach. 1976. Selection index programme. Seminar talk in El. Batan, Mexico.
12. H. J. Baratto, I. A. Bolanos and H. S. Cordova. 1991. Selection index program. Software operation guide, CIMMYT, Mexico.
13. P. K. Sahu, B. R. D. Yadav and B. Saratchndra. 1995. Evaluation of yield components in mulberry germplasm varieties. Acta Botanica Indica., **23**: 191-195.
14. K. Vijayan, A. Tikader, K. K. Das, S. P. Chakraborti and B. N. Roy. 1997. Correlation studies in mulberry (*Morus*.spp.). Indian J. Genet., **57**: 455-460.

15. R. Narayan Prakash, K. Periasamy and S. Radhakrishnan. 1985. Effect of dietary water content on food utilization and silk production in *Bombyx mori* L. (Lepidoptera: Bombycide). Indian J. Seric., **24**: 12-17.

16. L. Cappellozza, A. T. Coradazzi, B. Baldan and P. Marina. 1996. Studies on phenotypic variability of seven cultivars of morus alba L. and three of Morus multicaulis P. (*Moraceae*)-Part 2. Sericologia., **36**: 91-102.

17. B. K. Gupta, H. Sau, S. P. Chakraborti, G. Subba Rao, S. Sarkar and S. Chakraborti. 1988. Relationship of stomatal frequency and leaf characteristics of mulberry (*Morus* spp.). Bionature., **8**: 57-60.

18. B. N. Susheelamma, M. S. Jolly, K. S. Giridhar, N. K. Dwivedi, and N. Suryanarayan. 1988. Correlation and path analyses in mulberry under stress and non-stress conditions. Sericologia., **28**: 239-244.