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CYTOGENETICS OF *CROTALARIA*. VII. INDUCED CYTOCHIMERAL AND ASYNAPTIC MUTANTS OF *CROTALARIA JUNCEA* (SUNNHEMP)

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ABSTRACT

Using gamma rays and EMS or MMS separately and in various combinations, a cytochimeral and an asynaptic mutant were isolated in *Crotalaria juncea* (sunnhemp). The cytochimeral mutant was characterized by the presence of both 2x and 4x chromosome numbers in the same plant, while the asynaptic mutant was characterized by the presence of univalents in their PMCs. Both abnormalities led to considerable sterility. The possible causes of abnormalities are discussed.

Key words: *Crotalaria juncea*, Sunnhemp, cytochimera, asynaptic, mutant.

Many successful attempts have been made to improve crop plants through induced mutations. *Crotalaria juncea* (2 n = 16), commonly known as sunnhemp, is an important fibre and green manure plant of Leguminosae family. For its improvement, induced mutations through physical (gamma rays) and chemical (EMS, MMS) mutagens were tried individually and in various combinations. Various mutants have been isolated [1, 2]. Here we report a gamma-ray induced cytochimeral plant which showed two chromosome numbers (2x and 4x), and an asynaptic plant isolated in M_3 of an induced translocation heterozygote.

MATERIALS AND METHODS

Dry seeds of *C. juncea* (6% moisture) were irradiated with 5, 10, 20, 30, 40 and 50 kR gamma rays in ^{60}Co gamma cell at the Jawaharlal Nehru University, New Delhi, and later sown in field. Some seeds in each dose were also treated with 0.15% EMS or MMS for various durations and then sown in the field. Young flower buds were fixed in acetic-ethanol (1:3) for 24 h. Anthers stained in leuco basic fuchsin were squashed in 1% iron-acetocarmine. Photographs were taken from temporary preparations.

RESULTS

Control. The control plants behaved as normal diploids (2 n = 16) and had on an average 7.92 (7.08 ring + 0.84 rod) bivalents and 0.16 univalents. Number of chiasmata ranged from 13–23, mean 16.72. Anaphase I had normal distribution (8:8). Pollen stainability was 95% and seed set 8–10 per pod.

Cytochimeral plant. During the screening of mutants of *C. juncea* (2x = 16), a cytochimeric plant was isolated in M_1 of 5 kR gamma-ray treated seeds. This plant exhibited two chromosome numbers (16, 32) in its PMCs. Of the cells analysed at metaphase I of meiosis, 76% had 2x = 16 (Fig 1A, B) while the remaining 24% had 4x = 32 (Fig 1C, D). Both types of cells had either univalents or bivalents or both. The PMCs with 2x = 16 had, on an average, 3.71 bivalents and 8.52 univalents, range 0–8 and 0–16, respectively. The PMCs with 4x = 32 had an average of 8.0 bivalents and 16.0 univalents, range 0–16 and 0–32, respectively. Compared to control plants, rod bivalents predominated over ring bivalents in this plant (Fig. 1A). The aberrant chromosome numbers were observed at anaphase I also (Fig. 1E, F). The most common distribution was 16:16 (50%) followed by 8:8 (16.7%, Fig. 1E), 9:7 (8.3%), 17:15 (8.3%, Fig. 1F), and 15:1 (8.3%). One cell had 12:13 distribution with 7 chromosomes as laggards. Pollen grains were of two sizes and their stainability was 40%.

Asynaptic plant. This mutant was detected in the selfed progeny (M_3) of an induced translocation heterozygote which had been produced by 5 kR gamma rays + 0.15% MMS (8 h) treatment. The asynaptic plant was characterized by the presence of univalents at diakinesis/metaphase I of meiosis (Fig. 2A, B). Thirty cells were analysed at diakinesis/metaphase I. The most common association was 16 I (70%), followed by 1 II + 14 I, 2 II + 12 I, 4 II + 8 I, 3 II + 10 I, 6 II + 4 I and 8 II in 6.7, 6.7, 6.7, 3.3, 3.3 and 3.3% cells, respectively (Fig. 2A, B). The bivalents were rod shaped and chiasmata terminal. As compared to 7.92 bivalents and 0.16 univalents in the control, the asynaptic mutant had on an average of 1.03 (rod) II and 13.93 I per cell. Chiasma frequency was only 1.03 in the asynaptic plant. Among the cells analysed at anaphase I and II, 5 had one bridge–fragment configuration (Fig 2E). Significantly, a few cells had 32 chromosomes (Fig. 2C, D). The chromosome distribution at anaphase I was highly irregular. In two such cells, the distribution was 28:4 (Fig. 2D) and 27:5. Six cells had pentapolar distribution of chromosomes with a few laggards (Fig. 2F). The pollen stainability was 30% and seed set very poor. Only 10 seeds could be collected from the plants.

DISCUSSION

Gamma-ray induced cytochimeric plants showing two chromosome numbers have rarely been reported [3–5]. In the present case, it is evident from the meiotic data that the tetraploid sector in this plant constituted about 38.0% of the germinal tissue while the remaining 62.0% tissue was diploid. The probable cause of radiation-induced tetraploid cells

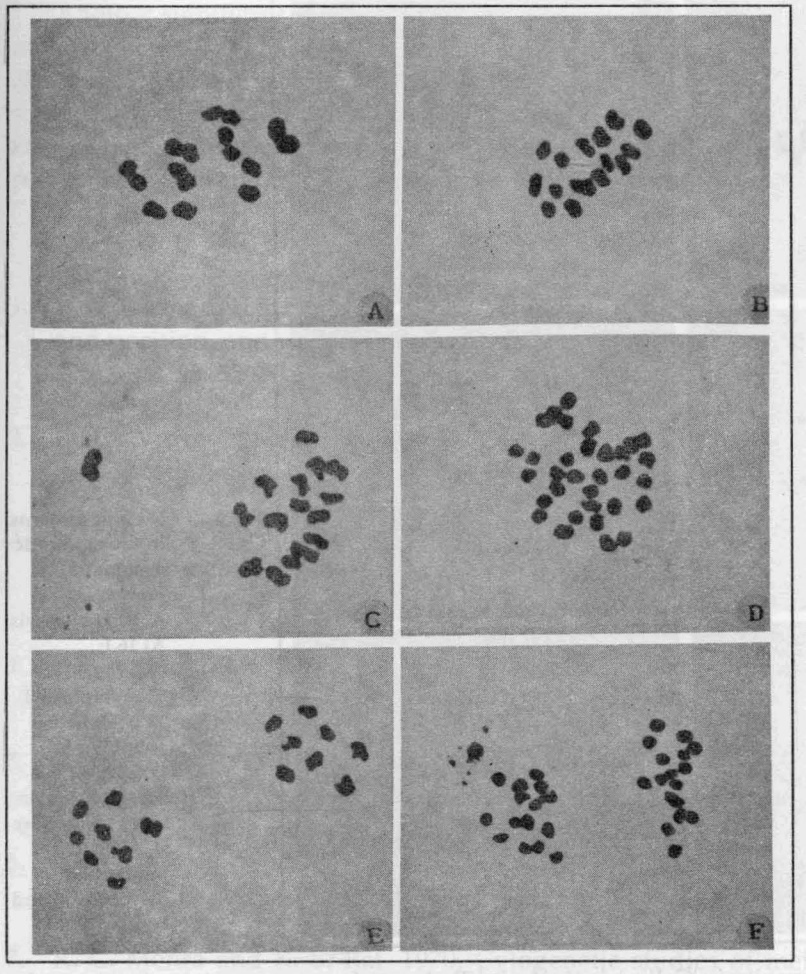

Fig. 1. Meiotic patterns in the cytochimeral mutant of *C. juncea*. A–D) Meiotic metaphase I: A) 6 II + 4 I; B) 16 I; C) 16 II; and D) 32 I. E, F) Meiotic anaphase I: E) 8:8 and F) 16:16.

was failure of normal separation of chromosomes at anaphase or formation of restitution nucleus following normal division due to failure of cytokinesis. This plant was partially asynaptic. Both diploid and tetraploid PMCs had variable number of univalents and bivalents. Some cells in both types had complete failure of pairing, resulting in either 16 or 32 univalents. Moreover, majority of bivalents in the cytochimeral plant were rod shaped. Such behaviour is indicative of mutation in gene/genes controlling chromosome pairing. Genes causing asynapsis may also result in polyploid gametes [6].

The plant showing partial asynapsis had 16 univalents (maximum possible) in 70% PMCs, while the other PMCs had different combinations of univalents and bivalents. There are only a few such reports where asynapsis was observed in the progeny of translocation heterozygotes. This has been reported in the third generation progeny of an X-ray induced

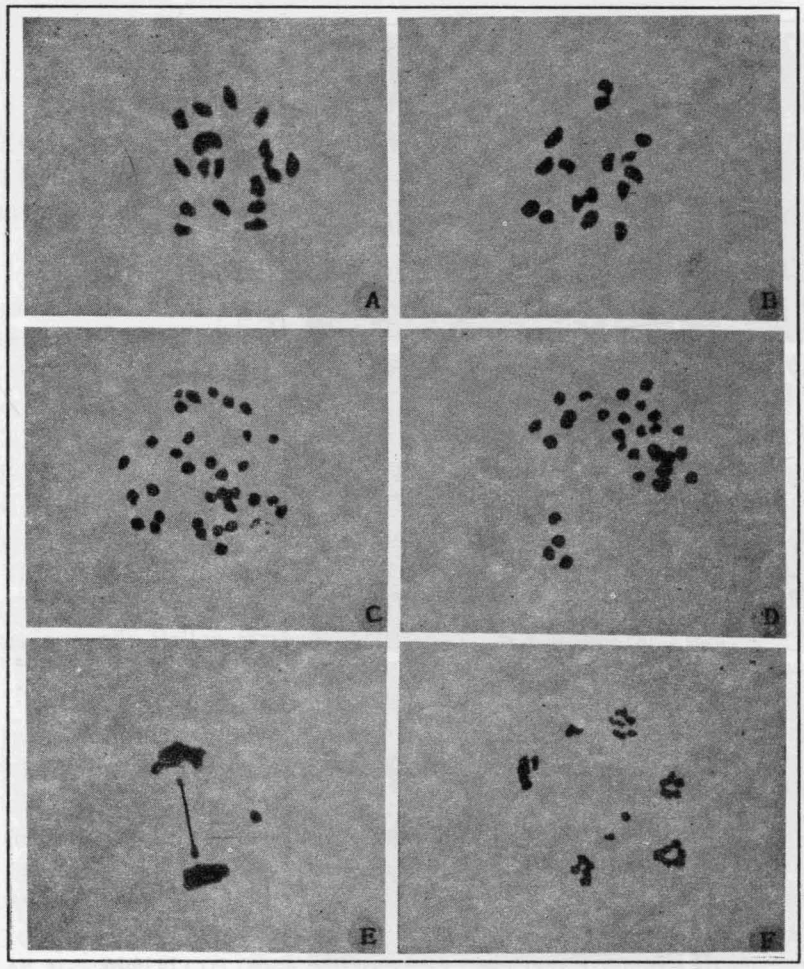

Fig. 2. **Meiotic patterns in the asynaptic mutant of *C. juncea*. A, B) Diakinesis: A) 16 I; B) 2 II + 12 I; C) metaphase I, 32 I; D, E) anaphase I: D) 28:4; E) bridge-fragment; and F) anaphase II. Note pentapolar distribution and laggards.**

translocation heterozygosity in *Collinisia tinctoria* [7]. Apart from asynaptic behaviour, the mutant in the present study was also characterized by rod bivalents with terminal chiasmata. At no stage of meiosis, a clear indication of chiasma in a bivalent was found. The association was always terminal, and no clearly observable exchange points were noticed even at diplotene/diakinesis. Such behaviour has been reported for quasi-bivalents [8], however, the present study did not reveal any quasi-bivalents.

The low chiasma frequency (1.03/cell) was due to low number of bivalents as well as considerable reduction in chiasmata per bivalent. It is postulated that the mutated gene does not govern chiasma formation but chromosome pairing which is interrupted as soon as it starts at the end of the chromosomes with the result that rod bivalents with terminal chiasmata are observed even at early stages. Thus, meiotic behaviour in the present case is due to partial asynapsis.

Another significant feature of this plant was the presence of tetraploid chromosome number (4x = 32) at anaphase I and highly irregular distribution of chromosome, such as, 28:4 and 27:5. The presence of polyploid cells could be a result of failure of cytokinesis in some cells. Genetic control of asynapsis is assumed to have operated in this case, as it is well known that pairing of homologous chromosomes in higher plants is controlled by a polygenic system and even if a single gene mutates pairing anomalies occur [9].

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