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EFFECT OF COLCHICINE ON MALE MEIOSIS OF *CARTHAMUS*

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Genetic variability is essential for any crop improvement programme. Experimentally induced mutation provides an important source of variability. During the present investigation the course of meiosis was analyzed in the plants raised after colchicine treatment. Seventeen accessions/species were explored for their cytogenetic attributes. Seeds of *Carthamus* were treated with 0.75% colchicine solution for 48 hours, which acts as more efficient mutagen. Colchicine induced high frequency of meiotic anomalies during first as well as second meiotic division. In addition to the presence of tetrads (four spores per PMC) monads, dyads, triads, and polyads were also noticed. Treatment with colchicine incited appreciable changes in diameter of pollen grains. Number of pollen grains per anther also decreased in treated set. Pollen sterility in all colchicine treated accessions increased significantly.

Keywords: *Carthamus*; Colchicine; Meiosis; Pollen Mother Cell.

Introduction

The genus *Carthamus* L. belonging to the tribe Cynareae (thistle), sub-family Tubifloreae of family Compositae has about 42 species with varying chromosome number of 2n=20 to 2n=64 and has a wide range of adaptation. *C. tinctorius*, commonly called safflower, is the only cultivated species of this genus. Commercially produced safflower seeds contain 32 to 52 percent oil. It is one of humanities oldest crop cultivated in India mainly for oil from the seeds and reddish and yellow dyes for clothing and food preparation from the flowers. Mutation induction is one of the established techniques for the production of genetic variability in plants. Different mutagens have been used in including variabilities, and become useful tools in mutation breeding programmes[1,2]. The present paper describes the meiotic course of colchicine treated accessions/species of *Carthamus* as compared to corresponding control sets.

Material and Methods

At least 100 seeds of seventeen accessions belonging to five species (Table 1) were soaked in 0.75% colchicine solution for forty-eight hours. After forty-eight hours these seeds were washed with water and allowed to grow, after germination, for three days. These were initially planted in polythene bags and subsequently transferred to field after fifteen days. The course of meiosis was analyzed in the plants raised after colchicine treatment as well as control sets. For investigating the male meiosis, floral heads of appropriate size were fixed in Carnoy's fluid II (6:3:1::absolute ethanol:chloroform:glacial acetic acid) for 24 hours and then transferred in 70% ethanol and stored in refrigerator. A little pinch of iron salt was added in Carnoy's II fluid for ensuring better staining. Anthers were smeared and squashed in 1.5% aceto-carmine for studying meiosis. All the observations and photomicrographs were taken from both unsquashed as well as squashed temporary preparations. Shrunken and unstained pollen grains were treated as sterile while well filled stained grains were considered as fertile.

Results and Discussion

All the accessions/species possessed 2n=24 (Fig. 1) except the accessions of *C. lanatus ssp. turkestanicus* 2n=64 (Fig. 2). These counts were established at metaphase-I and anaphase-I (Fig. 3, 4). Data related to the frequency distribution of chromosome configurations at metaphase-I, chiasmata/PMC are listed in Table 2. The meiosis with in anther was asynchronous and stages from prophase-I to telophase -II could be seen in the same anther. The coarse of male meiosis in the control sets were more or less regular with some exceptions having slight abnormal course as compared to respective controls. These accessions exhibited various types of meiotic anomalies such as formation of restitution nucleus during first division (Fig. 5), chromatin disintegration at metaphase-I (Fig. 7), late disjunction of bivalents/ chromosome at metaphase-I (Fig. 8) and metaphase-II

Table 1. List of accessions/species of *Carthamus,* which were used for colchicine treatment.

S.No.	Lab Code	Species	2n	Source Country
1.	G-1	*Carthamus glaucus*	24	Lebanon
2.	G-2	*Carthamus glaucus*	24	China
3.	L-1	*Carthamus lanatus*	24	E. Germany
4.	L-2	*Carthamus lanatus*	24	E. Germany
5.	L-4	*Carthamus lanatus*	24	E. Germany
6.	L-6	*Carthamus lanatus*	24	Belgium
7.	L-7	*Carthamus lanatus*	24	Portugal
8.	L-8	*Carthamus lanatus*	24	Former USSR
9.	L-9	*Carthamus lanatus*	64	Afghanistan
10.	L-11	*Carthamus lanatus*	24	Afghanistan
11.	O-1	*Carthamus oxycantha*	24	Former USSR
12.	P-1	*Carthamus palaestinus*	24	USA
13.	P-2	*Carthamus palaestinus*	24	USA
14.	P-4	*Carthamus palaestinus*	24	Israel
15.	P-7	*Carthamus palaestinus*	24	Israel
16.	T-10	*Carthamus tinctorius*	24	Afghanistan
17.	T-12	*Carthamus tinctorius*	24	Canada, Alberta

(Fig. 9), clumping of chromosomes at metaphase-I (Fig. 6) and metaphase-II (Fig. 11), more than one group of chromosomes at metaphase-I (Fig. 12) and metaphase-II (Fig. 13), lagging of chromosomes at anaphase-I (Fig. 14,15) and at anaphase-II (Fig. 16), unequal distribution at anaphase-I (Fig. 17), formation of micronuclei at telophase-I (Fig. 18)and at telophase-II (Fig. 19,20), formation of chromatin bridge telophase-I (Fig. 21) and at telophase-II (Fig. 22)), isobilateral tetrad (Fig. 23), tetrahedral tetrad (Fig 24) monad (Fig 25), dyad (Fig 26), triad (Fig 27) polyads (Fig. 28) and fertile and sterile pollen grains (Fig. 29,30) etc. The polyads had 5-12 spores per PMC. Treatment with colchicine occasionally incited appreciable changes in diameter of pollen grains. Sterile pollen grains were hyaline and empty as compared to fertile ones.

Induced mutation is now a proved means of creating or increasing genetic variability that is different from the kind obtainable through gene recombination. Mutagen induced chromosome abnormalities have also been reported by different workers in different plant materials after irradiation or chemical treatment[3-7]. The effect of colchicine on the anthers of safflower has been described by Krijthe[8] when colchicine-agar mixture was applied on the flower buds abnormal growth was caused. Krijthe[8] was probably the first to use colchicine on the anthers of a cultivated safflower to induce polyploidy. Schank and Knowles[9] induced autotetraploidy in several varieties of cultivated safflower and reported that most successful treatment was a 0.1% aqueous solution of colchicine applied four times daily to a cotton swab wedged between the cotyledons of young seedlings for a period of 3 days. Colchicine when applied on shoot apex caused high mortality at 0.2%. The mortality was 78% with no polyploids among the survivors. No visible growth was found in the shoot apex. Pillai[10], following the same method found 0.05 to 0.1% colchicine most efficient in safflower cv. 'IC11842'. Khidir and Knowles[11] recommended the application of colchicine on the growing tip of the seedling of *Carthamus* species with n=32, by mixing 0.1% solution with 1% tragacanth gum instead of cotton swab. The course of male meiosis in the control sets was more or less regular with some exceptions having slight abnormal course as compared to respective controls.

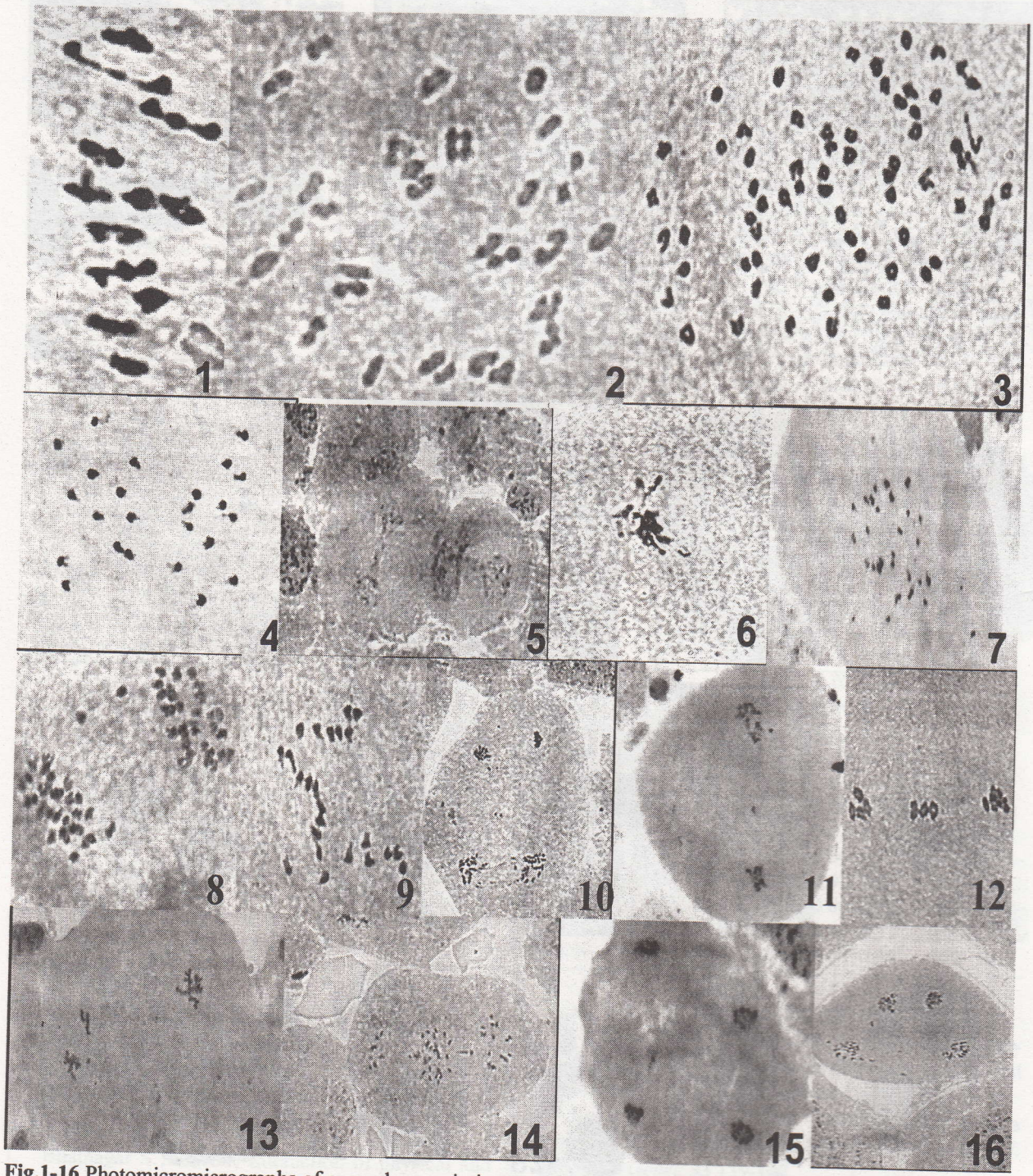

Fig.1-16 Photomicromicrographs of anomalous meiotic stages.1. Metaphase I showing 12 bivalents, 2. Metaphase I showing 32 bivalents, 3. Anaphase I showing 32:32 disjunction of chromosomes, 4. Anaphase I showing 12:12 disjunction of chromosomes, 5. Formation of restitution nucleus during first division, 6. Clumping at metaphase I, 7. Chromatin disintegration, 8-9. Late disjunction of bivalents at metaphase I, 10. Late disjunction of bivalents at metaphase II, 11. Clumping at metaphase II, 12. Bivalents arrange in > 1 group at metaphase I, 13. Chromosomes arrange in > 1 group at metaphase II, 14-15. Lagging at anaphase I, 16. Lagging at anaphase II.

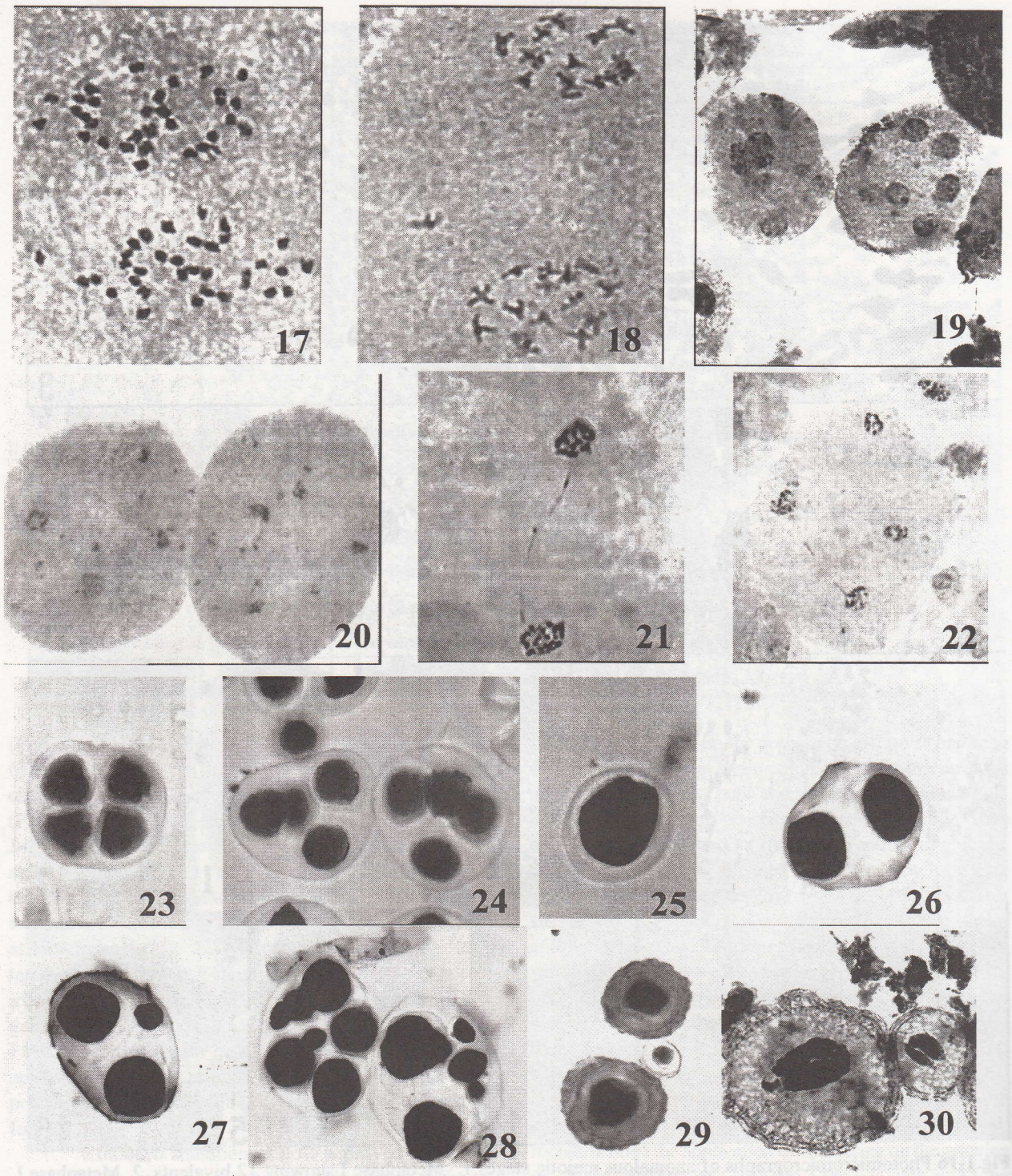

Fig.17-30 Photomicromicrographs of anomalous meiotic stages. 17. unequal distribution of chromosomes at anaphase I (31:33) 18. Micronuclei at telophase I, 19-20. Micronuclei at telophase II, 21. Chromatin bridge at telophase I, 22. Chromatin bridge at telophase II, 23. Isobilateral tetrad, 24. Tetrahederal tetrad, 25. Monad, 26. Dyad, 27. Triad, 28. Polyads, 29-30. Sterile and fertile pollen grains.

Table 2. Chromosome analyses at metaphase I in *Carthamus* accessions.

Acc.		Bivalents						X-ta/PMC		RC
		Ring			Rod					
		Mean±SE	Range	RC	Mean±SE	Range	RC	Mean±SE	Range	
G-1	Co	3.08 ± 0.45	1.00 - 5.00		8.92 ± 0.45	7.00 - 11.00		0.63 ± 0.02	0.54 - 0.71	
	Tr	6.71 ± 0.03	1.00 - 11.00	1.18	5.29 ± 0.03	1.00 - 12.00	-0.41	0.78 ± 0.00	0.50 - 0.96	0.24
G-2	Co	3.33 ± 0.47	1.00 - 6.00		8.67 ± 0.47	6.00 - 11.00		0.64 ± 0.02	0.54 - 0.75	
	Tr	6.21 ± 0.02	1.00 - 12.00	0.86	5.80 ± 0.02	1.00 - 11.00	-0.33	0.76 ± 0.00	0.54 - 1.00	0.18
L-1	Co	3.58 ± 0.48	2.00 - 6.00		8.42 ± 0.48	6.00 - 10.00		0.65 ± 0.02	0.58 - 0.75	
	Tr	5.26 ± 0.03	1.00 - 9.00	0.47	6.74 ± 0.03	3.00 - 11.00	-0.20	0.72 ± 0.00	0.54 - 0.88	0.10
L-2	Co	3.00 ± 0.48	1.00 - 6.00		9.00 ± 0.48	6.00 - 11.00		0.63 ± 0.02	0.54 - 0.75	
	Tr	5.72 ± 0.03	1.00 - 9.00	0.91	6.28 ± 0.03	3.00 - 11.00	-0.30	0.74 ± 0.00	0.54 - 0.88	0.17
L-4	Co	4.33 ± 0.63	1.00 - 8.00		7.67 ± 0.63	4.00 - 11.00		0.68 ± 0.03	0.54 - 0.83	
	Tr	5.80 ± 0.05	1.00 - 12.00	0.34	6.20 ± 0.05	1.00 - 11.00	-0.19	0.74 ± 0.00	0.54 - 1.00	0.09
L-6	Co	4.92 ± 0.63	2.00 - 9.00		7.08 ± 0.63	3.00 - 10.00		0.70 ± 0.03	0.58 - 0.88	
	Tr	5.07 ± 0.13	1.00 - 11.00	0.03	6.60 ± 0.03	1.00 - 11.00	-0.07	0.72 ± 0.00	0.54 - 0.96	0.04
L-7	Co	4.92 ± 0.63	2.00 - 9.00		7.08 ± 0.63	3.00 - 10.00		0.70 ± 0.03	0.58 - 0.88	
	Tr	4.64 ± 0.03	1.00 - 11.00	-0.06	7.36 ± 0.03	1.00 - 11.00	0.04	0.69 ± 0.00	0.54 - 0.96	-0.01
L-8	Co	3.50 ± 0.62	1.00 - 8.00		8.50 ± 0.62	4.00 - 11.00		0.65 ± 0.03	0.54 - 0.83	
	Tr	4.50 ± 0.02	1.00 - 10.00	0.29	7.50 ± 0.02	2.00 - 11.00	-0.12	0.69 ± 0.00	0.54 - 0.92	0.06
L-9	Co	19.67 ± 0.99	14.00 - 28.00		12.33 ± 0.99	4.00 - 18.00		0.81 ± 0.02	0.72 - 0.94	
	Tr	10.83 ± 1.07	5.00 - 17.00	-0.45	21.17 ± 1.07	15.00 - 27.00	0.72	0.67 ± 0.02	0.58 - 0.77	-0.17
L-11	Co	6.00 ± 0.62	2.00 - 9.00		6.00 ± 0.62	3.00 - 10.00		0.75 ± 0.03	0.58 - 0.88	
	Tr	5.56 ± 0.04	1.00 - 11.00	-0.07	6.44 ± 0.04	1.00 - 11.00	0.07	0.73 ± 0.00	0.54 - 0.96	-0.02
O-1	Co	4.33 ± 0.72	1.00 - 9.00		7.67 ± 0.72	3.00 - 11.00		0.68 ± 0.03	0.54 - 0.88	
	Tr	4.82 ± 0.02	1.00 - 9.00	0.11	7.18 ± 0.02	3.00 - 11.00	-0.06	0.70 ± 0.00	0.54 - 0.88	0.03
P-1	Co	3.33 ± 0.50	1.00 - 6.00		8.67 ± 0.50	6.00 - 11.00		0.64 ± 0.02	0.54 - 0.75	
	Tr	5.67 ± 0.07	1.00 - 12.00	0.70	6.53 ± 0.07	1.00 - 11.00	-0.25	0.73 ± 0.00	0.54 - 1.00	0.14
P-2	Co	3.92 ± 0.48	1.00 - 6.00		8.08 ± 0.48	6.00 - 11.00		0.66 ± 0.02	0.54 - 0.75	
	Tr	5.74 ± 0.04	1.00 - 11.00	0.47	6.26 ± 0.04	1.00 - 11.00	-0.23	0.74 ± 0.00	0.54 - 0.96	0.12
P-4	Co	4.25 ± 0.62	1.00 - 8.00		7.75 ± 0.62	4.00 - 11.00		0.68 ± 0.03	0.54 - 0.83	
	Tr	5.21 ± 0.05	1.00 - 12.00	0.23	6.62 ± 0.05	1.00 - 11.00	-0.15	0.72 ± 0.00	0.54 - 1.00	0.06
P-7	Co	3.50 ± 0.51	1.00 - 6.00		8.50 ± 0.51	6.00 - 11.00		0.65 ± 0.02	0.54 - 0.75	
	Tr	4.58 ± 0.03	1.00 - 12.00	0.31	7.40 ± 0.03	1.00 - 11.00	-0.13	0.69 ± 0.00	0.54 - 1.00	0.06
T-10	Co	3.67 ± 0.53	1.00 - 6.00		8.33 ± 0.53	6.00 - 11.00		0.65 ± 0.02	0.54 - 0.75	
	Tr	4.79 ± 0.02	1.00 - 10.00	0.30	7.21 ± 0.02	2.00 - 12.00	-0.13	0.70 ± 0.00	0.50 - 0.92	0.08
T-12	Co	3.92 ± 0.67	1.00 - 8.00		8.08 ± 0.67	4.00 - 11.00		0.66 ± 0.03	0.54 - 0.83	
	Tr	4.69 ± 0.03	1.00 - 9.00	0.20	7.31 ± 0.03	3.00 - 12.00	-0.10	0.70 ± 0.00	0.50 - 0.88	0.05

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References

1. Bhatnagar S M 1984, Interaction of physical and chemical mutagens in Kabuli chickpeas. *Int. Chickpea Newsl.* **11** 17.
2. Kharkwal M C 2000, Induced mutations in chickpea (*Cicer arietinum* L) I Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Ind. J. Genet.* **58** 159-167.
3. Rao G M and Rao V M 1983, Mutagenic efficiency and factors of effectiveness of physical and chemical mutagens in rice. *Cytologia* **48** 427-436.
4. Kumar S and Dubey D K 1998, Effect of gamma rays EMS DES on meiosis of *Lathyrus sativus* L. *J. Cytol. Genet.* **33** 139-147.
5. Dhamayanthi K P M and Reddy V R K 2000, Cytogenetic effects of gamma rays ethylmethane sulphonate in Chilli pepper (*Capsicum annum* L). *Cytologia* **65** 129-133.
6. Kumar S and Singh V 2003, Meiotic behavior of

induced translocation heterozygote in Pearl millet (*Pennisetum typhoides*). *Cytologia* **68** 245-248.

7. Borah S P and Talukdar J 2002, Studies on cytotoxic effects of extracts of caster seeds (*Ricinus communis* L). *Cytologia* **67** 235-243.
8. Krijthe J M 1942, On the influence of the colchicine on the anthers of *Carthamus tinctorius* L. *Proc. Acad. Sci. Amst.* **45** 283-287.
9. Schank S C and Knowles P F 1961, Colchicine induced polyploids of *Carthamus tinctorius* L *Crop Sci.* **1** 342-344.
10. Pillai RSN 1978, *Cytogenetic Studies in Safflower* (*Carthamus tinctorius* L). Ph.D. thesis, Banaras Hindu University, Varanasi, India.
11. Khidir MO and Knowles PF 1970, Cytogenetic studies of *Carthamus* species (Compositae) with 32 pairs of chromosomes II. Intersectional hybridization. *Can. J. Genet. Cytol.* **12** 90-99.