Indian J. Genet., 48(3): 305–311 (1988)

INDUCTION OF TETRAPLOIDY IN COLCHICINE-INDUCED MUTANT OF MULBERRY. I. MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN CULTIVAR KANVA-2

N. K. DWIVEDI,* A. K. SIKDAR, M. S. JOLLY, B. N. SUSHEELAMMA AND N. SURYANARAYANA

Mulberry Breeding & Genetics Section, Central Sericultural Research and Training Institute, Mysore 570008

(Received: January 24, 1987; accepted: January 15, 1988)

ABSTRACT

Autotetraploids have been produced by colchicine treatment in a colchicine-induced monoecious mutant of mulberry (*Morus alba* L.) cultivar Kanva-2. The most effective treatment was the treatment of axillary buds with 0.35 and 0.4% colchicine for 12 and 18 h, respectively. The autotetraploids produced large, thick and dark green leaves, broad petioles, long inflorescences and big floral parts and fruits. Considerable increase in respect of weight and water content of leaves, number of stomatal chloroplast and pollen diameter was observed in autotetraploids, while number of primary branches, sprouting and survival percentage and frequency of stomata per unit area were reduced. Meiosis was irregular. Various anomalies like unequal separation and precocious movement of chromosomes, chromatin bridge, micronuclei, laggards, cytomixis, etc., have been observed. The low pollen fertility and seed set may be due to irregular chromosomal pairing and separation rather than due to genic or physiological causes.

Key words: Tetraploidy, colchicine, mulberry, morphology, cytology.

Earlier, autotetraploidy was induced in the female plants of high yielding variety Kanva-2 of mulberry (*Morus alba* L.) by seed treatment with 0.2% colchicine [1]. Dwivedi et al. [2] reported expression of male and mixed inflorescences in it by 0.4% colchicine. Hazama [3] stated that due to slow growth of tetraploids the leaf yield is less than in diploid. But the superior nutritive qualities of leaves of tetraploid and triploid varieties were also reported [4, 5]. The present communication deals with morphological and cytological studies of colchicine-induced tetraploids in a colchicine-induced monoecious mutant of cultivar Kanva-2.

MATERIALS AND METHODS

Sprouting apical and axillary buds of 3-month-old saplings, raised in pots, were treated with 0.35, 0.40 and 0.45% colchicine (Loba, India), 6 and 8 h daily for 3 consecutive days. Five buds were taken in three replications in each treatment. The methods of colchicine treatment and screening the tetraploids and those pertaining

*Present Address: NBPGR Regional Station, CAZRI Campus, Jodhpur 342003.

to the study of diploids and tetraploids were the same as determined earlier [6]. Buds fixed in Carnoy's solution were squashed in 1% acetocarmine for studying meiosis.

RESULTS

Out of 60 treated buds in each treatment only 6 apical and 8 axillary buds were confirmed to be autotetraploids cytologically. The survival percentage of treated buds and frequencies of tetraploid plants were recorded (Table 1).

Table 1. Frequency of induced tetraploids by colchicine

Colchicine conc.(%)	Duration (h)	Apical buds		Vegetative buds	
		survival (%)	tetraploids (%)	survival (%)	tetraploids (%)
0.00	12	100	—	100	—
0.00	18	100	—	100	—
0.35	12	45	—	64	1.67
0.35	18	40	1.67	35	1.67
0.40	12	39	1.67	35	3.33
0.40	18	27	3.33	21	3.33
0.45	12	3	3.33	2	3.33
0.45	18	—	—	—	—

MORPHOLOGY

Comparative morphological data on colchiploid plants and their putative diploids are summarized in Tables 2 and 3. The tetraploids produced large, thick and dark green leaves, broad petioles, long inflorescences, and big floral parts and fruits. Considerable increase in respect of weight and water content of leaves, number of stomatal chloroplasts, and size of stomata and pollen grains, while reduction in primary branches, sprouting and survival percentage, frequency of stomata per unit area, pollen fertility and seed set percentage were observed in autotetraploids (Tables 2 and 3). Flowering was somewhat delayed and blooming duration prolonged in tetraploids.

CYTOLOGY

Meiosis was studied in diploid and autotetraploids. The data on chromosomal associations at metaphase I are presented in Table 4. The most common association in the diploid plants was 14 bivalents (Fig. 1). One bivalent was larger in size and occasionally quadri–, tri- and univalents were also observed (Table 4). In comparison, colchiploids were characterized by the presence of more multivalents along with bivalents. The range of hexavalents, quadrivalents, trivalents, bivalents and univalents

Table 2. Comparison of morphological characters in diploid and induced tetraploid

Character	Diploid	Tetraploid
Growth habit		
Height (cm)	221	213
Number of branches	14	9
Internodal distance (cm)	3.8	3.5
Sprouting (%)	82.0	80.2
Rooting (%)	79.0	52.0
Leaf		
Leaf size (cm^2)	182.7	193.3
Length of petiole (cm)	3.7	3.7
Width of petiole (cm)	0.3	0.4
Weight of 100 leaves (g)	405.0	480.0
Water content of leaf (%)	72.8	79.5
Length of stomata (μm)	15.9	20.3
Width of stomata (μm)	12.9	17.6
No. of stomata per unit area (μm^2)	48.8	37.6
No. of stomatal chloroplasts	14.2	21.8
Texture	Thin, green	Thick, dark green

was 0-1, 1-14, 0-3, 0-26 and 0-8, respectively (Fig. 2), their means being 0.028, 9.53, 0.90, 6.92 and 2.29, respectively (Table 4). The number of quadrivalents and bivalents varied from 1 to 14 and 0 to 26, respectively (Table 4). Out of 30 cells analysed at anaphase I, 15 cells were normal with equal distribution (28 : 28), and in the remaining 15 cells abnormalities like precocious movement of chromosomes at metaphase I and metaphase II, unequal distribution with univalent and bivalent laggards (Fig. 3), and formation of bridges were observed. Cytomixis was noticed in 2.34% PMC at metaphase I, 19.77% PMC at anaphase I, 1.89% PMC at diad, and 1.5% PMC at tetrads stages. Diads, pentads with two small degenerated microspores (Fig. 4), hexad and octal microspores were also observed in autotetraploids.

DISCUSSION

The sprouting bud treatment of established saplings with colchicine has proved to be effective for the induction of tetraploidy in mulberry, as also reported earlier [6].

Swanson [7] concluded that the slow rate of growth and development after colchicine treatment is due to physiological disturbances and slower rate of cell division. Increase in cell size has been regarded to be responsible for increase in

leaf thickness [6, 8]. The tetraploids in the present study are superior in most characters to their putative diploids except in sprouting and survival of cuttings, number of shoots, pollen fertility, and seed set. Sastry et al. [1] reported delayed sprouting in the vegetative buds of cuttings of induced tetraploids of female cultivar Kanva-2. However, in some crop plants autotetraploids have been reported to be inferior to their corresponding diploids [9–11]. Gigantism is one of the chief attributes of polyploidy [1, 8, 12]. Since the leaf is the economic product in mulberry, gigas character in its vegetative parts may be exploited for higher yield [6, 12, 13].

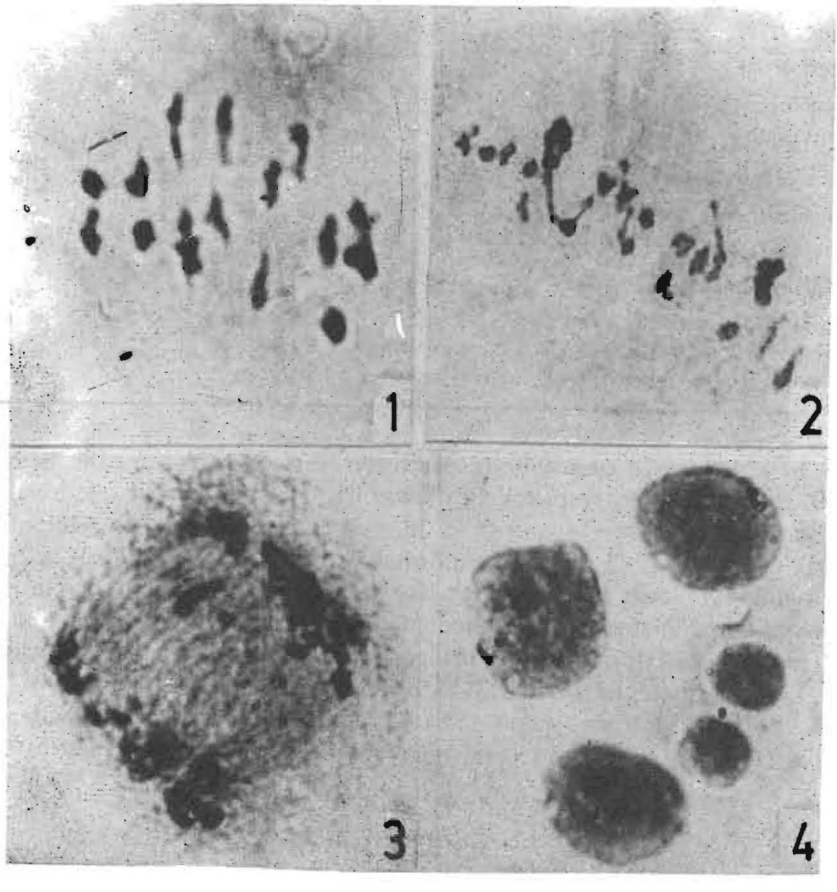

Fig. 1–4. 1. Metaphase I, 2x, showing 14 bivalents. 2. Metaphase I, 4x, showing different associations of chromosomes. 3. 4x, laggards. 4. 4x, pentad with two degenerated microspores. Magnification—Figs. 1, 2, 4: ×2000; and Fig. 3: ×1500.

Table 3. Comparison of morphological characters in diploid and induced tetraploid

Character	Diploid	Tetraploid
Male flowers		
Length of inflorescence (cm)	4.10	4.98
Diameter of inflorescence (cm)	0.84	1.33
No. of flowers per inflorescence	46.00	62.60
Length of flower (cm)	0.54	0.67
Diameter of flower (cm)	0.48	0.52
Length of stamen (cm)	0.50	0.64
Pollen diameter (μm)	18.83	23.94
Pollen stainability (%)	93.61	59.67
Female flower		
Length of inflorescence (cm)	2.40	3.80
Diameter of inflorescence (cm)	0.80	1.20
No. of flowers per inflorescence	31	45
Length of flower (cm)	3.75	4.60
Diameter of flower (cm)	1.75	2.80
Length of stigma (mm)	1.65	1.90
Length of style (mm)	0.40	0.60
Length of ovary (mm)	1.60	2.05
Fruit		
Sorosis length (cm)	2.25	3.80
Sorosis diameter (cm)	1.10	1.90
No. of seeds per fruit	26	22
Seed set (%)	81.07	18.89

Theoretically, the autotetraploids should form more quadrivalents at meiosis due to the presence of four homologous chromosomes, but it is not so as in cultivar RFS 135 [6]. Formation of 26 bivalents and 2 univalents in some PMC suggests that the presence of more than two homologous chromosomes is not the only prerequisite for multivalent association. This observation also support the genetic control of chromosome pairing [11, 14].

Table 4. Chromosome associations at metaphase I of diploid and induced tetraploid

Ploidy	No. of cells analysed	Associations									
		VI		IV		III		II		I	
		range	mean	range	mean	range	mean	range	mean	range	mean
2x	100	—	—	0–1	0.01	0–1	0.01	10–14	13.06	0–5	0.22
4x	72	0–1	0.028	1–14	9.53	0–3	0.90	0–26	6.92	0–8	2.29

Unequal distribution of chromosomes and/or laggard bivalents and univalents at anaphase I in autotetraploids are considered to be due to irregular distribution of multivalent associations [15]. Univalents have also been found to cause irregularities at anaphase I [11, 16, 17]. The reduction in pollen fertility and seed set can be ascribed to multivalent associations of chromosomes, presence of univalents, and various other meitotic abnormalities [6, 12, 15, 18] rather than genetics and/or physiological reasons.

REFERENCES

1. C. R. Sastry, S. V. Venkataramu and Azeez Khan. 1969. Induced tetraploid of an improved strain Kanva-2 of mulberry (*Morus alba* L.). Silkworm Inform. Bull., **1**(1): 95–99.
2. N. K. Dwivedi, A. K. Sikdar, S. B. Dandin and C. R. Sastry. 1984. Modification of sex expression in mulberry by colchicine. Curr. Sci., **53**(19): 1036–1037.
3. K. Hazama. 1968. Breeding of mulberry tree. JARQ: 15–19.
4. H. Seki and K. Oshikane. 1959. Studies on polyploid mulberry trees. III. The evaluation of breeded polyploid mulberry leaves and the results of feeding silkworms to them. Research reports of Faculty of Textiles and Sericulture, Shinshu University, No. 9: 6–15.
5. N. A. Dzhafurov and O. R. Alekperova. 1978. Fodder qualities of the leaves of inbred and interspecific polyploid mulberry hybrids. Shelk, No. 4: 5. From Referativny Zhurnal, **65**: 390, 1979 (in Russian).
6. N. K. Dwivedi, A. K. Sikdar, S. B. Dandin, C. R. Sastry and M. S. Jolly. 1986. Induced tetraploidy in mulberry. I. Morphological, anatomical and cytological investigations in cultivar RFS-135. Cytologia **51**(2): 393–401.
7. C. P. Swanson. 1957. Cytology and Cytogenetics. Prentice Hall, New Jersey, USA.
8. O. R. Alekperova. 1979. A useful autotetraploid form of mulberry. Genet. Selekts. Azerbidzhane, **3**: 97–103. From Referativnyl Zhurnal,1980 (in Russian).

9. N. K. Sen and R. V. Vidyabhushan. 1960. Studies of the induced polyploids of horsegram. Indian J. Genet., **20:** 212–233.

10. K. F. Schertz. 1962. Cytology, fertility and gross morphology of induced polyploid of *Sorghum vulgare*. Can. J. Genet. Cytol., **4:** 179–186.

11. R. Gupta and P. K. Gupta. 1975. Induced polyploidy in *Crotolaria* L. I. C. *juncea* and *C. retusa*. J. Indian Bot. Soc., **54:** 175–182.

12. B. C. Das, D. N. Prasad and A. K. Sikdar. 1970. Colchicine induced tetraploids in mulberry. Caryologia, **23**(3): 283–293.

13. I. Tojyo. 1966. Studies on the polyploidy in mulberry tree. I. Breeding of artificial autotetraploids. Bull. Sericult. Expt. Sta. Japan., **20**(3): 187.

14. R. Riley and V. Chapman. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature, **182:** 713–715.

15. C. D. Darlington. 1965. Cytology. J. and A. Churchill, London.

16. W. M. Myers and H. D. Hill. 1942. Variation in chromosomal association and behaviour during meiosis among plants from open pollinated population of *Dactylis glomerata*. Bot. Gaz., **104:** 171.

17. W. M. Myers and H.D. Hill. 1943. Increased meiotic irregularities accompanying in breeding of *Dactylis glomerata*. Genetics, **28:** 383.

18. W. Gottschalk. 1978. Open problems in polyploid research. Nucleus, **21:** 91–112.