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Colchicine Induced Polyploidy in Chickpeas (*Cicer arietinum* L.)

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Summary. The most successful induction of tetraploidy was obtained with 2 hour treatment by 0.25% aqueous colchicine solution of 18-hour watersoaked *desi* chickpeas material. However, *kabuli* types needed only 1½ hour treatment under similar conditions. Gigantism accompanied induction of polyploidy in *desi* as well as *kabuli* types but yield and fertility were greatly reduced. The meiotic abnormalities accompanying polyploidy were multivalent association of chromosomes followed by unequal disjunction, chromosome bridges, laggards etc. The percentage of stainable pollen, however, was at par between diploids and tetraploids. Gene control of percentage seed setting was observed in both levels of ploidy. A striking feature of the studies was the high seed setting percentage in $4n$ F_1 material resulting from diverse crosses, viz., *desi* × *kabuli*. A probable reduction in multivalent association coupled with yield increases in segregants from the later generation of tetraploids indicates the possibility of selection for higher yield and fertility from polyploids, particularly from some hybrid material.

Experience has shown (Stebbins, 1950) that in general plants with low chromosome number are a favourable material for polyploid breeding. *Cicer arietinum* (chickpeas) is an important pulse crop in India and is self-pollinated. It has only eight pairs of somatic chromosomes (Iyengar, 1939; Ramanujam and Joshi, 1941) and does not appear to be already a primary or secondary polyploid, so that it is likely to respond well to polyploidisation. Sterility has been the major handicap accompanying induced polyploidy in chickpeas (Ramanujam and Joshi, 1941). Selection for improved fertility following induction of tetraploidy, and the search for techniques to reduce initial sterility, therefore form the next step on which work may be concentrated. In some cases tetraploids from intervarietal crosses have shown higher fertility (Einset, 1947) and, perhaps for the same reason, greater success has been achieved with cross-pollinated species. The present study therefore used *desi* and *kabuli* varieties of chickpeas and their F_1 hybrids to evaluate the effectiveness and consequences of colchicine treatment in the induction of tetraploidy.

Material and Methods

Six improved types of *desi* chickpeas, namely Punjab 7, S 26, S 33, G 24, Greengram and Barachana, and three types of *kabuli* chickpeas, C 49, Punjab 1 and Rabbat, were selected for this study. In the first year of study, it was attempted to produce polyploids of the varieties only. Colchicine was administered in the form of 0.25 percent aqueous solution at room temperature (75 to 80 °F). The treatments for varieties were four: 1/2 hour, 1 hour, and 2 hours for pre-soaked seeds, and direct soaking of seeds in colchicine solution for 18 hours. During the second year, polyploids of some F_1 hybrids were also produced using the method found to be best during the first year. Each year, the material was laid out in a simple randomised-block design and data were recorded for some quantitative characters.

For cytological studies, the flower buds were fixed in Clarke's fixative between 8 a.m. and 9 a.m. The p.m.c's were studied by the squash method using acetocarmine stain. Pollen fertility was calculated as a percentage, again using acetocarmine stain.

Results

A. Optimum treatment with colchicine for induction of tetraploidy

Tetraploid seedlings produced by colchicine stood out markedly from diploids by virtue of their deep green foliage, larger leaflets, which were very closely spaced, and the larger size of flower standard. Random cytological checks in these plants confirmed that they were polyploids and their pollen grains and stomata were conspicuously large. The data regarding percentage of surviving tetraploids (given in Table 1) indicate that the 1 hour treatment of presoaked seeds was the most uniform and gave the maximum number of polyploids, i.e. nearly 55%. It can be seen that *kabuli* types were less variable over different treatments than *desi* types and also yielded less polyploids than the latter.

B. Observations on C_1 and C_2 generations for quantitative characters

The data on the mean values of different characters for controls and treated plants are set out in Table 2. On average, the diploids took 8.8 days to germinate compared with 17.7 for C_1 tetraploids and 15.0 for C_1 treated diploids, the differences being statistically significant. However, the C_2 of tetraploids and

Table 1. *Number of surviving tetraploids (%) in some chickpea varieties under different treatments of colchicine*

Variety	Control	Polyploids (%) (Mean of two years)			
		½ hour	1 hour	2 hours	18 hours
Desi					
Punjab 7	0	21.6	50.0	75.5	72.0
G 24	0	19.2	52.0	37.5	41.5
S 26	0	20.1	53.5	63.2	60.3
S 33	0	26.0	50.2	71.1	67.6
Greengram	0	37.2	54.5	60.7	40.7
Barachana	0	37.5	56.7	59.1	39.3
Kabuli					
C 49	0	28.0	53.5	31.1	39.7
Punjab 1	0	30.5	58.8	22.4	39.6
Rabbat	0	32.5	62.5	23.2	38.4
Mean	0	28.07	54.52	49.31	48.79
Mean *desi* types		26.9	52.7	61.2	53.6
Mean *kabuli* types		30.3	58.3	25.6	39.2

X^2_3 (heterogeneity) *desi* vs. *kabuli* 14.238 ($p = 0.01$ to 0.001)

Table 2. *Data on some quantitative characters of normal and polyploid chickpeas material (Mean of all varieties)*

Character	Control		Treated diploid		Tetraploid	
	Ist Yr.	2nd Yr.	C_1	C_2	C_1	C_2
Days to germinate	8.8	9.0	15.0	9.16	17.7	9.3
Days to flower	94	111	98	111	105	109
Length of longest branch (cm)	37.9	49.9	30.1	50.5	23.0	58.9
Number of primary branches per plant	3.9	3.8	2.7	3.7	2.0	3.7
Number of secondary branches per plant	12.2	18.1	9.2	18.4	7.4	21.6
Percentage setting	48.6	50.6	40.3	49.8	12.4	17.6
Grain yield (gms/plot)	38.2	276.1	20.6	241.1	7.3	56.8

treated diploids were at par with the control. The situation for days to flower was essentially the same as that for days to germinate. The controls had longer branches compared with C_1 tetraploids. In C_2, the opposite was true. The number of primary branches in C_2 polyploids of the varieties was similar to that of their diploid progenitors, though there was a reduction in the C_1 generation. The situation for secondary branches was the same as for primary branches. The *desi* and *kabuli* types, as groups, reacted similarly with regard to quantitative characters.

For fertility, the polyploids fared badly during the C_1 as well as C_2 generations, with mean fruit settings of 12.4% and 17.6% respectively as against 48.6% and 50.6% for diploids. Also, the grain yield of tetraploids was quite poor in the C_1 as well as C_2, being only about 1/5th that of diploids. The varieties S 26 and Rabbat exhibited differential behaviour, the former having top rank as diploid but only fifth as tetraploid, while the latter was fifth among diploids and first among tetraploids.

C. *Behaviour of unaffected (diploid) treated seedlings*

For almost all the characters studied, it was found that the immediate effect of colchicine treatment (C_1) is an upset, making plant behaviour abnormal. There is a delay in germination and flowering; growth, branching, fertility and yield are also reduced. Normality is restored, though not fully, in the C_2 generation.

D. *Comparative performance of parents and F_1 hybrids in C_1 generation*

Among the quantitative characters studied, setting percentage and grain yield merit special consideration. Mean values for these characters for each hybrid and parent are set out in Table 3.

Table 3. *Data on setting percentage and grain yield of controls, treated diploids and tetraploids of some chickpea varieties and intervarietal crosses in C_1 generation*

Variety/Hybrid	Percentage setting			Grain yield (gms/plot)		
	C	*T*	*P*	*C*	*T*	*P*
Punjab 7	54.5	46.3	17.5	81.0	23.8	10.3
G 24	56.8	46.5	19.0	81.5	24.5	10.8
Pb 7 × G 24	57.3	46.8	20.3	85.5	25.0	10.8
S 26	55.0	43.8	18.5	80.5	23.8	10.0
Pb 7 × S 26	56.5	44.3	19.3	86.0	22.0	11.0
S 33	56.3	46.3	19.8	82.8	23.3	10.5
Pb 7 × S 33	56.8	45.3	19.8	85.8	25.0	11.5
C 49	49.3	43.8	19.8	88.3	28.3	10.8
Pb 7 × C 49	49.5	44.0	20.0	105.0	39.8	13.0
Punjab 1	46.3	34.0	30.0	53.8	22.3	2.3
Pb 7 × Pb 1	46.8	32.8	36.8	71.3	23.5	20.5
Rabbat	45.8	33.0	9.3	54.3	21.5	6.5
Punjab 7 × Rabbat	46.8	31.0	38.5	46.5	22.8	22.0
Greengram	39.8	32.8	8.0	8.8	5.5	1.0
Pb 7 × Greengram	39.0	32.8	10.3	12.5	10.8	1.5
Barachana	40.3	32.5	8.8	9.8	6.5	1.5
Pb 7 × Barachana	40.8	30.3	9.8	14.0	8.3	1.3
Mean	49.2	40.4	17.9	61.6	21.0	9.1
C.D. at 5%		±2.3			±5.9	

C = Control; *T* = Treated diploid; *P* = Autotetraploid.

Setting percentage: The polyploids had a considerably lower setting percentage (17.94%) than the diploids (49.23%). The general trend of parents and hybrids was similar among diploids and tetraploids, with two exceptions, *Pb 7* × *Pb 1* and *Pb 7* × Rabbat. These two had 36.75% and 38.5% setting respectively compared with 8 to 20% in the rest of the polyploid material, the differences being significant.

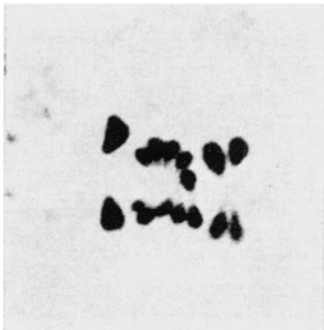

Fig. 1
Anaphase I in diploid. Equal disjunction of 8 *vs* chromosomes 8

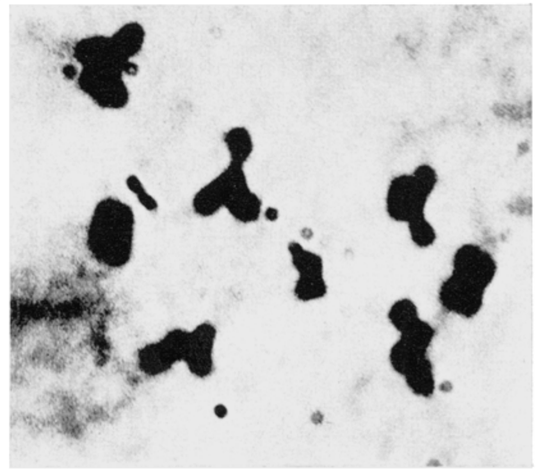

Fig. 2
Metaphase I with 8 IV
(C_1 tetraploid)

Fig. 3. Metaphase I.4 IV + 8 II, the most common figuration in C_1 and C_2

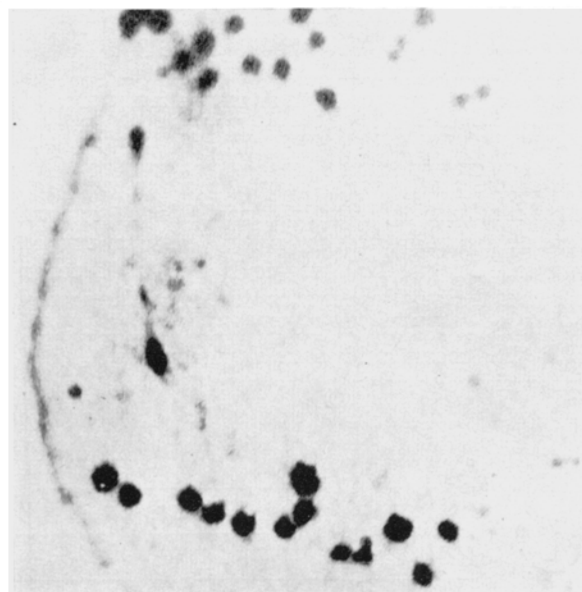

Fig. 4. Anaphase I late disjunction of a bivalent and 18 *vs* 14 disjunction (C_1 generation)

Fig. 5
Metaphase I.2 IV + 12 II
(C_2 generation)

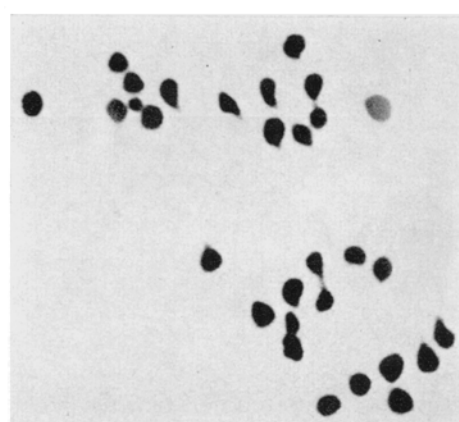

Fig. 6
Anaphase II in an aneuploid ($4\,n - 2$) plant showing precocious disjunction of bivalents

Figures 1—6. Microphotographs of meiosis in diploid and autopolyploid chickpeas

Grain yield: The yield results were similar to setting percentage: the crosses *Pb* 7 × *Pb* 1 and *Pb* 7 × Rabbat gave much higher yields than the rest of the polyploids. It is interesting that neither *Pb* 1 nor Rabbat were high-yielding by themselves but when crossed with *Pb* 7 their hybrids gave very high yields.

The data on some other quantitative characters of C_1 tetraploids, intervarietal crosses and their parents indicated that the mean number of days taken by F_1 tetraploids to germinate was slightly longer than for varietal tetraploids. As diploids, or tetraploids, the number of days taken by hybrids to flower was generally midway between that of the parents. The mean length of branch in 2 *n* for *Pb* 7, *Pb* 1 and Rabbat was 39.5, 51.5 and 55.5 cm, while for the crosses it was 47.8 and 49.5 cm; the corresponding values in 4 *n* were 29.3, 42.5, 42.8, 39.0 and 39.8 cm respectively. This indicates that the hybrids *Pb* 7 × *Pb* 1 and *Pb* 7 × Rabbat approached their long branch parents in 2 *n* as well as 4 *n*. Other F_1's, however, were nearer to their respective short branch parents in both the stages of ploidy.

The mean number of primary branches for hybrids was significantly more than the mean midparental value for diploids, but for tetraploids the two values were on a par. However, in the 4 *n* stage, two of the crosses, *Pb* 7 × Rabbat and *Pb* 7 × Greengram, excelled their midparental value. For the secondary branches, the diploid hybrids were statistically at par with the mean midparental performance, but as tetraploids they were below par.

against 0.85, 8.82, 0.13 and 3.27 respectively in the C_2. Apparently, the frequency of quadrivalents decreased and that of bivalents increased during C_2. The most common association in the C_1 and C_2 was 8 II+4 IV, occurring in 18.7 and 16.5 percent of cells respectively. However, more cells in the C_2 exhibited a combination of various associations. Mostly, the trivalents were found in the same cells as univalents, but trivalents and univalents occurred independently in 5.5 percent C_2 cells and in 8.3 percent C_1 cells. The size and number of univalents was not always identical. *A* I behaviour of tetraploids was irregular to a greater extent in the C_1 than in C_2. Some types of irregularities, such as laggards, delayed separation, unequal disjunction and late or early disjunction of certain multivalents were observed in about 30 percent of cells of the C_1. 17 *vs* 15 disjunction was the most commonly occurring irregularity (12.75%). In the C_2, the irregularity rate seemed to have fallen: unequal disjunction occurred in 7.6 percent of cells only. Some aneuploid plants were also encountered.

Although meiotic behaviour of induced tetraploids was irregular, the percentage stainable pollen of diploid *vs* C_1 tetraploid turned out to be just significant at the 5% level. The difference of diploids from C_2 tetraploids was non-significant.

Discussion

Ramanujam and Joshi (1941) reported that because of the high degree of sterility, the induced tetraploids of chickpeas were not useful economically, and there is evidence that after 10—12 generations of selection, the fertility of autotetraploids can not be increased to a satisfactory level. The present study on varietal tetraploids and F_1 tetraploids was taken up to find out if genetic diversity was useful in polyploid breeding as far as chickpeas are concerned. *Desi* and *kabuli* types of chickpea are known to be genetically diverse (Anon., 1965). The present study reveals that while the maximum number of polyploids in *desi* chickpeas was obtained with 2 hour treatment of presoaked seeds, that for *kabuli*

Table 4. *Chromosome pairing at diakinesis/Metaphase I and pollen fertility in induced tetraploids of some chickpea varieties in C_1 and C_2 generations*

Values for	% stainable pollen		Chromosomal Association			
	2n	4n	I	II	III	IV
C_1 generation						
Overall mean	91.0	89.4	0.18	7.37	0.15	4.15
Range of varietal means	85.0—97.0	80.0—93.0	0.15—0.22	7.00—8.25	0.08—0.25	3.66—4.35
C_2 generation						
Overall mean	91.7	91.3	0.85	8.82	0.13	3.27
Range of varietal means	86.0—96.0	85.0—95.0	0.28—1.64	7.68—10.33	0.06—0.18	2.46—3.62

E. Cytological studies

The meiotic behaviour of varietal diploids and tetraploids was observed during the C_1 and C_2 generations at diakinesis/metaphase I and anaphase I and II stages. Diploids were essentially regular at meiosis, giving 8 II at MI and 8 *vs* 8 disjunction at *A*I. In the tetraploids, 5 to 10 plants of each strain in each generation were studied and the data taken from 35 to 50 cells per plant. Table 4 shows that the mean univalent, bivalent, trivalent and quadrivalent frequencies in the C_1 were 0.18, 7.37, 0.15 and 4.15 as was obtained with 1 hour treatment. Interestingly, the best treatment for *desi* was the most detrimental to *kabuli* chickpeas.

The effect of induced autotetraploidy on morphological characters in all the varieties was generally the same as that given already by Ramanujam and Joshi (1941). However, it may be mentioned that the primary and secondary branches in C_2 tetraploids were respectively at par with and superior to diploids. Also, the polyploids had about 35 percent greater seed size. Chandra (1960) has shown that branching and seed size are among the most important yield-contributing factors in chickpeas and in this context the performance of tetraploids is encouraging.

The response of eight intervarietal crosses to induced tetraploidy exhibited the same general trend as the varieties for most of the characters studied. The dominance relationships in F_1 were not disturbed by tetraploidy. It was observed that over the two years for which data are reported here, most of the yield-contributing quantitative characters showed wide variation, which is an indication of their strong susceptibility to environmental variation. But the setting percentage was more or less unaffected by the year, and is therefore of interest to the breeder. However, the setting percentage of even the C_2 tetraploids was very much reduced compared with diploids. The increased yield potential presented by increased branching and seed size in the former was off-set by the reduced fertility to such an extent that the average seed yield of tetraploids was only about 1/5th that of the diploids. Nevertheless, higher yielding diploids also had higher yielding C_2 tetraploids and *vice-versa*, with two exceptions, S 26 (*desi*) and Rabbat (*kabuli*). As diploid, S 26 yielded higher than Rabbat during both years, but as tetraploid, the latter outyielded the former in the C_2 generation. This may be an indication that certain varieties give a better response than others to the induction of autotetraploidy.

Compared with other F_1 hybrids, there was a significant increase in fertility of the autotetraploids produced from two crosses between *desi* and *kabuli* types, Punjab 7 × Punjab 1 and Punjab 7 × Rabbat. The yield performance of these hybrids was also the best among the tetraploid material. High fertility in the C_1 generation of these crosses, as distinct from others having parents with about the same initial fertility, is very interesting.

Before predicting the usefulness of these findings in practical work, it is necessary to consider the factors responsible for fertility in this material. The fertility of pollen, as judged by its ability to take stain, was not related to setting percentage. A higher rate of sterility in C_1 tetraploids was associated with a higher mean number of multivalents and the consequent production of unbalanced gametes. In the C_2 generation, the fertility increased while multivalent frequency decreased, implying that multivalent frequency is related to sterility. The occurrence of aneuploid plants may have had a role in the higher sterility of polyploids. Meiotic abnormalities thus appear to be one of the causes of sterility. That genetic factors must also be involved is apparent from these facts: varieties with low initial fertility gave rise to tetraploids with lower fertility; the dominance of low setting percentage over high at diploid as well as tetraploid level in some crosses; and the over-dominance in $4n$ stage for higher fertility in the diverse genetic background of *desi* × *kabuli* crosses. In the present study, the meiotic behaviour of hybrids could not be tested, and therefore it is difficult to say whether genetic or cytological factors are the more important. Studies on these aspects would appear necessary. However both factors are likely to be important in this crop plant.

Swaminathan and Shulbha (1959) have indicated that induced autotetraploids are likely to approach the cytological diploidization faster if the initial coefficient of multivalent formation is low. This would be the situation in chickpeas which has a small chromosome size. Hilpert (1957) has shown that selection for tillering in tetraploid rye was effective in bringing about a shift towards more regular meiosis. It is possible that in chickpeas a selection for yield-contributing factor(s) may do the same. Moreover, Stebbins (1950) has shown that it would be experimentally possible to induce a shift from the multivalent to bivalent type of synapsis by using irradiation techniques. Also, the hybrid material in a self-pollinated plant may produce segregates for higher fertility. Again, the material with strikingly high fertility at C_1 level promises useful recombinants particularly if over-dominance is due to additive effects. In view of this, and the encouraging performance of some hybrid tetraploids, polyploidy may not be entirely ruled out as a breeding tool for chickpeas. Besides, visual observation of tetraploids revealed that they have stronger and deeper root systems and harder stems, which make them resistant to lodging, as well as thicker pods which impart resistance to pod borer. Thus polyploid chickpeas may still be considered as a future possibility.

References

1. Anonymous: Annual Report of the Economic Botanist Millets, Ferozpur (Punjab) India (1965). — 2. Chandra, S.: Economic evaluation of some indigenous and exotic varieties of gram. M. Sc. dissertation, Punjab University (1960). — 3. Einset, J.: Aneuploidy in relation to partial sterility in autotetraploid lettuce. American J. Bot. **34**, 99—105 (1947). — 4. Hilpert, G.: Effect of selection for meiotic behaviour in autotetraploid rye. Hereditas (Lund) **43**, 318—322 (1957). — 5. Iyengar, N. K.: Cytological investigations on genus *Cicer*. Annals Bot., London, **3**, 271—306 (1939). — 6. Ramanujam, S.,

Joshi, A. B.: Colchicine-induced polyploidy in crop plants 1. Gram (*Cicer arietinum* L.). Indian J. agric. Sci. **9**, 835—849 (1941). — 7. Stebbins, G. L.: Variation and evolution in plants. Columbia University Press (5th print, 1963); pp. 643 (1950). — 8. Swaminathan, M. S., Shulbha, K.: Multivalent frequency and seed fertility in raw and evolved tetraploids of *Brassica campestris* var. *toria*. Z. Vererbgsl. **90**, 385—392 (1959).

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