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Induced autotetraploidy in chickpea (*Cicer arietinum* L.)*

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Summary. In chickpea, out of three colchicine concentrations and two treatment durations used (combinations of 0.25, 0.05, 0.025% colchicine and 4 and 6 h duration), seed treatment with 0.25% for 4 h proved to be the most effective in producing autotetraploids. Colchicine treatment on seedlings failed. The induced tetraploidy was accompanied by larger leaves, flowers, stomata, pollen grains and seeds. Mean percentage stainable pollen and podset were reduced, but some plants had relatively normal meiosis and produced as many pods as the diploid parent, indicating the potential of induced autotetraploids in chickpea improvement.

Key words: Chickpea – Colchicine – Tetraploids

Introduction

Chickpea (*Cicer arietinum* L.) has only eight pairs of somatic chromosomes (Iyengar 1939; Ramanujam and Joshi 1941; Ladizinsky and Adler 1976) and as it does not appear to be a primary or secondary polyploid, it is likely to respond well to polyploidization. Artificial induction of tetraploidy has been reported in chickpea (Ramanujam and Joshi 1941; Sohoo et al. 1970; Phadnis and Narkhede 1972). In the present study, an attempt was made to induce autotetraploidy in chickpea using colchicine with the objective of creating more genetic variability and of testing the material for seed size and pod production. Wild *Cicer* species possess useful attributes. *Cicer judaicum* has *Fusarium* wilt immunity (van der Maesen et al. 1980) and high methionine content (U. Singh, pers. commun.). This species could not be crossed with cultivated chickpea. By inducing tetraploidy in both species hybrids may possibly be obtained.

Materials and methods

The experiments were conducted on chickpea cv. 'Annigeri', at the ICRISAT Center, Patancheru, during the postrainy seasons of 1979–80 and 1980–81. 'Annigeri', a short duration genotype (maturing in about 100 days), is a commercially released cultivar found in the Karnataka State of India. The plants have a spreading growth habit, the seeds are yellow-brown in color and it is a typical local Indian type cultivar (desi).

Seed treatment

Well developed seeds, numbering 500 in each treatment, were submerged in three different concentrations of aqueous colchicine solution; 0.25, 0.05, and 0.025% for 4 and 6 h. After treatment, the seeds were thoroughly washed in running water and sown in the field.

Seedling treatment

Seedlings were raised in small earthenware pots filled with soil and farmyard manure (3 : 1). After seedling emergence, a small absorbant cotton wad was placed around the apical meristem and the colchicine solution was applied at regular intervals to keep the wad moist. Two colchicine concentrations, 0.25 and 0.125% were used on 100 seedlings for each treatment, with a soaking duration of 24 h.

The leaf stomata size and leaf area of each C_1 plant were measured before flowering. At flowering, pollen grain stainability of each plant was assessed by staining with 2% potassium ioide mixed with a drop of glycerine. For chromosome examination, young buds were collected in bulk from suspected tetraploids in the early morning and fixed in 6 : 3 : 1 v/v (alcohol : chloroform : acetic acid) fixative. Smears were prepared using 1.0% acetocarmine and observations of metaphase I of meiosis were made at 1000× magnification. Ob-

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servations on yield components were also taken. Pod set was recorded as % of flowers which form pods. Number of seeds per pod was expressed as a ratio of total number of seeds to total number of pods on a single plant. The statistical difference of means of the traits of normal and autotetraploid plants were computed by the 't' test.

Results

C_1 generation

Seedling treatment. Following colchicine treatment the seedlings became dark green and thick. The apical meristems remained stunted. Lateral branches arose below the apical meristems but despite their removal, the apical meristems failed to develop and only a single branch on one plant was found to be tetraploid.

Seed treatment. The effects of concentration and duration of colchicine seed treatment on percent germination and number of tetraploid seedlings are shown in Table 1. At a concentration of 0.05 and 0.025%, germination was near normal and only one tetraploid plant was observed. At a concentration of 0.25% for 4 h, the seed germination was reduced to 2.8% and nine tetraploid plants were obtained. The same concentration extended to 6 h proved to be more lethal, germination was reduced to 0.4% and only two tetraploid seedlings were obtained.

The tetraploid seedlings were usually distinguishable from diploids by their dark green, large and closely spaced leaflets and enlarged flower size. Stomatal and pollen grain measurements and observations of meiosis confirmed the plants to be tetraploids.

Table 1. Germination percentage and tetraploid production after colchicine treatment of 500 seeds

Colchicine concentration (%)	% Germination		No. of tetraploid plants	
	4 h	6 h	4 h	6 h
0.025	90.0	89.0	0	0
0.05	85.0	80.0	0	1
0.25	2.8	0.4	9	2

Table 2. Number of diploid and tetraploid plants in C_2 progenies

Progeny	Total	2X	4X
1	14	12	2
2	2	1	1
3	1	0	1
4	2	2	0
5	14	0	14
6	35	34	1
7	4	0	4
8	73	67	6
9	2	2	0

C_2 generation

Progenies of nine tetraploids segregated into diploids and tetraploids as shown in Table 2. Three progenies were all tetraploids, two were all diploids, and four progenies consisted of both diploid and tetraploid plants.

Morphologically the C_2 tetraploids were very similar to those of the C_1 generation. The observations recorded on C_2 plants (Fig. 1 A) have been summarized in Table 3. The stomatal size was about 33% larger in tetraploids than in diploids (Fig. 1 C, D). Pollen grain stainability was unexpectedly high, ranging from 79.2 to 90.2%, with a mean value of 84.4% compared to an average of 96.0% in the diploid material. Mean pollen diameter was 29% larger in tetraploids.

Table 3. The ranges and means for different characteristics of autotetraploid and diploid plants of chickpea cv. 'Annigeri'

Characteristic	Tetraploid		Diploid		't' value
	Range	Mean	Range	Mean	
Leaf size (sq. cm)	4.00 – 5.59	4.55 ± 0.28	1.63 – 2.41	1.99 ± 0.15	8.10**
Flower vexillum size (sq. cm)	–	0.61	–	0.43	–
Stomata length (μm)	32.5 – 37.5	36.0 ± 0.61	25.0 – 30.0	27.5 ± 0.79	8.49**
Stomata width (μm)	27.5 – 32.5	31.0 ± 0.61	22.5 – 23.0	23.0 ± 0.50	10.11**
Percent stainable pollen	79.2 – 90.2	84.4 ± 1.16	94.0 – 97.0	96.0 ± 0.62	6.71**
Pollen diameter	38.3 – 41.5	40.3 ± 0.30	27.5 – 35.0	31.3 ± 1.30	9.12**
Canopy height (cm)	20.0 – 35.0	28.7 ± 4.05	22.0 – 27.0	25.0 ± 0.83	0.46
Percent pod set	6.0 – 21.8	13.7 ± 1.72	42.0 – 48.2	44.6 ± 1.13	13.06**
Pods per plant	2.0 – 65.0	21.5 ± 19.93	35.0 – 53.0	43.0 ± 3.42	0.55
Seeds per pod	1.00 – 1.33	1.15 ± 0.08	1.00 – 1.14	1.03 ± 0.02	0.76
100-seed weight (g)	25.8 – 34.9	30.9 ± 2.88	17.2 – 19.1	18.5 ± 0.34	2.60*

*, ** Significant at 5% and 1% respectively

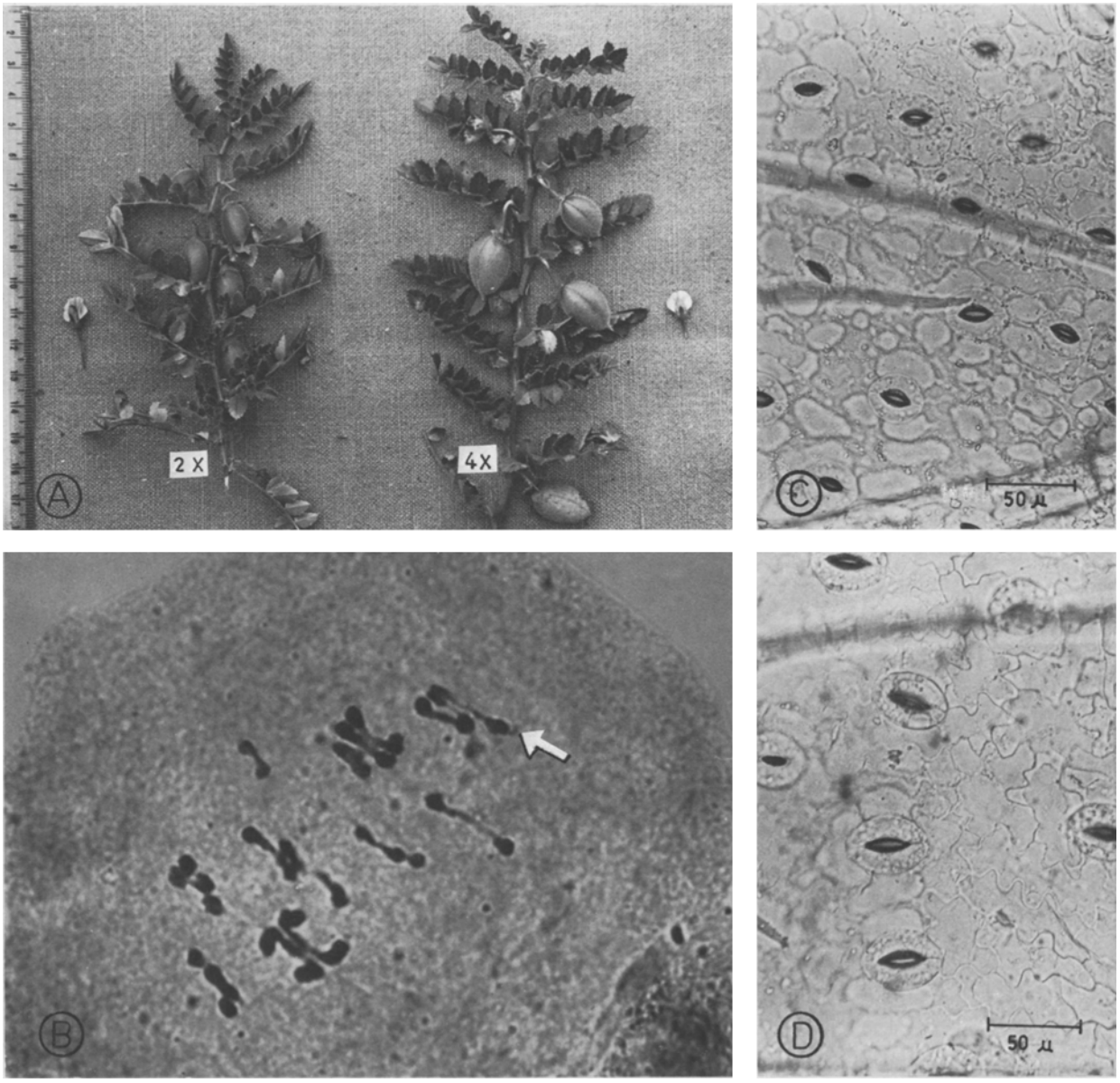

Fig. 1. **A** Diploid (2x) cv. 'Annigeri' and autotetraploid (4x) derivative; **B** Metaphase I in autotetraploid, arrow indicating quadrivalent; **C** Stomata of diploid; **D** Stomata of tetraploid

Pod set was poorer in the tetraploids (13.7%) than in the diploids (44.6%) but in the tetraploids there was a wide range of pod numbers varying from 2 to 65 with a mean value of 21.5 per plant. The average number of seeds per pod was similar. The most conspicuous difference was in seed size. In tetraploids, seed weight varied from 25.8 to 34.9 g per 100 seeds compared with a mean of 18.5 g in the diploid material.

Twenty five pollen mother cells were observed at metaphase I of meiosis. Quadrivalent associations ranged from one to seven in different cells. Twelve percent of the cells had three to four univalents. Trivalents were rare; only one such association in 25

Table 4. Chromosomal associations at Metaphase I of meiosis of the C_2 autotetraploids

Quadrivalent	Trivalent	Bivalent	Univalent	No. of cells
7	–	2	–	1
6	–	4	–	2
5	–	6	–	2
3	–	10	–	5
2	–	12	–	6
2	–	10	4	2
1	–	14	–	6
1	1	11	3	1
Mean 2.52	0.04	10.36	0.44	

cells was observed. A high frequency of bivalents was observed ranging from 2 to 14 in different pollen mother cells (Table 4, Fig. 1 B).

Discussion

In the present study, seed treatment with 0.25% colchicine for 4 h was more effective than the other combinations for producing autotetraploids. The same concentration for 6 h proved to be lethal, lower concentrations had very little effect and produced only one tetraploid plant. Ramanujam and Joshi (1941) found a 0.25% concentration for half-an-hour duration on presoaked seeds to be most effective, while Sohoo et al. (1970) reported 0.25% concentration for a 2 h duration to be the best. In the present study longer durations were chosen in order to be able to use dry seeds (Ladizinsky, pers. commun.). Results may further differ due to differences in genotypes. Phadnis and Narkhede (1972) also found 0.25% to be the most effective concentration, while at 0.50% concentration, seeds did not germinate. Colchicine treatment of seedlings proved ineffective because in chickpea seed germination is hypogeal and the epicotyl between the treatment region and cotyledons produces diploid branches which eventually suppress tetraploid meristems.

The progenies of tetraploid plants, consisted of various proportions of diploids and tetraploids. These might have resulted from the presence of either both 2 x and 4 x shoots in C_1 plants or reversion of tetraploids to diploid level. A similar situation was observed by Ramanujam and Joshi (1941) and they explained that it was because of the mixed tissues in the C_1 tetraploid plants. In the present study also some C_1 plants had diploid and tetraploid branches. Reversion to diploidy may occur due to chance fusion of gametes with 8 chromosomes formed by unequal separation of chromosomes at Anaphase I during meiosis. The formation of diploid tissues in tetraploids in pre-meiotic stages also might lead to reversion (Hagberg and Ellerstrom 1959). Thompson (1962), on the other hand, pointed out that occurrence of multipolar spindles results in complement fractionation leading to diploidy in progenies. This needs to be investigated in detail in chickpea.

The increased vigour of the autotetraploids compared to their diploid counterparts, confirmed the earlier observations of Ramanujam and Joshi (1941) and Sohoo et al. (1970). Cases have been reported in several crops, including pulses, that the tetraploids were inferior or only equal to the diploids with respect to particular morphological characters. The reports of Kumar (1945) in green gram, Bhattacharya (1956) in pigeonpea, Kumar Sen and Chedda (1958) in black gram, indicate that autotetraploids do not express gigantism in all characters and that they are often shorter than the diploids and have fewer branches.

The autotetraploids obtained in the present study produced a high proportion of fertile pollen grains but pod set was poorer than in the diploids. Similar observations were made by Sohoo et al. (1970). At meiosis in pollen mother cells quadrivalent formation was low and the number of bivalents were near normal. This accounts for the high pollen fertility and indicates the possibility of developing full functional diploidization in subsequent generations, as Hilpert (1957) had demonstrated by selection for tillering, or Stebbins (1950) by irradiation, in tetraploid rye. The fertility and the morphological features of the tetraploid chickpea suggest that induced polyploidy may have some application in chickpea improvement to enlarge diversity, and even yield potential.

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