260

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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

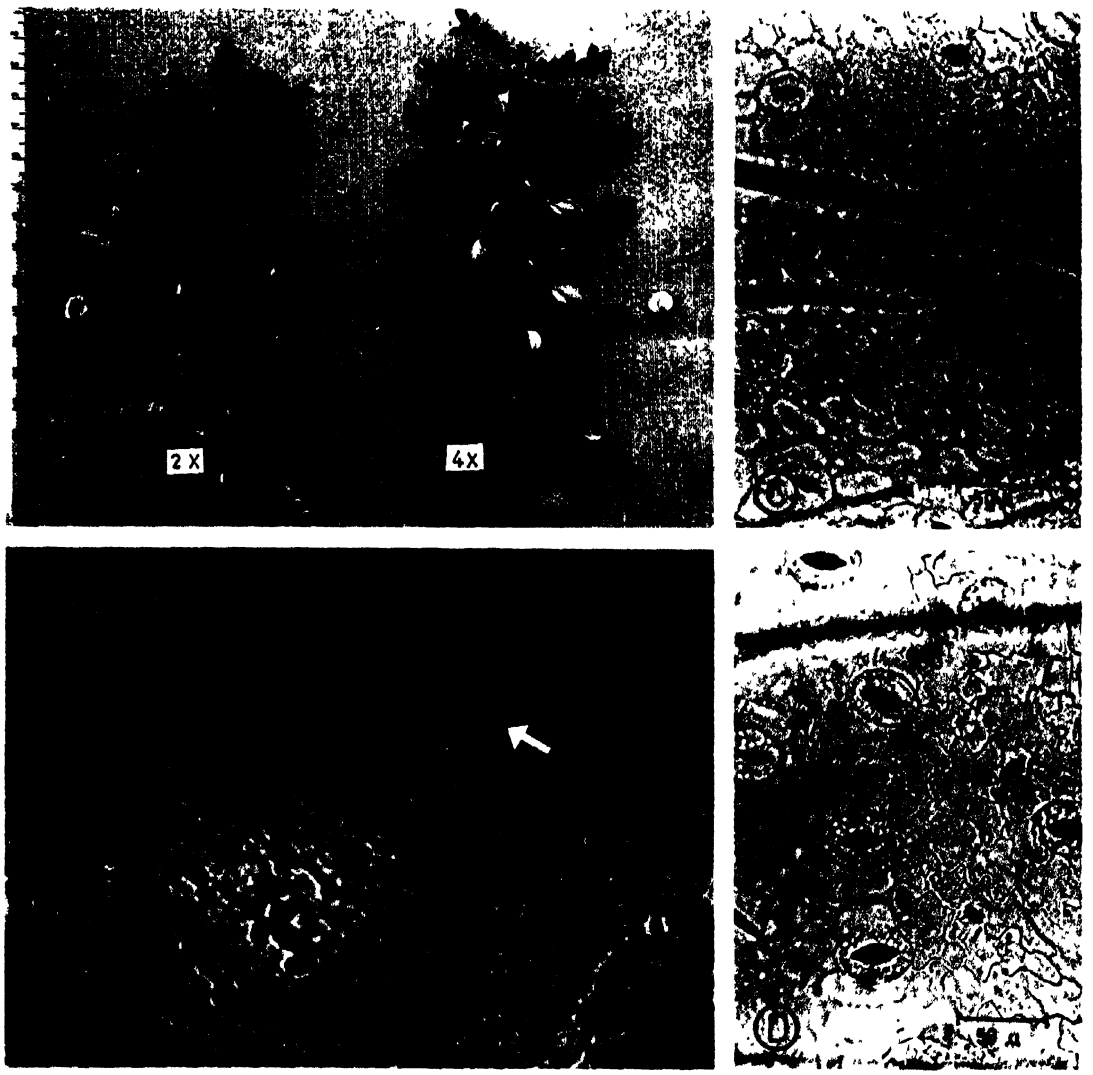


**Fig. 1.** A Diploid (2x) cv 'Annigeri' and autotetraploid (4x) derivative; **B** Metaphase I in autotetraploid, arrow indicating quadrivalent; **C** Stomata of diploid; **D** Stomata of tetraploid

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 | |

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# GENETIC ANALYSIS OF SOME LEAF CHARACTERISTICS IN PIGEONPEA [*CAJANUS CAJAN* (L.) Millsp.]

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## ABSTRACT

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A diallel cross involving nine parent types, representing three diverse agronomically promising cultivars each divided into early, medium and late maturity groups, was used to study the inheritance of leaf area, leaf weight, specific leaf weight, petiole length and petiole weight in pigeonpea (*Cajanus cajan* (L.) Millsp.). Estimates of genetic parameters suggested a primary effect of additive gene action for all the characteristics studied. However, dominance was also significant for specific leaf weight and petiole weight. Ratios computed using these genetic parameters indicated the presence of partial dominance and unequal distribution of positive and negative alleles in the parents. The correlation between *Wr + Vr* and *Yr* was positive and significant only for petiole weight. This together with the position of the parents along the regression line clearly showed that high petiole weight was under the control of recessive genes. In the case of specific leaf weight and petiole length the correlations were non-significant but the position of parents along the regression line gave some indication that large and heavy petioles were controlled by recessive genes.

## INTRODUCTION

Considerable genetic variation exists for leaf characteristics in pigeonpea (*Cajanus cajan* (L.) Millsp.). Recently, Rawson and Constable (1981) suggested that selection for large leaves along branches should result in high yield. Leaf area per plant (Singh et al., 1977) and specific leaf weight and leaf fresh weight (Saxena and Sharma, 1981) were found to have positive but moderate association with seed yield ($r$ = 0.47**, 0.49** and 0.42*, respectively). Specific leaf weight in alfalfa (Pearce et al., 1969) and leaf blade size, petiole length and petiole weight in soybean (Auckland and Lambert, 1974) were recommended as selection criteria for fodder and seed yield respectively. For effectiveness of selection for various leaf characteristics it is im-