Cytomorphology and Stability of Induced Variants in *Zinnia linearis*

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Zinnia is one of the many genera of the Compositae prized among ornamentals. The species, *Z. linearis* Benth. is a common garden annual of the plains frequently grown for edging and for beds because of its short, compact-stature, small-leaves, profuse and thin-branches and the mass of little, bright-yellow flowers. Two varieties are recognised, the yellow and white-flower types (Bailey 1957). As forms of this species with larger flowers would enhance their value in floriculture, tetraploid plants with yellow flowers were induced by colchicine treatment, doubling the chromosome number from 22 to 44 (Madhava Menon *et al.* 1969). Attempts were also made to synthesise autotriploids by intercrossing the 4n and 2n plants. The comparative effects of two new levels of ploidy on the expression of ornamental attributes and stability are outlined below.

Material and methods

The ray florets of the autotetraploid were pollinated with pollen from the diploid plant. The progenies consisted of diploids and triploids, the former being predominant. Data on the metric characters were collected at the time of peak flowering of the three chromosomal forms. Meiosis was examined in temporary acetocarmine smears of the pollen mother cells after fixing the flower buds in Carnoy's fluid (3: 4: 1) with a trace of ferric acetate added.

Observations

The morphological characteristics of the three chromosomal races of *Z. linearis* are given in Table 1. Compared to the diploid, the tetraploids were taller, had thicker stems, larger leaves and capitula of greater diameter. The ray florets were larger, but the number of whorls did not show any increase. The length of disc floret and ovary and size of seed were also slightly increased. Differences observed in the size of stomata and pollen grains between diploids and tetraploids were significant, those of the latter being larger. The number of stomata per unit area was, however, less in the tetraploid.

In respect of height of plant, size of leaf and ray floret and diameter of capitulum, the triploid was almost intermediate between the parental forms (Fig. 1). The thickness of stem, number of branches and stomata and length of peduncle showed exaggerated expressions compared to the tetraploid. A reduction from the

level of expression of the diploid was noticed in case of thickness of peduncle and number of stomata, while the increase in the length of disc floret and ovary was negligible. The length of stomata was greater than in the diploid but the width was not appreciably increased. The triploid and tetraploid were later than diploids in the commencement of flowering but the period of blooming was much longer.

Table 1. Characteristics of diploid, triploid and tetraploid plants of *Z. linearis* (Mean values in cm)

Characters	Diploid	Triploid	Tetraploid
Height of plant	57.30	60.00	74.00
Thickness of stem	0.34	0.52	0.41
Number of branches	4	12	5
Size of leaf (Length $\times$ breadth)	4.20 $\times$ 0.65	4.80 $\times$ 0.97	6.0 $\times$ 1.30
Length of peduncle	2.10	3.50	2.80
Thickness of peduncle	0.38	0.19	0.30
Diameter of capitulum	2.70	3.30	4.20
Ray floret—size of petal (Length $\times$ breadth)	1.20 $\times$ 0.60	1.35 $\times$ 0.75	1.80 $\times$ 0.90
Disc floret—length of floret	0.90	0.92	1.03
length of ovary	0.37	0.36	0.40
Stomata—number/range	52.0/45–62	36.7/27–49	33.1/29–44
size (μ)	31.2 $\times$ 24.4	37.8 $\times$ 24.8	39.9 $\times$ 26.9
Pollen—size (μ)	22.3	22.0	28.2
Seed—size of ray floret	0.27 $\times$ 0.12	—	0.20 $\times$ 0.15
disc floret	0.21 $\times$ 0.11	—	0.26 $\times$ 0.15

Fig. 1. Capitulum of diploid (left), autotriploid (centre) and autotetraploid (right) forms of *Z. linearis*. 'nat' size.

Cytology: Chromosome associations ranging from 5_{IV} to 22_{II} were observed in the tetraploid in contrast to 11_{II} in the diploid suggesting the haploid complement of the latter to be constituted of two basic sets of 5 and 6 chromosomes. The set of 5 chromosomes has identical homologues while the other set of 6 has only partially homologous partners in the diploid complement leading to the expression of preferential pairing in the tetraploid in case of the latter set. The formation of

only bivalents in conjunction with quadrivalents and the absence of univalents in cells where bivalents alone are expressed, point to the fact that univalents result only from precocious separation of members of a quadrivalent (Figs. 2 and 7-11). The lagging of univalents occurred at almost the same rate in later progenies (24 percent in C_1 and C_2 and 20 in C_3). The fertility of pollen showed an increase

Figs. 2-11. Meiosis in diploid, triploid and tetraploid races of *Z. linearis*. 2, eleven bivalents in the diploid. 3, MI in the triploid showing $5_{III}+6_{II}+6_I$. 4, precocious disjunction in trivalents and bivalents leading to univalents. 5, cell with 10/17 distribution and an accessory plate of 6 chromosomes. 6, unequal distribution of chromosomes (16/14) and laggards. 7, cell showing an association of $2_{IV}+3_{III}+9_{II}+9_I$ in the tetraploid. 8, prometaphase with 22 bivalents. 9, precocious separation of members of quadrivalents and bivalents leading to $2_{IV}+9_{II}+18_I$. 10, unequal distribution with 20/24 chromosomes. 11, cell showing 22/22 distribution. Figs. 2, 4 and 7, $\times 1400$. Figs. 3 and 8, $\times 1800$ and rest $\times 1100$.

from C_1 to C_3 . Seed fertility conformed to a slightly different pattern in variation from generation to generation (Table 2). An interesting feature was the occurrence of a considerable proportion of diploid segregants in the C_2 and C_3 progenies which, however, resembled the normal diploid plants. The tetraploid plants of the progenies continued to maintain the larger size of the vegetative organs and floral parts.

In the triploid, the chromosomes associated themselves into trivalents, bivalents and univalents. Analysis of premetaphase cells indicated a minimum of 5 and maximum of 8 trivalents. The average association was represented by $5.46_{III} + 3.83_{II} + 8.93_I$. The set of 5 formed only trivalents, while the other set of 6 gave 1-3 trivalents and 3-5 bivalents and univalents in about 33 percent of cells and 1-6 bivalents plus 6-16 univalents only in the rest, the mean association being $0.46_{III} + 3.83_{II} + 8.93_I$. Most AI figures showed lagging chromosomes. Whole and dividing univalents were observed at this stage. At TI, distributions as 16/16, 15/14, 15/15, 15/17, 14/16, 13/17, 13/13, 12/17, 12/15, 12/12 and 10/20 were noticed. Although laggards and occasional bridges were observed at the second division, there was very little evidence of disintegration of cells (Figs. 3-6). Quartets appeared normal except for the occurrence of micronuclei. The few seeds extracted from the capitula were shrivelled and incapable of germination.

Table 2. Chromosome association and fertility in 2n, 3n and 4n plants of *Z. linearis*

Ploidy	Chromosome association								Fertility (%)	
	Maximum				Average				Pollen	Seed
	IV	III	II	I	IV	III	II	I		
Diploid	—	—	11	—	—	—	11	—	91.3	82.3
Autotriploid	—	8	3	3	—	5.46	3.83	8.93	62.9	—
Autotetraploid										
C ₁	5	—	12	—	2.5	0.3	16.2	0.7	67.7	63.3
-do- C ₂	3-5	—	12-16	—	1.6-2.5	0.0-0.5	16.2-	0.3-	65-85	51-82
							18.5	0.7		
-do- C ₃	3-4	—	14-16	—	0.9-2.3	0.1-0.9	13.3-	0.2-	63-76	49-64
							19.6	0.7		

Discussion

Induced triploid and tetraploid strains of ornamental plants have been introduced in floriculture with reasonable success (Bali and Tandon 1956, Elliot 1958, Jain *et al.* 1962, Raman 1972). Triploid forms in species of *Canna*, *Fritillaria*, *Jasminum*, *Tulipa* etc. which possess greater ornamental appeal have established themselves in garden practice because of the ease in propagation through asexual means. Tetraploid snapdragons, zinnias and marigolds are favoured for their larger flowers and longer flowering period and other attributes of value in floriculture. Reduced seed fertility in the tetraploids is a drawback to be reckoned with in sexually propagated ornamental annuals, which could be overcome by selection and other breeding techniques.

The effects of induced autotetraploidy in *Zinnia linearis* are more significant in the intensification of expression of height of plant, size of leaf and ray floret and diameter of capitulum, while the triploid is intermediate in respect of these. The observed increase in size of parts can be attributed to an increase in cell size which, however, has a deterrent effect on the rate of differentiation of plant parts, as evidenced from the reduction in the number of florets. The greater vigour and larger

number of flowers of the triploid confer a definite advantage for its success as an improved form in floriculture.

The variation in levels of seed fertility in the progenies of the tetraploid indicates the scope for selection towards higher fertility, yet the extent of diploid segregants needs to be kept at a minimum or eliminated. Therefore, progeny analysis with reference to the two features viz., seed fertility and proportion of diploids should be undertaken on an intensive scale for stabilisation of tetraploid lines by selection. The tendency for parthenogenetic development of eggs is a common feature in species of the Compositae. The high frequency of diploids in the progenies of the tetraploid can, therefore, be considered as an intensified expression of the genetic potentialities for this apomictic phenomenon in the tetraploid.

The genus consists of about fifteen species most of which are distributed in North and South America. The sub-genus *Diplothrix* includes species with 20, 40, 42 and 84 ($2n$) chromosomes which are caespitose and perennial while those of *Zinnia* are annuals with erect habit and possess 24 somatic chromosomes (Torres 1962). The importance of hybridisation and polyploidy is well evident both in the evolution of the 22 chromosome species and in the diversification of species of the *Diplothrix*.

Two basic species with $2n=10$ and $2n=12$ may have gone into the formation of the present day diploids with 22 chromosomes. A workable hypothesis would be, three parents, A ($2n=10$) B and C ($2n=12$) hybridising to form primary hybrids AB and AC and subsequently resulting in 22 chromosome derivatives. These (AABB and AACC) would show normal chromosome pairing and fertility due to the high differentiation of the constituent genomes. Hybridization between the two can give rise to plants comprising two genomes of parent A, and one each of B and C, the chromosomes of the latter showing small structural variations. Further selection in sexual progenies would have helped in establishing the cultivated forms of *Z. linearis*. The absence of any recognisable aberrations would mean that the structural differences between chromosomes of the sub-genomes derived from parents B and C as indicated above are minute and incapable of affecting the phenotype of either the parent or of its progenies.

Summary

Colchicine induced autotetraploids of *Zinnia linearis* were taller, had thicker stems, larger leaves and ray florets and capitula of greater diameter than diploids. The autotriploids presented increased values in respect of thickness of stem, number of branches and length of peduncle compared to the tetraploid but were more or less intermediate between the parental forms with regard to height of plant, size of leaf and ray floret and diameter of capitulum.

No linear relationship could be traced between the meiotic aberrations and sterility in the tetraploids. Factors of a genetic nature seem to be superimposed over those of cytological origin in determining sterility. The differences in pollen and seed fertility and the occurrence of parthenogenetic diploids are impediments to quick progress in selection. In the triploid, the concomitant sterility enhances the vigour and ornamental value.

The haploid complement of *Z. linearis* appears to be constituted of two basic sets of 5 and 6 chromosomes, the former possessing identical homologues while the latter having only partially homologous partners in the diploid complement as reflected in the expression of preferential pairing in the tetraploid. This differential pairing behaviour is also evident in the meiosis of the autotriploid. It is probable, that hybridisation between two putative types with 5 and 6 (n) chromosomes could have contributed to the development of this ornamental species.

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