J Indian bot Soc Vol 76 (1997) 73-76

CYTOMORPHOLOGY OF INDUCED AUTOPOLYPLOIDS OF *GAMOLEPIS TAGETES* (L.) DC.

M.S. SIDHU*, B.S. GILL AND R.C. GUPTA
Department of Botany, Punjabi University, Patiala-147002.
*Guru Kashi College, Damdama Sahib-151302, Punjab.
(Accepted May, 1997)

Autotetraploidy in *Gamolepis tagetes* was induced by colchicine through seed-cum-seedling treatment while the autotriploid was produced from the seeds of open pollinated autotetraploids of C_1 generation which is intermediate between diploid and autotetraploid so far as the morphological characters are concerned. Autotetraploids have become stabilized after C_2 generation. Meiosis in the autotriploid is characterised by trivalents and univalents besides bivalents but frequency of trivalents is less than expected. Maximum number of trivalents reported is five. Distribution of chromosomes is irregular with 11:10 and 12:9 chromosome distributions more common than other types. Microsporogenesis is abnormal with high frequency of polyads and tetrads with micro-nuclei besides normal tetrads. As a sequel, achene fertility is highly reduced.

Key Words : Autotetraploidy, *Gamolepis tagetes*, autotriploid, colchicine.

Autotetraploidy was induced in *Gamolepis tagetes* (2n=14), a winter ornamental composite with the purpose of bringing some improvement in floral features, to see its effect on reproductive capabilities and its subsequent stabilization. The open pollinated progeny of C_1 generation of large number of autotetraploids produced two tetraploids (2n=28) and one triploid (2n=21) besides large number of diploids. The present paper deals with cytomorphological studies of these autopolyploids particularly the autotriploid.

MATERIAL AND METHODS

Autotetraploidy was induced by seed-cum-seedling treatment with various aqueous concentrations of colchicine (0.10% to 0.40%). Seeds collected from open pollinated autotetraploids were sown to get the next progeny. Cytological level of plants of progenies was confirmed through meiotic analysis using standard acetocarmine technique. Pollen fertility was estimated by mounting them in 50% glycero-acetocarmine.

OBSERVATIONS

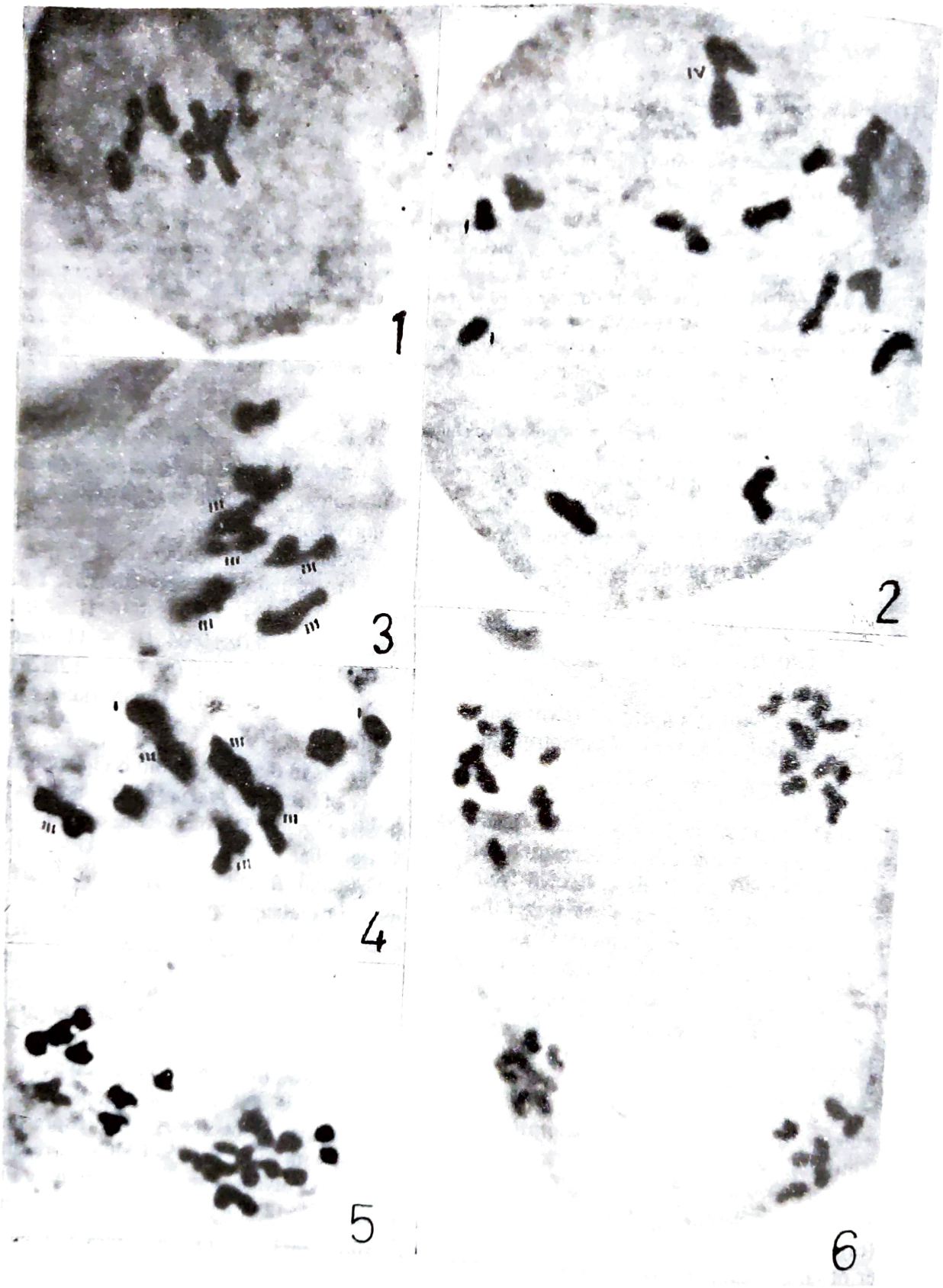
The species is diploid with 12 metacentric and 2 submetacentric chromosomes with secondary constriction present in one pair. During meiosis 7 bivalents are regularly constituted with regular distribution at anaphases (Fig. 1) and more than 90% pollen fertility. The number of chiasmata per PMC varies from 8 to 10 with an average frequency of 8.82 per PMC and 0.63 per chromosome.

The autotetraploids have relatively low frequency of multivalent formation (Fig. 2) which goes on decreasing with the advancement of generations. The average chromosome associations in the autotetraploids of C_1, C_2 and C_3 generations are 1.82±0.17 IV + 0.02±0.02 III + 10.24±0.36 II + 0.18±0.18I, 1.24±0.18IV + 0.08±0.04 III + 11.36±0.35 II + 0.08±0.04 I and 0.74±0.09 IV + 12.32±0.21 II + 0.40±0.11 I, respectively. Quadrivalents are mostly ring and chain type.

Meiosis in the autotriploid is characterised by the presence of trivalents and univalents besides bivalents. (Table 1, Figs. 3, 4). Analysis based on 25 trivalents indicates that 'chain' type (48.00%), 'fork' type (44.00%) and 'frying pan' type (8.00%) are common shapes of trivalents. The chiasma frequency per PMC ranges from 11-16 with average per PMC and per chromosome being 13.55±0.35 and 0.65, respectively. Anaphases are quite abnormal due to presence of laggards (42.36% at A-I/T-I & 44.50% at A-II/T-II) and irregular distribution of chromosomes. (Figs 5,6). Analysis shows that more common types of distribution are 11:10 and 12:9 observed in 48.57% and 25.72% cells, respectively. Microsporogenesis is abnormal with only 36.67% PMCs representing normal tetrads. Polyads and tetrads with micronuclei are reported in 15.00% and 48.33% PMCs, respectively.

Autotetraploids of C_3 generation show more enhancement in the ornamental characters like plant height (33.5±3.79 cm), number of capitula (97±27.41),

Received December, 1996



Figures 1-6.
Fig. 1. Diploid, M-I, 7_{II} 2. Autotetraploid M-I, $1_{IV} + 11_{II} + 2_{I}$. 3-6. Autotriploid. Fig. 3. M-I, $5_{III} + 3_{II}$. Fig. 4. M-I, $5_{III} + 2_{II} + 2_{I}$. Fig. 5. A-I showing 13:8 chromosomes. Fig. 6. A-II showing irregular distribution of chromosomes.

Table 1. Analysis of chromosomal associations at diakinesis/M-I in the autotriploid (2n=21) of *Gamolepis tagetes*.

PMCs Observed		Chromosomal associations		
Number	%age	III	II	I
6	30.00	2	6	3
5	25.00	5	2	2
3	15.00	5	3	—
2	10.00	1	7	4
2	10.00	1	6	6
1	5.00	3	4	4
1	5.00	4	3	3
Total 20	100.00	63	88	55
Average frequency/PMC		3.15±0.36	4.40±0.42	2.75±0.35
Range		1 - 5	2 - 7	0 - 6
Chromosomes involved (% age)		45.00	41.90	13.10

size of capitula (34.0±2.75 mm) and number of ray florets (11±1.10) than those of autotriploid and diploid (see : Table 2). Though achene setting is reduced in the autotetraploids yet it shows improvement with the advancement of generations.

Autotriploid show some enhancement in some of the characters like plant height, size of capitulum, ray floret, etc. and microcharacters like pollen and stomatal size. While number of branches, capitula and plant spread, etc. show reduction as compared to the diploid. Achene setting is very poor with an average of 2 achenes per capitulum. Three single trisomics and two diploids were recovered from the progeny of autotriploid plant.

DISCUSSION

Induced autotetraploids of *Gamolepis tagetes* have been stabilized after C_2 generation and are much superior to the diploids in floral characters. However, the autotriploid does not show much improvement over diploid and is midway in the diploid and autotetraploid in many characters. In composites, autotriploid of *Chrysanthemum coronarium* (Gupta & Gill, 1984) and *Zinnia linearis* (Raman *et al.*, 1976) are also reported to be intermediate between diploid and autotetraploids.

In the autotriploid of present species, frequency of trivalents is low than expected. The highest number (5/PMC) is observed in 40.00% of the PMCs. Frequency of trivalents in the artificially produced autotriploids is quite variable. Those of *Oryza sativa* (Rao & Reddi, 1971), *Luffa acutangula* (Roy & Dutt. 1972), *Zinnia linearis* (Raman *et al.*, 1976) and

Table 2. Morphological comparison of diploid and autotriploid in Gamolepis tagetes.

Character	Diploid (2n=14)	Autotriploid (2n=21)
1	2	3
1. Plant height (cm)	(16.5 - 23.5) 19.5 ± 1.78	- 26.5
2. Leaf size (cm)	(3.2x2.0 - 6.8x3.3) 5.0±0.87 x 2.6±0.29	(3.2x1.2 - 4.5x3.0) 3.8±0.28 x 2.5±0.42
3. Number of main branches per plant	10 - 12 11 ± 0.41	- 9
4. Capitulum characters :		
i) Diameter (mm)	(20.0 - 25.0) 23.0 ± 0.94	25.0 - 29.0 27.0 ± 0.71
ii) Number of capitula per plant	(20 - 42) 32 ± 4.78	- 26
iii) Number of ray florets per capitulum	(8 - 12) 19 ± 0.9	8 - 10 9 ± 0.41
iv) Number of disc florets per capitulum	(44 - 52) 48 ± 1.68	36 - 40 37 ± 0.70
v) Size of ray floret (mm)	(14.0x2.5 - 17.0x3.5) 16.0±0.71 x 3.0±0.20	(16.0x1.5 - 18.0x3.0) 17.0±0.41 x 2.30±0.32
vi) Size of disc floret (mm)	(5.0x1.0 - 7.0x2.0) 6.0±0.58 x 1.5±0.20	(8.0x1.0 - 8.5x2.5) 8.3±0.12 x 1.8±0.32
5. Number of achenes set per capitulum	(30 - 40) 35 ± 2.38	(1 - 4) 2 ± 0.22
6. Plant spread (cm)	13.0 x 10.0	17.5 x 6.0
7. Blooming period of capitulum intact on plant (h)	96	100
8. Duration of flowering period of plant (days)	75	60
9. Pollen size (µm)	(21.0 - 24.5) 22.4	(17.5 - 28.0) 22.5
10. Pollen fertility (%)	93.5	33.5
11. Stomatal characters : (Lower epidermis)		
i) Size (µm) Length	(20.9 - 37.5) 31.1	(37.5 - 56.2) 46.5
Breadth	(16.7 - 22.9) 19.8	(18.8 - 29.2) 25.4
ii) Frequency per unit area	(4 - 6) 5.0	(2 - 4) 2.9
iii) Index	(11.4 - 15.8) 13.7	(10.5 - 20.0) 15.7

*Figures in the parentheses indicate range values and those outside are average values.

Chrysanthemum coronarium (Gupta & Gill, 1984) have low trivalent frequency while those of *Amaranthus tricolor* (Madhusoodanan & Pal, 1984), *Hyoscyamus muticus* (Tyagi & Dubey, 1989) and

Morus species (Basavaiah *et al.*, 1990) have high frequency. Univalents ranging 0-6 (average 2.75) in the autotriploid of *G. tagetes* might have originated either due to the failure of one chromosome of homologous sets of three chromosomes to pair, leading to a bivalent and a univalent or all the three chromosomes of a set remaining as univalents due to competetive pairing.

The chromosome configuration like $2_{III} + 6_{II} + 3_{I}$, $5_{III} - 3_{II}$ and $1_{III} + 7_{II} + 4_{I}$ having low number of univalents than expected might be due to pairing in some nonhomologous chromosomes. 'Chain' and 'fork' type trivalents are more frequent than other shapes. However, high frequency of 'chain' and 'V' shaped trivalents by Pandey & Pal (1983) and of 'chain' and 'frying pan' shaped trivalents by Tyagi & Dubey (1989) is reported in autotriploids of *Tagetes erecta* and *Hyoscyamus muticus*, respectively.

Besides other factors, meiotic abnormalities such as high incidence of laggards at anaphases and highly abnormal microsporogenesis seem to be responsible for poor achene fertility in the autotriploid of present species. In the induced autotriploids of composites, even non viable achenes are also reported by Gupta & Gill (1984) and Raman *et al.* (1976).

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