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Induced Autotetraploidy in *Zinnia linearis*

by

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Artificial doubling of the chromosome number has been found to be a fruitful approach in breeding new types of ornamental plants. Its value in floriculture has been well recognized and attempts have been made to produce strains of sexually and asexually propagated ornamental plants with reasonable success (Emsweller and Ruttle, 1941; Bali and Tandon, 1956; Jain *et al.*, 1962). Tetraploid snapdragons, zinnias and marigolds have been introduced in garden practice (Elliott, 1958) and are favoured for their large 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. Attempts were made to induce tetraploidy in *Zinnia linearis* and the observations are presented in this paper. This species is a common garden annual of the plains frequently grown for edging and for beds because of its short, compact stature, small leaves, profuse thin-branches and the mass of little, bright-yellow flowers. Two varieties are recognized viz., the yellow and white-flowered types (Bailey, 1957). Forms of this species with larger flowers would be of greater appeal to the floriculturist.

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Material and Methods: Seeds of *Zinnia linearis* (yellow-flowered form) were soaked for 12 hours in aqueous solution of colchicine at concentrations of 0.01, 0.05, 0.1 and 0.2 %, washed in water and sown in pots. Fifty seeds were used in each treatment. Significant differences were not evident at the early seedling stages due to the effects of different concentrations of colchicine, though there was a higher mortality at the highest concentration. At flowering, three plants among the group raised from seeds treated with 0.05 % concentration, showed larger flowers (capitula) than their counterparts. Preliminary examination of these showed them to be tetraploids and detailed studies were taken up thereafter. Meiosis was examined in temporary acetocarmine smears of the P M. cells after fixing the flower buds in 3:4:1 Carnoy's fluid, with a trace of ferric acetate added.

Observations: The morphological characteristics of the tetraploids and diploids are given in the Table 1 below for comparison.

TABLE 1. *Characteristics of diploid and autotetraploid plants of Z. linearis (Mean values in cm)*

Characters	Diploid	Tetraploid
Height of plant	57.3	74.0
Thickness of stem	0.34	0.41
Number of branches	4.00	5.0
Size of leaf (length × breadth)	4.20 × 0.65	6.00 × 1.30
Length of peduncle	2.1	2.8
Thickness of peduncle	0.28	3.00
Diameter of capitula	3.1	4.3
Ray floret - No. per head	84.2	71.4
Size (length × breadth)	1.2 × 0.9	.6 × 1.0
Length of ovary	0.37	0.43
Disc floret No. per head	8.9	6.8
Length of floret	0.90	1.03
Length of ovary	0.37	0.40
Size of stomata (μ)	31.2 × 24.4	39.9 × 26.9
Distribution / unit area	52.0	33.1
Size of pollen grains (μ)	22.3	28.2

The tetraploids were taller, had thicker stems, larger leaves and capitula of greater diameter. The ray florets were larger, but their number was less and the number of whorls of ray florets did not show any increase. The size of the ovary and the length of disc florets were more than in diploids, their number, however, being reduced. Differences observed in the size of stomata and pollen grains between diploids and the tetraploids were significant, those of the latter being larger. However, the distribution of stomata per unit area was less in tetraploids. The tetraploids were later than diploids in the commencement of flowering by 15 days but the period of blooming was much longer.

Meiosis: In the diploid types 11_{II} were regularly observed at diakinesis and Metaphase I. The subsequent stages of meiosis were regular. Pollen and seed fertility was high.

In tetraploids (2n:44) the chromosome associations ranged from a maximum of $5_{IV}+12_{II}$ to 22_{II} (Table 2). Univalents were observed only in conjunction with trivalents, due to the early disjunction in the quadrivalents. Unpaired chromosomes, however, could not be observed when the association in a cell was as bivalents only. At AI separation was 22/22 in 80% of the cells. Unequal separation such as 23/21 and 20/22 were infrequent, though few laggards were observed in many cells. The lagging univalents mostly were included in the dyad nuclei. The second division also showed lagging chromosomes with bridges at anaphase. In addition to normal tetrads, supernumerary spores were observed, besides micronuclei in the cells of the tetrad of spores. Pollen fertility in the tetraploids was 67.7% compared to 91.3% in the diploids. Seed-setting was 63.3% in the 44-chromosome plants while it was 82.3% in the diploids.

TABLE 2. *Chromosome association in diploid and autotetraploid Z. linearis*

		No. of p.m. cs	Chromosome association at diakinesis							
			Maximum		Minimum		Mean			
			IV	II	IV	II	IV	III	II	I
Diploid		50	–	11	–	11	–	–	11	–
Autotetraploid										
C_1		20	5	12	–	22	2.5	0.3	16.2	0.7
C_2	1	50	5	12	–	22	1.6	0.5	17.3	0.5
	2	25	3	16	–	22	2.2	0.4	16.8	0.4
	3	10	3	16	–	22	1.6	–	18.5	0.3
Mean							2.0	0.3	17.3	0.5
C_3	1	20	4	14	–	22	1.5	0.5	18.4	0.5
	2	20	4	14	–	22	1.6	0.1	18.6	0.2
	3	20	3	16	–	22	1.3	0.7	18.1	0.5
	4	20	4	14	–	22	1.7	0.2	18.3	0.2
	5	30	4	14	–	22	2.0	0.2	19.5	0.7
	6	20	4	14	–	22	2.0	0.1	17.7	0.4
	7	20	3	16	–	22	1.4	0.2	19.0	0.2
	8	20	4	14	–	22	2.3	0.3	17.0	0.2
	9	20	3	16	–	22	1.4	0.5	18.4	0.3
	10	20	3	16	1	20	0.9	0.2	19.6	0.7
Mean							1.5	0.3	18.4	0.4

Breeding behaviour: The C_1, C_2 and C_3 progenies of the tetraploids were studied. An interesting feature was the occurrence of a considerable proportion of diploid plants (2n:22) in the progenies. These constituted from 56 to 90 %. The tetraploid plants of the progenies continued to maintain the larger size of the vegetative organs and floral parts, their flowers being showy compared to the diploid, facilitating their quick detection while in bloom. The diploid segregants were similar to the normal diploid plants in every respect. The fertility of the pollen showed an increase from 67.7 % in C_1 to 65 to 85 % in C_2 and 63 to 76 % in C_3. Seed fertility showed a slightly different pattern in variation from generation to generation. Though 63.3 % fertility of seed was noted in C_1, it ranged from 51 to 82% in C_2 and 49 to 64% in C_3.

Discussion: The species *Z. linearis* Benth. is characterised by the chromosome number n:11 which is different from that of the more common species *Z. elegans* Jacq. (n:12). The former species is highly stable in its diploid state with distinct morphological characteristics. However, the variability in this form is considerably limited compared to the species *Z. elegans* where many varieties with different plant and flower types and flower colour are known in nature and tetraploids with larger flowers have been induced and introduced in culture. *Z. linearis* with its frail plant type, small, yellow or white capitula is less common in gardens, probably due to its limited variability. The induced tetraploidy of the species *Z. linearis* had larger showy capitula and an enhancement of expression of most of the morphological characters. The identification of the tetraploid nature of these plants could also be done easily by the larger size of the stomata and pollen grains compared to those of the diploids. The observed increase in size of parts can thereby 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 number of florets, leaves, branches *etc.*

Meiosis in the tetraploids was disturbed by the formation of multivalents as could be expected. But the number of quadrivalents formed reached a maximum of only five as against a higher frequency that could have been possible in view of the duplication of all the chromosomes of the diploid set. The formation of only bivalents without any univalents in cells where quadrivalents alone are noticed and the absence of univalents in cells where bivalents association without any higher associations are expressed, point to the fact that univalents result only from a precocious separation of members of a quadrivalent. As such, there appears to be no upset in the genetic balance controlling the synapsis and separation of chromosomes at meiosis. The low frequency of quadrivalents (5_{IV}) and formation of 22_{II} in a recognizable number of cases of the cells analysed, would suggest the diploid complement of 22 chromosomes to have only partial structural homology. The haploid complement of 11 may, therefore, be constituted of two basic sets of 5 and 6 chromosomes. The set of 5 is to be considered as having identical homologues in the diploid complement (2n:22) while the other set constituted of 6 finds only partially homologues partners in the genome of the diploid.

It is also pertinent to point out that in the genus *Zinnia*, species with n=10 and n=12 occur (Torres, 1962). The species *Z. linearis* with n=11 bring out the fact that hybridisation between ancestral diploids (2n=10 and 2n:12) could have led to the origin of the new basic number. The absence of any recognizable aberrations in the diploid would mean that the structural differences between chromosomes of one of the sub-genomes indicated above are minute and incapable of affecting its own phenotype or of the progenies.

Pollen and seed-fertility in the tetraploids cannot be considered to be high in the C_1 generation (67.7 and 63.3 % respectively) in spite of the high frequency of bivalent formation (average of only 2.8_{IV} per cell) and low frequency of univalents. The lagging of univalents at anaphase occurred almost at the same rate in later progenies (C_2: 24 %, C_3 = 20 %, compared to C_1 = 24 %) even though the pollen fertility differed significantly in individuals of these populations (C_2=65.10 to 85.03, C_3=63.60 to 76.02). This points to the fact that no linear relationship can be traced between the meiotic aberrations and the sterility in the tetraploids. Particularly in the present case, genic factors seem to be playing a more significant role in conditioning sterility in the 'raw' tetraploid, as is evidenced from the variation in fertility in the progenies.

The trends in variation of seed-fertility from the C_1 to the C_3 generation appear to be quite different from that of pollen fertility. Though in the C_1 seed fertility was only 63.3%, in C_2 it ranged from 51.4 to 81.5% and in C_3 from 49 to 65.9 %. This feature is further to be examined with the fact that the 'reverted' diploid segregants occur in the progenies of the tetraploid. In the tetraploids the seeds which were considered fertile could be expected to be constituted of two kinds, *viz.*, those with tetraploid embryos from sexual fusion and with diploid embryos. Only further study of the individual progenies of plants with varying levels of seed fertility and the diploid-tetraploid composition of the families can throw light on the situation.

The occurrence of diploid segregants in the progenies make complex the the procedure for selection towards the improvement of fertility in tetraploids. The variation in levels of seed fertility in C_2 and C_3 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.*, (i) seed fertility and (ii) proportion of tetraploids to diploids should be undertaken on an intensive scale for stabilisation of tetraploid lines by selection. The occurrence of diploid segregants in tetraploid lines has been observed by different investigators (Randolph and Fischer. 1939; Stebbins, 1957).

The tendency for parthenogenetic development of eggs is a common feature in species of the compositae (Stebbins, 1950). The high frequency of diploids in the progenies of the tetraploids can, therefore, be considered as an enhanced expression of the genetic potentialities for this apomictic phenomenon in the tetraploids.

Summary: Tetraploidy was induced in *Zinnia linearis* by treatment of seeds with colchicine at concentration of 0.05 % in acqueous solution. The tetraploids were taller, had thicker stems, larger leaves and capitula of greater diameter than diploids. The tetraploids were later to bloom than diploids but the periods of blooming was much prolonged. The differences in size of pollen grains and stomata in diploids and tetraploids were significant, those of the latter being larger.

Chromosome associations ranging from $5_{IV}+12_{II}$ to 22_{II} were observed in the tetraploid (2n : 44) in contrast to 11_{II} in the diploid. Pollen fertility was 67.7% in tetraploid compared to 91.3% in diploid. Seed setting amounted to 63.3% in the tetraploid and 82.3% in the diploid. The high frequency of bivalents and maximum multivalent association of 5_{IV} in a recognicable number of p.m.c's suggested the haploid (n : 11) complement of *Z. linearis* to be constituted of two basic sets of 5 and 6 chromosomes. It is probable that hybridisation between two putative basic types with n : 5 and n : 6 could have contributed to the development of this ornamental species.

In C_2 and C_3 progenies a high percentage of dilpoid plants (2n : 22) was observed. There was also an increase in the pollen and seed fertility. The trend of increase in seed fertility from C_1 to C_3, however, was different from that of pollen fertility. The higher maximum realised in case of seed fertility could be attributed to the parthenogenetic development of seeds with diploid embroys. Variation in frequency of occurrence of diploids in the progenies of selected plants brought out the need for directed selection to stabilise the tetraploid lines.

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Studies on the Floral Biology and Fruit-set in Onion (*Allium cepa* L.) - I

by

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Inrtoduction : The importance of floral biological studies in projects aiming at crop improvement work is well known. The exact technique to be employed in breeding programme for the evolution of strains with desirable attributes is dependent on the selfed or open pollinated nature of the crops concerned, which itself is determined by the genetic and physical barriers existing in those crops. The present studies were therefore, carried out with four different cultivars of cepa or common onion from commercial sources with the same object in view.

Review of Literature : Onion is one of the few crops which has received considerable attention at the hands of plant breeders and geneticists (Jones and Emsweller, 1934; Jones, 1937; Jones and Clarke, 1947; Trofimec, 1940; Becker, TH., 1943–44; Ustinova, 1950 and Agati, (1952), Hawthorn and Pollard (1953) and Jones and Mann (1963) have reviewed excellently the various aspects of research carried out with this crop. Although several of the European and American varieties of onion have been introduced into our country and grown for over six decades, only recently crop improvement work has been initiated in this crop. The earlier work in onion in our country was primarily concerned with agronomic aspects of production and floral biology in this crop, does not appear to have been studied.

Jones (1937), Jones and Mann (1963) have dealt in great detail with the anthesis and pollination in onion grown for seeds, besides describing the floral structures. According to him the pollen of a single flower is shed over a period of 24 to 72 hours from the time of flower opening and long before

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