# CYTOGENETIC STUDY OF THE INDUCED TRANSLOCATION HETEROZYGOCITY IN CARTHAMUS 

A. MALIK<br>Department of Botany, C. C. S. University, Meerut-250 004, India.

Genetic variability is essential for any crop improvement programme. Experimentally induced mutation provides an important source of variability. Fifty-one accessions of Carthamus belonging to five species were given colchicine treatment out of the $M_{1}$ population raised from the treated seeds, two plants, one each belonging to $C$. glaucus and $C$. palaestinus exhibited translocation heterozygosity. Translocation heterozygosity was transferred to $\mathrm{M}_{2}$ generation despite the presence of abnormal course of male meiosis and significant amount of pollen sterility. These plants exhibited more or less regular formation of one quadrivalents at metaphase-I.

Keywords: Carthamus; Colchicine; Translocation heterozygosity.

## 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 $2 \mathrm{n}=20$ to $2 \mathrm{n}=64$ 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, reddish and yellow dyes for clothing and food preparation from the flowers.During the cytological screening of treated lines, translocation heterozygotes were isolated. The present paper documents the cytological features of these translocation heterozygotes.

## Materials and Methods

At least 100 seeds of different accession were soaked in $0.75 \%$ colchicine solution for forty-eight hours. Then 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. 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 to $70 \%$ 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. The various cytological
parameters, related to male meiosis, that were analyzed included: (a) Chromosome configuration at metaphase I/ diakinesis, (b) including the chiasmata frequency, (c) Types and frequency of various meiotic anomalies, (d) Number of spores per tetrad, (e) Pollen size, (f) Pollen sterility, (g) Number of pollen per anther, and (h) Asynchrony for meiotic divisions within an anther.

Pollen stainability as an index of fertility was noted from the anthers of mature flowers. Shrunken and unstained pollen grains were treated as sterile while well filled stained grains were considered as fertile.

## Results and Discussion

Two plants of $\mathrm{M}_{1}$, one belonging to C. glaucus and one to C. palaestinus, exhibited translocation heterozygosity. These plants were selfed and the seeds were raised to get $M_{2}$ plants. These plants exhibited more or less regular formation of one quadrivalents at metaphase-I. The data pertaining to metaphase I configurations are tabulated in Table 1.

Table 2 has data for percent asynchrony in translocation heterozygote plants. These plants had no significant increase in the asynchrony as compared to their control plants.

Different types of meiotic anomalies recorded during first and second meiotic division. Translocation heterozygote plànts exhibited significant increase in meiotic anomalies such as quadrivalent formation (Fig. 14), formation of restitution nucleus during first division (Fig. 5), chromatin disintegration (Fig. 6,9,10), late disjunction of chromosome at metaphase II (Fig. 7), clumping at metaphase ( Fig .8 ), lagging of chromosomes at anaphase-I (Fig. 11), lagging of chromosomes at

Table 1. Chromosome analyses at metaphase I in translocation heterozygotes.

| Acc. |  |  | $\mathrm{G}-\mathrm{I}(\mathrm{Co}$ ) | G-1 (M-1) | G-1(M-2) | P-7(Co) | P-7(M-1) | P-7(M -2) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| II | $\begin{aligned} & \text { O} \\ & \text { a } \end{aligned}$ | Mean | 3.08 | 2.20 | 3.12 | 3.50 | 3.33 | 3.11 |
|  |  | SE | 0.45 | 0.37 | 0.05 | 0.51 | 0.29 | 0.01 |
|  |  | M in | 1.00 | 0.00 | 1.00 | 1.00 | 2.00 | 1.00 |
|  |  | Max | 5.00 | 5.00 | 22.00 | 6.00 | 5.00 | 6.00 |
|  | $\stackrel{\infty}{\underset{\sim}{\infty}}$ | Mean | 8.92 | 9.27 | 7.85 | 8.50 | 7.73 | 7.90 |
|  |  | SE | 0.45 | 0.48 | 0.01 | 0.51 | 0.47 | 0.01 |
|  |  | M in | 7.00 | 5.00 | 2.00 | 6.00 | 5.00 | 4.00 |
|  |  | Max | 11.00 | 12.00 | 11.00 | 11.00 | 10.00 | 11.00 |
| IV | $\stackrel{\infty}{\underset{\sim}{\infty}}$ | Mean | 0.00 | 0.20 | 0.42 | 0.00 | 0.40 | 0.32 |
|  |  | SE | 0.00 | 0.11 | 0.00 | 0.00 | 0.13 | 0.00 |
|  |  | M in | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | Max | 0.00 | 1.00 | 2.00 | 0.00 | 1.00 | 1.00 |
|  | $\begin{aligned} & \underset{\sim}{\Xi} \\ & \underset{U}{U} \end{aligned}$ | Mean | 0.63 | 0.00 | 0.12 | 0.00 | 0.07 | 0.17 |
|  |  | SE | 0.02 | 0.00 | 0.01 | 0.00 | 0.07 | 0.01 |
|  |  | M in | 0.54 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | Max | 0.71 | 0.00 | 1.00 | 0.00 | 1.00 | 1.00 |
| X-ta |  | Mean | 0.63 | 0.91 | 0.88 | 0.65 | 0.86 | 0.87 |
|  |  | SE | 0.02 | 0.02 | 0.00 | 0.02 | 0.01 | 0.00 |
|  |  | M in | 0.54 | 0.79 | - 0.58 | 0.54 | 0.79 | 0.63 |
|  |  | Max | 0.71 | 1.00 | 2.08 | 0.75 | 0.92 | 0.96 |

$\mathrm{Co}=$ Control, $\mathrm{M}-1=$ Mutant $1, \mathrm{M}-2=$ Mutant 2 .
Table 2. Frequency (\%) distribution of PMCs in an anther at different stages of meiosis.

| Acc. | $\mathrm{P}-\mathrm{I}$ | $\mathrm{M}-\mathrm{I}$ | $\mathrm{A}-\mathrm{I}$ | $\mathrm{T}-\mathrm{I}$ | $\mathrm{P}-\mathrm{II}$ | $\mathrm{M}-\mathrm{II}$ | $\mathrm{A}-\mathrm{II}$ | $\mathrm{T}-1 \mathrm{l}$ | $\% \mathrm{AS}$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{G}-\mathrm{I}(\mathrm{Co})$ | 31.82 | 3.03 | 9.09 | 4.55 | 3.03 | 7.58 | 6.06 | 34.85 | 65.15 |
| $\mathrm{G}-1(\mathrm{M}-1)$ | 31.58 | 15.79 | 0.00 | 26.32 | 15.79 | 0.00 | 10.53 | 0.00 | 68.42 |
| $\mathrm{G}-1(\mathrm{M}-2)$ | 14.35 | 16.17 | 19.40 | 17.91 | 14.19 | 6.27 | 5.82 | 5.88 | 67.14 |
| $\mathrm{P}-7(\mathrm{Co})$ | 28.00 | 8.00 | 4.00 | 6.00 | 10.00 | 10.00 | 6.00 | 28.00 | 72.00 |
| $\mathrm{P}-7(\mathrm{M}-1)$ | 11.76 | 17.65 | 29.41 | 11.76 | 23.53 | 5.88 | 0.00 | 0.00 | 70.59 |
| $\mathrm{P}-7(\mathrm{M}-2)$ | 14.25 | 18.58 | 18.44 | 14.09 | 10.30 | 9.80 | 7.38 | 7.15 | 70.70 |

$\mathrm{Co}=$ Control, $\mathrm{M}-\mathrm{I}=$ Mutant $\mathrm{I}, \mathrm{M}-2=$ Mutant2, $\mathrm{P}-\mathrm{I}=$ Prophase $\mathrm{I}, \mathrm{M}-\mathrm{I}=$ Metaphase $\mathrm{I}, \mathrm{A}-\mathrm{I}=$ Anaphase $\mathrm{I}, \mathrm{T}-\mathrm{I}=$ Telophase I , P-II=Prophase II, M-II=Metaphase II, A-II=Anaphase II, T-II=Telophase II, \% AS= Percent Asynchrony.
anaphase-II (Fig. 12), tetrahedral tetrad (Fig. 13), monad (Fig. 14), dyad (Fig. 15), triad (Fig. 16), polyads (Fig. 17,18) and fertile and sterile pollen grains (Fig. 19). The frequencies distributions of PMCs with different number of spores are tabulated in Table 3. The polyads possessed

## 5-12 spores per PMC.

All the translocation heterozygote plants were also analyzed for the frequency distribution of pollen sterility, number of pollen grains per anther and pollen diameter. Data related to these are given in Table 4. Pollen


Fig.1-19. Photomicrographs of anomalous meiotic stages. 1. Metaphase I showing ring quadrivalents, 2, 3, 4. Metaphase I showing chain quadrivalents, 5. Formation of restitution nucleus during first division, 6. Chromatin disintegration, 7. Late disjunction of chromosome at metaphase I, 8. Clumping at metaphase I, 9, 10. Chromatin disintegration, 11. Lagging at anaphase I, 12. Lagging at anaphase II, 13. Tetrahederal tetrads, 14. Monad, 15. Dyad, 16. Triad, 17, 18. Polyads, 19. Sterile and fertile pollen grains.

Table 3. Frequency distribution (\%) of PMCs with different number of spores.

| Acc. | 1 | 2 | 3. | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Spore/PMC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| G-I(Co) | 0.00 | 0.00 | 0.00 | 100.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.93 |
| G-I(M-1) | 4.17 | 10.42 | 10.42 | 10.42 | 12.50 | 4.17 | 12.50 | 6.25 | 4.17 | 10.42 | 4.17 | 10.42 | 6.35 |
| G-1(M-2) | 3.91 | 3.91 | 7.74 | 7.56 | 6.59 | 11.40 | 8.24 | 8.82 | 10.14 | 10.61 | 11.07 | 10.02 | 7.34 |
| P-7(Co) | 0.00 | 0.00 | 0.00 | 92.86 | 0.00 | 7.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.24 |
| P-7(M-1) | 0.00 | 2.44 | 12.20 | 3.28 | 7.32 | 0.00 | 12.20 | 17.07 | 12.20 | 12.20 | 12.20 | 12.20 | 8.12 |
| P-7(M-2) | 4.41 | 4.52 | 6.97 | 8.29 | 7.75 | 7.18 | 9.69 | 10.03 | 12.07 | 11.48 | 8.64 | 8.96 | 5.67 |

$\mathrm{C} 0=$ Control, $\mathrm{M}-1=$ Mutant $1, \mathrm{M}-2=$ Mutant2.
Table 4. Pollen per anther and pollen diameter.

| Acc. | \%PS | Pollen per Anther |  |  |  | Pollen diameter |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Mean $\pm$ SE |  | Range |  | Mean $\pm$ SE | Range |  |
| G-I(Co) | 5.15 | 995.6 | $\pm 15.15$ | 896.00 | - 1044.00 | $45.95 \pm 0.51$ | 42.00 | - 49.00 |
| G-1(M-1) | 71.10 | 460.40 | $\pm 12.21$ | 392.00 | - 500.00 | $23.70 \pm 4.20$ | 4.00 | - 50.00 |
| G-1(M-2) | 52.72 | 503.84 | $\pm 17.41$ | 423.09 | - 589.27 | $27.93 \pm 2.97$ | 5.36 | - 48.27 |
| P-7(Co) | 0.00 | 1070.80 | $\pm 7.26$ | 1036.00 | - 1096.00 | $41.85 \pm 0.49$ | 38.00 | 46.00 |
| P-7(M-1) | 47.52 | 589.20 | $\pm 17.94$ | 500.00 | - 652.00 | $25.55 \pm 3.11$ | 10.00 | - 55.00 |
| P-7(M-2) | 50.83 | 561.56 | $\pm 19.86$ | 465.80 | - 646.20 | $28.85 \pm 2.95$ | 7.45 | - 51.75 |

$\mathrm{Co}=$ Control, $\mathrm{M}-1=$ Mutant $1, \mathrm{M}-2=$ Mutant 2 .
sterility in all these translocation heterozygote increased remarkably while number of pollen grains per anther notably decreased.

Translocation have been induced and studied by some workers. Schank and Knowles' analyzed 20 of the possible of 21 hybrids produced from intercrossings seven Carthamus species with $\mathrm{n}=10$ and found that three, C. dentatus (Turkey), C. glaycus (Iran and Syria), had naturally occurring chromosome interchange. Estilai and Knowles ${ }^{2}$ evidenced that $C$. leucocaulos had a chromosome arrangement similar to that of $C$. dentatus. Khidir and Knowles ${ }^{3}$ found a reciprocal translocation in $\mathrm{F}_{1}$ hybrid of polyploids C. baeticus x C. turkestanicus. Pillai' induced translocation in cultivated safflower. Pillai et al. ${ }^{5}$ isolated translocation homozygotes and identified each chromosome involved in interchange through karyotype analysis.
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