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Induction of polyploidy in Pride-of-India (*Melia azedarach* Linn), an agroforestry tree

N. D. Jambhale[1], S. C. Patil and S. V. Pawar

Plant Biotechnology Centre, Department of Agril. Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722

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Abstract

Polyploidy was induced in Pride-of-India (*Melia azedarach* Linn) a small tree grown as a hedge plant and is important for agroforestry having timber, fodder and therapeutic and insecticidal uses. Colchicine 0.5% treatment for 3 consecutive days to shoot apices of three to four day old seedlings was effective. Polyploid plants have smaller leaves with dark green, thick and overlapped leaflets, larger stomata, smaller inflorescence, reduced pollen fertility and less number of drupes than that of diploids. They achieved faster growth in three-and-a-half years. Anther squashes of the two polyploid C_1 plant showed chromosome number of 2n = 56 in the polyploids as against 2n = 28 in diploid.

Key words : Pride-of-India, *Melia azedarach*, induced polyploidy, colchicine

Introduction

Polyploidy has played an important role in the development of new varieties of forest trees. Poplars (*Populus*), alders (*Alnus*), redwood (*Sequoia*), semul (*Bombax*) and numerous other groups have polyploid members [1, 2]. It is estimated that one third of the hard wood species are polyploid derivatives [3]. Chromosome doubling may also play a role in the improvement of other genera where natural polyploids do not exist.

Pride-of-India, (*Melia azedarach* Linn), a tree of north eastern India naturalized in many subtropical countries is important for its timber, fodder, therapeutic and insecticidal uses. It is planted as shade tree in betelvine gardens, on bunds and in different agroforestry models. No polyploid species has so far been identified in the genus *Melia*. This paper deals with an induced polyploidy in *M. azedarach*.

Materials and methods

Freshly collected drupes from 15 trees were sown in green house in polybags filled with sand during August 1995. Three to four day old seedlings were treated with colchicine. The colchicine treatments of 0.1, 0.5 and 0.75% concentration were given for 3 consecutive days. For this, a cotton swab soaked in colchicine was kept between the two emerged cotyledons to wet the shoot apex. The cotton swab was kept wet by adding drops of colchicine by means of a dropper as and when required during day time. The shoot apices were then washed free of colchicine. The colchicine treated seedlings were then transferred in earthen pots and studied in April 1996. Plants with swollen shoot, slow growth and dark green foliage were suspected to be polyploids. Such plants were critically studied for stomatal variations. Two out of the three putative polyploid plants (C_1) were transferred in the field during June 1996 and studied in August 1999. Cytological studies were carried out by fixing floral buds of the two C_1 plants to 1 acetic acid : 3 absolute alcohol fixative for 24 h and squashing anthers in 1% aceto-carmine during May 1999.

Seeds obtained on the three year old suspected polyploid plants were further sown in sand during June 1999 to raise C_2 generation. Root tips of the C_2 plants were cut and prefixed in 0.1% cochicine for 4 h, fixed in 3 absolute alcohol : 1 acetic acid fixative for 24 h, hydrolysed in 1 N HC1 for 9 minutes and, squashed in 1% aceto-orcin and observed under microscope.

Results and discussion

Survival of the seedlings was normal in treatments with 0.1 and 0.5% colchicine. However, colchicine treatment of 0.75% concentration was lethal as no seedling could survive. Three seedlings from 0.5% colchicine treatment showing swollen shoots with irregular shaped thick dark green leaves were suspected to be polyploids (Table 1).

The three suspected polyploid plants were transferred in earthen pots and studied for one year. One of the plants died. Observations of the two polyploid

[1]Present address: Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli 415 712

Table 1. Effect of colchicihine treatment to the seedling shoot tips of *Melia azedarach*.

Dose (%)	No. of seedlings treated	Survival (%)	Remarks
Control	50	100	Normal growth
0.10	50	100	Normal growth
0.50	50	100	Shoots exhibited swelling with slow growth, dark green foliage, three seedlings with thick leaves were suspected to be polyploids, one of them died subsequently.
0.75	50	0	Lethal dose

plants named as MAP-1 and MAP-2 alongwith control recorded after 8 months are given in Table 2. Polyploid plants (Fig. 1) exhibited slower growth initially than control (diploid). Leaves of diploid were larger than that of polyploids. Leaflets of the compound leaf in diploid were free without overlapping, while those were overlapped in polyploids (Fig. 2). Polyploids had thicker, dark green leaves with larger stomata. However, frequency of stomata was less in polyploids.

Table 2. Observations of eight-month plants of *Melia azedarch* growing in pots

Sr. No.	Character	Control (Untreated)	Suspected Polyploid plants	
			MAP-1	MAP-2
1.	Height (m)	1.53	1.51	1.14
2.	Stem colour	Greenish brown	Greenish brown	Greenish brown
3.	Leaf length	2.30	1.85	1.63
4.	Leaf type	Bi-tri pinnate	Bipinnate	Bipinnate
5.	Leaf shape	Ovate with serrate margin	Ovate with serrate margin	Ovate with serrate margin
6.	Leaflet size (cm)	5.70 ± 0.39	4.49 ± 0.25	3.20 ± 0.31
7.	Leaf breadth (cm)	2.33 ± 0.20	2.55 ± 0.24	2.15 ± 0.12
8.	Leaflet arrangement in leaf	Free leaflets	Leaflets overlapping	Leaflets overlapping
9.	Leaf colour	Dark green	Dark green	Dark green
10.	Length of stomata (μ)	17.05 ± 0.13	19.26 ± 0.09	21.28 ± 0.04
11.	Breadth of stomata μ)	11.97 ± 0.08	13.66 ± 0.05	14.93 ± 0.04
12.	No. of stomata/microscopic field	32.78 ± 0.2	29.0 ± 0.20	28.0 ± 0.13

One year old polyploid plants growing in pots were transferred in the field and observed after two years of field growth (Table 3). The polyploid plants flowered after three and a half years. Polyploid plants have smaller leaves, smaller infloresence, reduced pollen fertility and less number of drupes than that of diploids (Fig. 3). The two C_1 plants resembled each other in most of their morphological traits.

Table 3. Characters of 3 year old plants of *Melia azedarach*

Sr. No.	Character	Control	Tetraploid	
			MAP-1	MAP-2
1.	Height (m)	5.0	5.8	5.5
2.	Stem girth (cm)	20	27.0	28.0
3.	Leaf			
	i) Leaf of petiole (cm)	36	23	23
	ii) Length of terminal leaflet (cm)	7.0	4.0	4.2
	iii) breadth of terminal leaflet (cm)	1.5	1.5	1.5
	iv) No. of secondary branches	8	10	10
	v) Length of secondary rachis (cm)	10	7	7
	vi) No. of leaflets/ secondary rachis	9	9	-
4.	Inflorescence			
	i) Length of peduncle (cm)	28.5	22.2	20.8
	ii) No. of secondary branches	12.8	5.2	4.9
	iii) Pollen fertility	98.2	82.8	83.1
5.	Fruit			
	i) No. of drupes/peduncle	(10-30)	(4-11)	(4-15)
	ii) Diameter of drupe	1.53	2.0	1.97
	iii) Diameter of stone	0.93	0.86	0.96
	iv) No. of seeds/dupe	4	4	4
	v) Length of seed (cm)	0.8	0.8	0.8

The drupes obtained from the polyploid plants were collected, seeds separated and sown. The seeds of both the polyploid plants had normal germination. Morphological features of the polyploid plants in C_2 generation was similar to that observed in C_1.

Root type mitosis of normal diploid *M. azedarach* plants revealed chromosome number of 2n = 28 with normal separation. Anther squashes of normal diploid plants showed chromosome number of n = 14 in PMCs at diakinesis and metaphase I, forming only bivalents. At anaphase I regular and normal separation of 14-14 chromosomes was observed in all PMCs.

Anther squashes of the two polyploid C_1 plants (MAP-1 and MAP-2) showed chromosome number of 2n = 56 at diakinesis and metaphase I having various associations and configurations of chromosomes (Table 4). Quadrivalent and bivalent configurations were observed in 88% of the PMCs while 12% PMCs had formation of trivalents and univalents. At anaphase I regular and normal separation of 28-28 chromosomes was observed in maximum cells, might be in the PMCs with bivalent and quadrivalent configurations. Some

Fig. 1. Eight months old plant of *Melia azedarach*: (a) induced polyploid (C1); (b) normal diploid

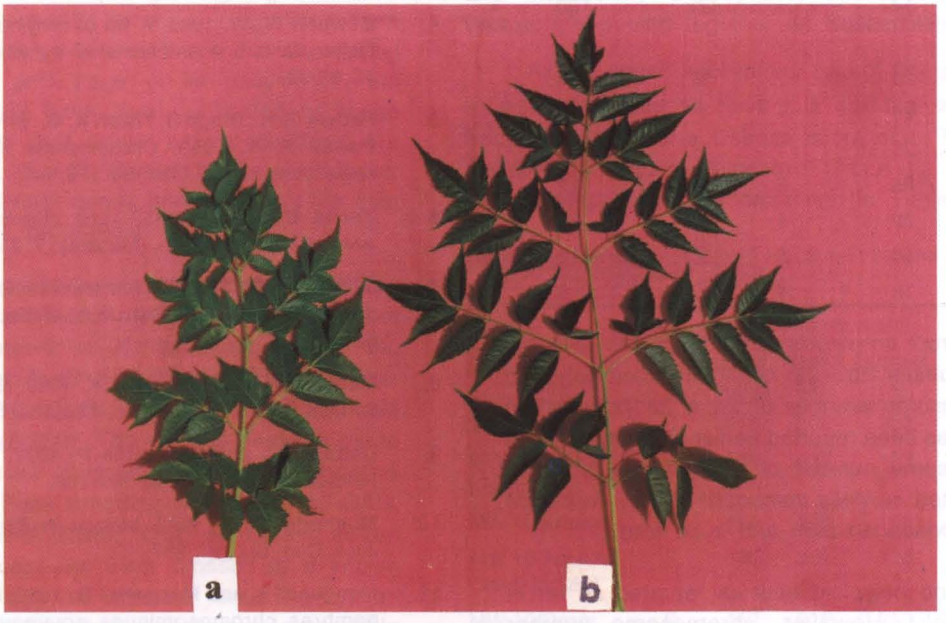

Fig. 2. Leaves of *Melia azedarach*: (a) induced polyploid (C1); (b) normal diploid

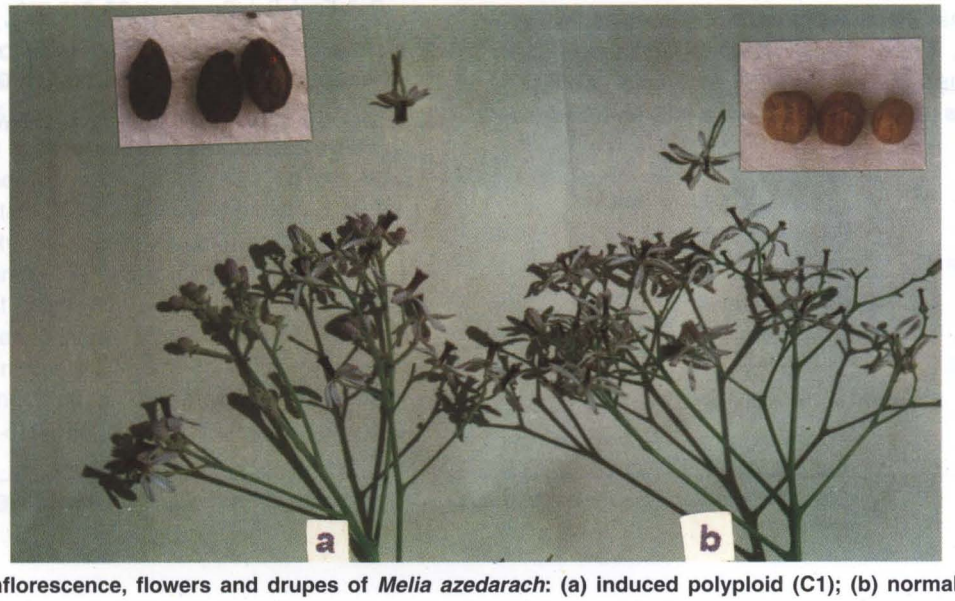

Fig. 3. Inflorescence, flowers and drupes of *Melia azedarach*: (a) induced polyploid (C1); (b) normal diploid

cells exhibited unequal separation of chromosomes leaving laggards which may be in PMCs having univalent and trivalent configurations. Trivalent and univalent configurations observed in metaphase I cause anaphasic irregularities of meiosis leading to unbalanced chromosome numbers in pollen. The reduced pollen fertility (82.8%) observed in the polyploid plants may be due to unequal separation of chromosomes in different chromosome configurations.

Table 4. Frequency of types of chromosome configurations in diploid and tetraploid cells of *Melia azedarach*

Ploidy level	Chromosome configurations				No. of PMCs studied	Percentage PMCs showing the configurations
	I	II	III	IV		
Diploid	-	14	-	-	20	100
Tetraploid						
	-	28	-	-	6	24
	1	26	1	-	1	4
	-	26	-	1	6	24
	-	24	-	2	3	12
	-	20	-	4	4	16
	-	18	-	5	3	12
	-	18	1	4	2	8

Study of root tip mitosis of 10 C_2 plants revealed chromosome number 2n = 56 confirming their polyploid nature. Chromosome number of 2n = 28 (x = 14) in *M. azedarach* has been reported earlier by many workers [4-13]. Chromosome number of n = 14 and 2n = 28 has been reported in *Melia composita* [8], *M. floribunda* curr. and *M. toosandan* sieb and zucc also [7].

There is no polyploid species reported so far in the genus *Melia*. However, chromosome number comparable to the induced polyploid *Melia azedarach* with 2n = 58 has been reported in different genera of Meliaceae *viz.*, 2n = 58 in *Turraea floribunda* and *Nymania capensis* [7] and 2n = 56 in *Toona ciliata* [8, 12], *T. sincensis* [7], *Soymida febrifega* [13] and *Cedrela mexicana* [7].

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