Induced Trisomics in *Chrysanthemum coronarium* Linn.

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Chrysanthemum coronarium, a commonly cultivated winter ornamental, is diploid ($n=9$) with fairly high incidence of structural heterozygosity. As many as 16 chromosomes are sometimes involved in reciprocal translocations with maximum of eight chromosomes in a single association (Gill and Gupta 1981). Colchicine treatment was given to seeds and seedlings for the artificial induction of autopolyploidy with the purpose to understand its effect on ornamental characteristics and structural heterozygosity. As a consequence of this two hypertriploids ($2n=28$ and 30) and one mixoploid ($2n=16, 18$) were obtained, besides many autotetraploids ($2n=36$). Two singly trisomics ($2n=19$) and one doubly trisomic ($2n=20$) were recovered from the open pollinated progeny of one hypertriploid ($2n=30$). The present communication deals with the transmission of trisomy and the cytomorphology of these trisomics.

Material and methods

Smears for appropriate sized capitula were made in 1% acetocarmine. Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine.

Results

Morphology

Both the singly as well as doubly trisomics are not found to have any appreciable morphological difference as compared with the normal plants. Comparison of stomatal and pollen characters of these trisomics is given in Table 1. It could not be ascertained whether the two singly trisomics have trisomy for the same or for different chromosomes. Therefore, it is preferred to refer them as trisomic A and B.

Cytology

In trisomic A, besides bivalents, univalents and multiple associations of 3 and 4 chromosomes exist with an average frequency per PMC of 8.32, 0.54, 0.50 and 0.08, respectively (Table 2) (Figs. 1-3). Chiasma frequency which ranges from 16-18, is 17.036 per cell and 0.897 per chromosome.

In trisomic B, the number of bivalents and univalents ranges 6-9 and 0-3 with average frequency being 7.38 and 0.42, respectively. Single multiple association which is represented in 88.46% of PMCs, has variable number of chromosomes

Table 1. Stomatal and pollen characters of diploid and trisomics

Character	Diploid	Trisomics		
		Singly (2n=19)		Doubly (2n=20)
		A	B	
Stomatal size				
Upper epidermis	42.9–56.5 × 29.2–39.0 μm	35.1–46.8 × 27.3–33.1 μm	—	35.1–46.8 μm 29.2–33.1 μm
Lower epidermis	39.0–62.4 × 27.3–50.7 μm	31.2–40.9 × 27.3–31.2 μm	31.0–54.0 × 23.0–35.0 μm	39.0–62.4 × 27.3–50.7 μm
Stomatal frequency				
Lower epidermis	103.1/mm ²	146.4/mm ²	88.5/mm ²	122.2/mm ²
Stomata index				
Lower epidermis	23.8	24.2	22.2	27.2
Pollen size	27.3–31.2 μm	27.3–39.0 μm	22.4–33.1 μm	25.3–42.9 μm
Pollen fertility	95%	90%	85%	92%

Table 2. Chromosomal associations at diakinesis and metaphase I

Taxa		Chromosomal associations					
		VII	V	IV	III	II	I
Trisomic A (PMCs observed 50)	Range	—	—	0–1	0–1	6–9	0–3
	Total	—	—	4	25	416	27
	Average	—	—	0.08	0.50	8.32	0.54
	frequency/PMC	—	—	—	—	—	—
	% age of chromosomes involved	—	—	1.68	7.89	87.58	2.84
Trisomic B (PMCs observed 26)	Range	0–1	0–1	0–1	0–1	6–9	0–3
	Total	2	9	4	8	192	11
	Average	0.08	0.35	0.15	0.31	7.38	0.42
	frequency/PMC	—	—	—	—	—	—
	% age of chromosomes involved	2.95	9.21	3.16	4.89	77.68	2.21
Doubly trisomic (PMCs observed 51)	Range	—	—	0–2	0–2	6–10	0–2
	Total	—	—	3	54	403	40
	Average	—	—	0.06	1.06	7.90	0.78
	frequency/PMC	—	—	—	—	—	—
	% age of chromosomes involved	—	—	1.20	15.90	79.00	3.90

Figs. 1–16. 1–3. Singly trisomic A. 1, MI, I_{III}+8_{II}. 2, MI, 9_I+1_I. 3, diakinesis with 'frying pan' trivalent and 8 bivalents. 4–7. Singly trisomic B. 4, MI, I_V+7_{II}. 5, MI, I_V+7_{II} with one chromosome of a bivalent fragmented. 6, MI, I_{IV}+7_{II}+1_I. 7, MI, diakinesis, I_{VII}+6_I. 8, 9: Doubly trisomic. 8, MI, 2_{III}+7_{II}. 9, MI, 1_{III}+8_{II}+1_I. 10–12: Trisomic A. 10, AII with 10 : 10 : 9 : 9 chromosomes at the poles. 11, MII with 9 two chromatids chromosomes

and 1 one chromatid chromosome in each nuclear plate. 12, TI with laggards. 13, 14: Trisomic B. 13, TII with chromosome bridge. 14, TII with laggards. 15-16: Doubly trisomic. 15, AI with 10 : 10 chromosomes. 16, AI with 11 : 9 chromosomes.

(Table 2). The average frequency per PMC for multiple association of 7, 5, 4 and 3 chromosomes is 0.08, 0.35, 0.15 and 0.31, respectively (Figs. 4–7). Chiasma frequency has a range of 13–17 with an average of 14.962 per cell and works to 0.787 per chromosome.

The number of quadrivalents, trivalents and univalents in the doubly trisomic ranges from 0–2 (Figs. 8, 9) with an average frequency of 0.06, 1.06 and 0.78, respectively (Table 2). The average frequency for bivalents is 7.90 per cell. Chiasma frequency which ranges from 15–19, is 16.659 per cell and 0.833 per chromosome.

Analysis of trivalents in these three trisomics shows that four different shapes, namely, 'chain' type, 'frying pan' type, 'fork' type and 'ring' type are represented. Of these 'chain' type is the most common followed by 'frying pan' type (Table 3).

In both the singly trisomics, the chromosome distribution is mostly 10 : 9 at AI and 10 : 10 : 9 : 9 (Fig. 10) at AII. Rarely the extra chromosome divides into two chromatids with one included to each pole (Fig. 11). Laggards are present very rarely at AI/TI (Fig. 12) and AII/TII (Figs. 13, 14). In the doubly trisomic, out of 50 PMCs analysed at A-I, there is equal distribution of 10 : 10 (Fig. 15) in 44% and 11 : 9 (Fig. 16) in 50%. In the remaining 6%, one univalent divides

Table 3. Frequency of different shapes of trivalents

Taxa	Trivalents observed	Types			
		'chain'	'frying pan'	'ring'	'fork'
Trisomic A	Number 40	25	11	—	4
	% age	62.5	27.5	0.0	10.0
Trisomic B	Number 38	29	7	2	—
	% age	76.3	18.4	5.3	0.0
Doubly trisomic	Number 60	51	7	2	—
	% age	85.0	11.7	3.3	0.0

into two chromatids, with one going to each pole. Similar type of distribution exists at AII. Laggards are not present at anaphases and telophases.

Progeny of trisomics

Open pollinated progenies of singly and doubly trisomics were morphologically identical except for some natural variations in capitulum characters. In the progeny of trisomic A, there were 11.4% singly trisomics which were triple for the same chromosome. The progeny of trisomic B, consisted of only diploids. The progeny of the doubly trisomic was analysed to have 12.0% singly trisomics and 88% diploids. These trisomics might have either of the extra chromosomes of the doubly trisomic. Of the three trisomics from the progeny of doubly trisomic, analysed cytologically, two are found to be free from structural heterozygosity whereas the third had a multiple association of four chromosomes.

Discussion

Prior to the present studies, there is no report of experimentally produced tri-

somics in the family Compositae. Those of *Verbena tenuisecta* (Arora and Khoshoo 1969), *Trigonella corniculata* (Singh *et al.* 1978) and many other species (*vide*: Khush 1973) are morphologically distinguishable from the normal diploids. However, all the trisomics obtained presently in *Chrysanthemum coronarium* are indistinct morphologically. Such trisomics are known to have been obtained in *Solanum tuberosum* (Lam and Erickson 1971), *Amaranthus tricolor* (Madhusoodanan and Pal 1976) and some other species (*vide*: Khush 1973). As a limited number of trisomics were obtained during the present work, the possibility cannot be ruled out that trisomics of other members of the complement may behave differently as has been the case with *Gossypium hirsutum*, *Zea mays* and species of *Petunia* where phenotypic differences are recorded only for some of the trisomics (*vide*: Khush 1973).

On the basis of the chromosome number and the chromosomal configurations of $I_{IV}+8_{II}$, $2_{IV}+6_{II}$ and 10_{II} , the aneuploid with $2n=20$ might be taken as tetrasomic. But such associations are hardly represented in 7.84% of the PMCs. Whereas in 92.16% PMCs configurations are $I_{III}+8_{II}+1_I$, $2_{III}+7_{II}$ and $9_{II}+2_I$, which not only warrant such a consideration but at the same time are suggestive of doubly trisomic constitution. The formation of multiple associations of four chromosomes though at a very low rate (0.06 per PMC), is due to the structural heterozygosity involving eight chromosomes other than those representing trisomy.

The trisomic B has multiple associations of four, five and seven chromosomes. It is interesting to note that the multiple associations of odd number such as those of five and seven are represented in 42.31% of the PMCs and there is complete absence of trivalent or univalent in such cells. These type of associations are suggestive of the fact that trisomy is represented in a pair which is involved in structural interchanges.

In the presently studied trisomics, there is almost equal frequency for the extra chromosome to form a trivalent or to remain as univalent. However, tremendous variation in the frequency of trivalent in relation to one bivalent and one univalent is reported in the different trisomics of *Trigonella foenum-graecum* (Singh and Singh 1976). In the trisomics of *Amaranthus tricolor* (Madhusoodanan and Pal 1976) and *Raphanus raphanistrum* (Kamala 1980), the extra chromosome mostly remains in the form of a univalent, whereas in the nucleolar trisomic of maize (Ghidoni 1975), there is higher frequency for the extra chromosome to form a trivalent rather than remaining as univalent.

Shape of the trivalent, which depends upon the extent of pachytene pairing, and number and position of chiasmata is quite variable. Mostly these are of 'chain' type followed by 'frying pan' type. Rarely 'ring' type and 'fork' type may also be represented. Similar trend is found in the autotriploid ($2n=27$) and hypertriploid ($2n=30$) taxa of the species (Gupta 1981). The higher frequency of 'chain' type trivalents, in spite of large size of the chromosomes in the species, might be attributed to the distal localization of chiasmata.

Equal frequency of trisomics and diploids in the progeny of singly trisomics and about 25% doubly trisomics, 50% singly trisomics and 25% diploids in that of doubly trisomic are expected if anaphase distribution in meiosis is on the pattern of microsporogenesis. However, all the individuals of the progeny of one

of the singly trisomic were diploids and the frequency of trisomics was rather low (12%) in those of doubly and other singly trisomic. The low rate of transmission of the extra chromosome cannot be attributed to its elimination during meiosis. Probably the reduced transmission is due to the functional inviability of the aneuploid gametes caused by extra chromosome (Satina *et al.* 1938, Khush and Rick 1969). Besides, non-viability of $2n+1$ zygote embryo might be the other reason for the low incidence of trisomics (*vide*: Khush 1973). As such there seems to be little chances of exploiting aneuploidy as a method of improving the floriculture characteristics of the species in which structural heterozygosity might not favour the establishment of aneuploidy even at diploid level.

Summary

Two singly trisomics ($2n=19$) and one doubly trisomic ($2n=20$) were obtained in the open pollinated progeny of the colchicine induced hypertriploid ($2n=30$) of *Chrysanthemum coronarium*. Open pollinated progeny of one singly trisomic consisted of diploids only whereas the frequency of trisomics was rather low (12%) in the progeny of other trisomics. Trisomy is found to have no morphological effect. In about 50% of the PMCs, the extra chromosome constitutes a trivalent. In one singly trisomic, interchanges also involve the triple chromosome leading to the formation of multiple association of five and seven chromosomes.

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