Cytogenetical Studies of Artificially Induced Autohexaploids of *Tabernaemontana divaricata*

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Received November 12, 1974

Tabernaemontana divaricata, an ornamental member of family Apocynaceae, is represented by single and double flowering varieties (vars. 1 and 4), which on cytological investigations were found to be at $2n$ and $3n$ level (Raghuvanshi and Chauhan 1969). Besides these our intensive screening of different populations resulted in isolation of two more varieties one of which was diploid (var. 2) and other single flowering triploid (var. 3). This paper presents the results of our efforts to alter the level of ploidy through colchicine treatment, in a single and double flowering triploid varieties. However, data of $2n$ and their artificially induced tetraploids have also been included for comparison (Chauhan and Raghuvanshi 1971).

Material and methods

Young shoots of rooted cuttings of vars. 3 and 4 were treated with .2% colchicine for 3 days, daily for 6 hrs. This initial treatment was found suitable for the production of hexaploid tissue but due to high reversion frequency (Chauhan and Raghuvanshi 1970), treatment of reverted shoots had to be repeated. When stabilised polyploids were obtained, buds of appropriate size were fixed in 1:3 acetic alcohol, fortified with iron, for 24 hrs. Anthers were squashed in acetocarmine later slides were made permanent by ethanol-butanol schedule.

Observations

Morphological

Comparative study of morphology of diploid, triploid, tetraploid and hexaploid has been presented in Table 1. Polyploids are characterised by having larger vegetative and floral parts. Rate of growth in these polyploids was lower but there was significant increase in width of leaves and petals, and the size of stomata.

Flowers in *T. divaricata* have five epipetalous anthers. This character is well stabilised in two diploid varieties and single flowering triploid variety but in double flowering triploid variety, the number of anthers varies from 1-5. At hexaploid level the number of anthers in single flowering var. 3 was unaffected but in double flowering var. 4 instability in number of anthers was further enhanced. The anthers were absent in most of the flowers examined and rarely one anther could be seen in a flower. Further, various stages of reduction in anthers were also metwith. In

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some cases reduced yellowish lobe was found attached on the corolla tube. When this was crushed in acetocarmine, pollen grains were seen.

Cytology

Triploid

Single flowering var. 3: The chromosome number was $2n=33$. At MI only bivalents and univalents were observed (Fig. 1). Presence of univalents resulted in disturbed meiosis. At AI, unequal groupings, dividing univalents, strays and lagging chromosomes were noted. Subsequent stages were abnormal. Number of spores in a sporad varied from 3–7. Pollen fertility was 15%.

Table 1. Morphological characters of diploid, triploid, tetraploid and hexaploid in *Tabernaemontana divaricata*

Characters	2n	3n	4n	6n
Length of leaves (cm)	14.73	15.17	16.35	16.4
Length of petiole (cm)	1.0	1.0	1.2	1.4
Width of leaves (cm)	4.72	5.3	7.40	7.45
Length of blade (cm)	13.73	14.3	15.15	15.0
Length of stomata (μ)	22.88	33.484	39.875	47.984
Breadth of stomata (μ)	4.686	5.94	6.38	8.316
Length of aperture (μ)	13.948	21.186	22.99	25.63
Number of stomata per field	20.12	10.24	8.0	6.0
Spread of flower (cm)	4.5	5.64	5.10	6.0
Length of petal (cm)	2.2	2.84	2.4	3.0
Breadth of petal (cm)	1.2	2.74	2.0	3.5
Length of corolla tube (cm)	2.3	2.36	2.7	3.0
Length of sepal (cm)	.4	.36	.4	.4
Length of anther (cm)	.2	.3	.34	.35
Length of stigma (cm)	.1	.1	.1	.1
Length of style (cm)	1	1	1	1
Length of ovary (cm)	.1	.1	.1	.1

Double flowering var. 4: Meiosis was fairly similar to that of var. 3 (Fig. 2). The number of dividing univalents ranged from 1–8. Break-down of spindle, strays, laggards etc. were also observed. In one cell 19 univalents were present. Number of spores in a sporad varied from 2–7. Pollen fertility was 3% only.

Hexaploid

Single flowering var. 3: Hexavalent was observed in one cell (Fig. 3). Frequency of ring and chain quadrivalents was .54 and .05 per cell. Chromosomes at metaphase I showed fairly normal behaviour. Frequency of univalents was 1.5 per cell. Unequal segregation, stray chromosomes, break-down of spindle, were observed. Number of spores in a sporad varied from 1–7. Pollen fertility was 78.96%.

Double flowering var. 4: Stabilised hexaploids were obtained but their detailed cytology could not be studied because flowers were mostly devoid of anthers except in rare case, where one anther was present. However, chromosome number was determined by analysing PMCs which clearly indicated hexaploid condition (Fig. 4).

Discussion

Triploids, tetraploids and hexaploids were sturdy and had thicker leaves and flowers. The number of anthers in single flowering triploid variety is stabilised which is not effected by change in ploidy, however, in double flowering triploid variety variability in anthers is prevalent at triploid level where normal development of varying number of anthers is effected. This abnormal development of anthers becomes further enhanced at hexaploid level when most of the flowers appear to be devoid of any fertile anthers.

Figs. 1-4. *T. divaricata*., 1, 11 bivalents and 11 univalents in single flowering var. 3 ($3n$). 2, 11 bivalents and 11 univalents in double flowering var. 4 ($3n$). 3, 1 hexavalent, 1 quadrivalent, 27 bivalents and 2 univalents in single flowering var. 3 ($6n$). 4, 2 quadrivalents, 28 bivalents and 2 univalents.

Presence of low number of multivalents in polyploids appears to be general feature of *Tabernaemontana divaricata* and also of some other species of family Apocynaceae. In natural occurring triploid varieties multivalents were not seen but in artificially induced tetraploids quadrivalents were observed although in low frequency (Chauhan and Raghuvanshi 1971). Similarly in artificially induced hexaploids, quadrivalents were observed but hexavalent was seen in one cell only. In triploids meiosis was disturbed due to the presence of univalents while at hexaploid level it became comparatively normal due to the increased chromosomal

association. Chiasma frequency in single flowering triploid was 18.03 per cell and in hexaploid it was 41.6 per cell. Number of chiasma per chromosome in triploid is .54 and in hexaploid .63. The increase in value at hexaploid level is attributed to increased chromosomal association and reduction in number of univalents (Table 2). In artificially induced tetraploid this value is .66 which is higher than triploids and hexaploids. In diploid var. 1 the synapsis is effected by change in temperature during different seasons (Raghuvanshi and Chauhan 1970).

Table 2. Chromosome association per cell at MI in diploid, triploid, tetraploid and hexaploid

plants	Hexavalent	Quadrivalent		Bivalent		Univalent	Chiasma frequency	Chiasma per chromosome
		ring	chain	ring	rod			
Var. 1	—	—	—	.024	2.7	16.0	2.8	.13
2n	—	—	—	0-1	0-5	12-20	0-5	
Var. 3	—	—	—	7.2	3.34	11.3	18.03	.54
3n	—	—	—	3-11	0-7	11-15	13-22	
Var. 4	—	—	—	6.08	4.6	11.44	16.7	.5
3n	—	—	—	2-11	0-9	11-15	10-22	
Var. 1	—	.26	—	7.62	12.44	1.16	29.38	.66
4n	—	0-2	—	2-12	8-17	0-12	23-38	
Var. 3	.02	.54	.05	8.2	22.7	1.5	41.6	.63
6n	0-1	0-3	0-1	3-12	15-30	0-6	36-51	

Müntzing and Prakken (1940) found in autotriploids of *Phleum pratense* a genotypically controlled tendency to form bivalents only. Similarly natural occurring autotriploids of *T. divaricata* and *Plumeria alba* (Raghuvanshi and Chauhan 1969, 1971) are devoid of multivalents. Riley and Chapman (1958), Sears and Okamoto (1958) suggested that regular bivalent formation in *Triticum aestivum* was under control of a single gene or block of genes situated in one chromosome pair. There is enough evidence to suggest (Swaminathan 1961) that autopolyploids, both natural occurring and induced may acquire either gradually or suddenly the cytological behaviour of a diploid. This may also explain the absence of multivalents in natural occurring triploids while their presence in low frequency in induced tetra- and hexaploids. Furthermore, spindle functions normally in these autopolyploids which generally is not the case. In other autopolyploids like, *Catharanthus roseus*, (Raghuvanshi and Chauhan 1969), *C. pusillus*, *Rauvolfia serpentina*, *R. canescens*, *Nerium indicum* and *Thevetia peruviana* normal bipolar spindle was observed. However, in mixoploids, hexaploid and octoploids of *Catharanthus roseus* (Raghuvanshi and Chauhan 1974) multiple and multipolar spindles have been frequently observed.

Pollen fertility in single flowering triploid was 15% while in induced hexaploid it was 78.96%. This increased fertility may be due to formation of spores which had balanced number of chromosomes. In triploid percentage of 4 spores at tetrad stage was 70.6 (Table 3) but these may have varying number of chromosomes due to irregular meiosis. So these spores also contributed to the production of sterile

Table 3. Percentage of varying number of spores in a sporad in diploid, triploid, tetraploid and hexaploid

No. of spores level of ploidy	1	2	3	4	4	5	6	7	8	9	10	11	12	14	16	20
	—	—	.	.	unequal	.	.	.	—	—	—	—	—	—	—	—
Var. 1, 2n	—	—	6.3	10.9	19.48	34.79	24.6	4.24	—	—	—	—	—	—	—	—
Var. 3, 3n	—	—	1.7	70.6	5.8	19.6	2.3	.58	—	—	—	—	—	—	—	—
Var. 4, 3n	—	.002	.004	78.9	—	16.09	2.82	.007	—	—	—	—	—	—	—	—
Var. 1, 4n Fertile	1.2	.8	1.2	60.4	1.2	25.4	5.97	2.0	.4	.4	—	—	—	—	—	—
Var. 1, 4n Sterile	1.89	—	—	11.7	—	14.2	10.3	12.5	12.3	10.1	5.3	2.68	8.2	4.9	4.53	1.03
Var. 3, 6n	.76	—	—	87.6	—	8.4	2.3	.76	—	—	—	—	—	—	—	—

pollen. In hexaploid percentage of 4 spores at tetrad stage was 87.6 (Table 3). In this case due to regular separation at second division equal number of chromosome may have formed four nuclei, thus, producing fertile pollen grains.

Summary

The paper presents comparative studies of diploids, tetraploids, triploids and hexaploids of *Tabernaemontana divaricata*. Hexaploids were artificially induced from natural occurring triploids. They are characterised by enhancement in plant parts but slow growth rate. The instability in number of stamens observed at triploid level is further enhanced in artificially induced hexaploids. These hexaploids show regular meiosis and low multivalent frequency. Pollen fertility was increased from 15% at $3n$ to 78.96% at $6n$ level.

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