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A REPORT ON THE INDUCTION OF A FERTILE MULTIPLE TRANSLOCATED PLANT FOLLOWING GAMMA IRRADIATION TO SEEDS FROM HOMOZYGOTE STOCK OF *RHOEO SPATHACEA* VAR. *CONCOLOR*

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A fertile multiple translocated plant (P_2) demonstrating chromosomal interchanges (multivalents varied from trivalent to dodecavalent) in 74.1 percent PMCs has been induced following gamma irradiation (10 KR) to seeds from homozygote stocks of *Rhoeo spathacea* var. *concolor* (2n=12). Meiosis, pollen stainability and seed setting were studied in the translocated plant and in standard normals and the results are discussed.

Keywords: Gamma irradiation; Homozygote; Multiple translocation. *Rhoeo spathacea* var. *concolor*.

Introduction

The complex-interchange heterozygosity has enjoyed a revival of interest as it represents a model of surveying genic and chromosomal changes[1]. Furthermore, a fertile translocation stock has selective advantage for breeding behaviour of the species. Present paper reports on the meiotic chromosome behaviour, pollen stainability and seed setting in the standard normal plants and in the gamma ray induced multiple translocated plant from the homozygote stock of *Rhoeo spathacea* var. *concolar* (Commelinaceae), a garden ornamental of West Bengal plains.

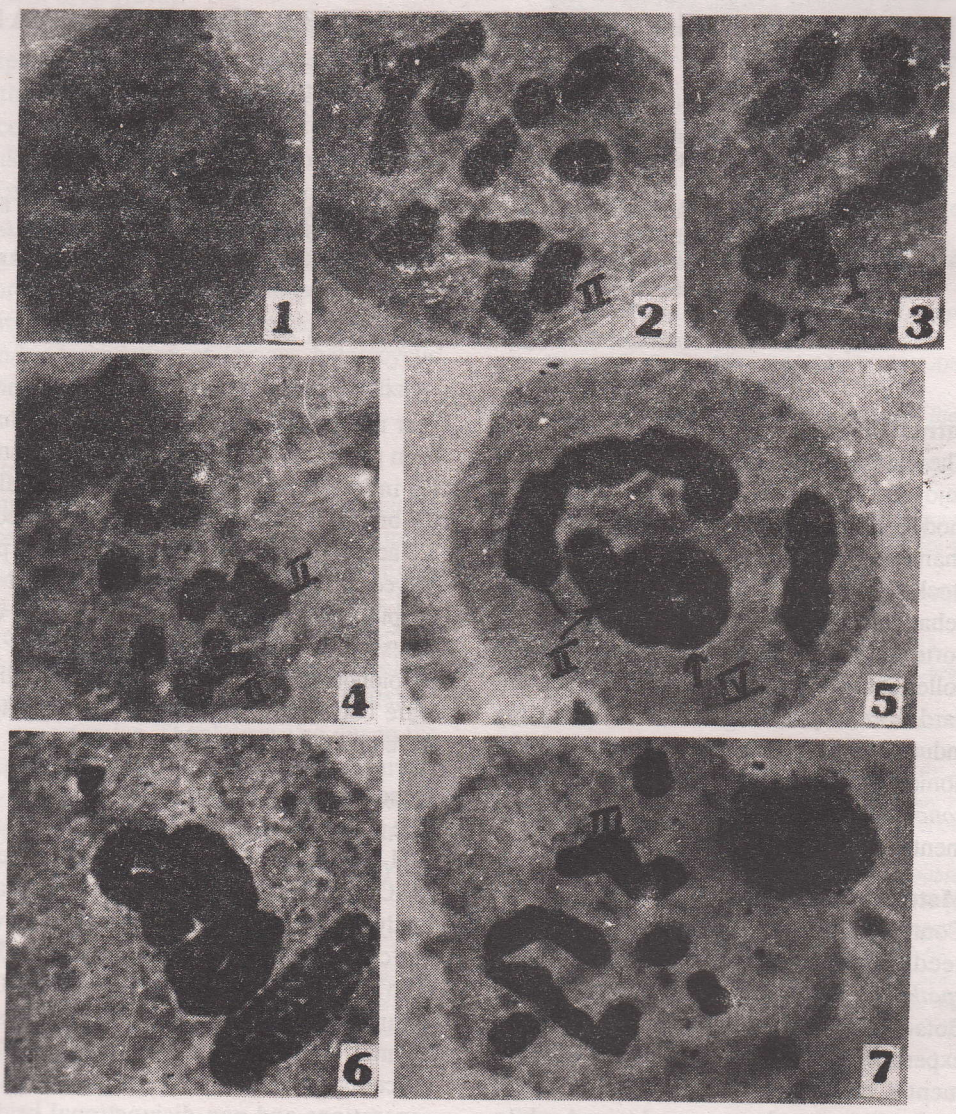
Materials and Methods

Control (untreated) 10 Kr gamma irradiated seeds of homozygote stocks of *Rhoeo spathacea* var. *concolor* (obtained from Kew Botanical Garden, London) were planted in experimental garden under uniform environmental conditions. Out of 100 untreated seeds 18 mature plants were obtained; while, 4 seedlings were raised from 62 gamma irradiated seeds (10 Kr) of which one plant (P_2) was found to have chromosomal interchanges detected through meiotic studies.

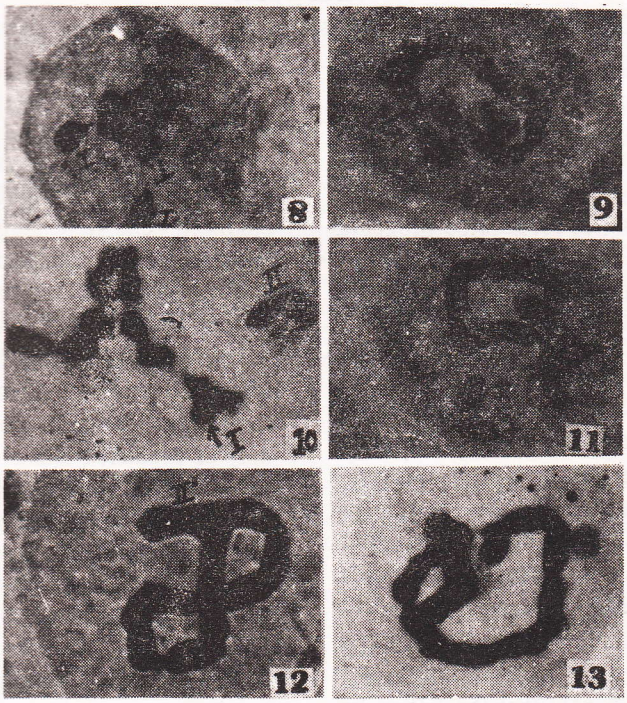
Meiosis and pollen stainability were observed in all the 18 standard normal plants and in the marked plant (P_2) throughout the year and pooled data over the seasons have been presented in the text. For meiotic studies inflorescences (after careful removal of the bracts) from the plant types were fixed in 1:3 acetic alcohol (twice in each month), preserved in 70% ethanol and squashed in 1% propiono-carmine. Pollen stainability was assessed by staining pollen grains in 1% propionocarmine solution and stained pollens were considered fertile. Photomicrographs were taken from suitable preparations.

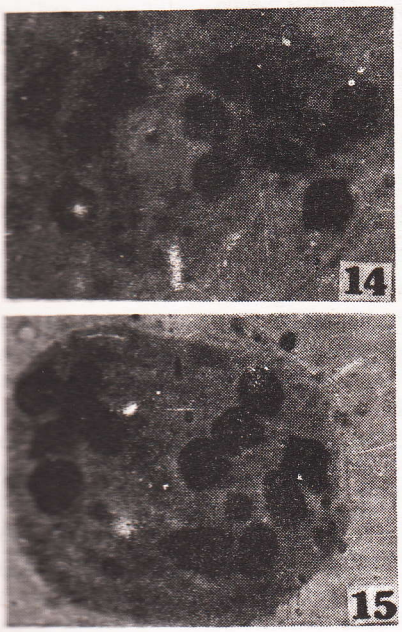
Observations

The standard normal plants demonstrated 6 II formation (Fig.1) in 79.46 percent PMCs and the pooled data for all the plants analysed over the seasons showed an average chromosome association per cell of 5.60 II to 5.83 II (3 to 6) + 0.32 I to 0.78 I(0-6) estimated from 1638 PMCs. In anaphase I, normal 6/6 separation was noted in 96.7 percent cells and the rest demonstrated 5/7 separations and non-disjunctional bridges (1106 cells scored). No abnormalities were recorded in A II cells (610 cells scored). Average pollen stainability in the standard normals was noted to be 74.33% (46.9% - 92.1%) and the plants set seeds (12-19 seeds/inflorescence; 108-171 seeds/plant). Correlation of pollen stainability with 6/6



Figs. 1-13: Diplotene and M I showing chromosomal associations in normal and in translocated plant of *R. spathacea* var. *concolor*.1. 6 II; 2. 1 III + 1 II +7 I; 3. 1 IV + 3 II + 2 I; 4. 1 0 IV + 2 II + 4 I; 5. 1 0 IV + 1 IV + 2 II; 6. 3 0 IV; 7. 1 V + 1 III + 4 I; 8. 1 0 V + 1 II + 5 I; 9. 1 VI + 1 V + 1 I; 10. 1 IX + III +1 I; 11. IVIII + 1 II + + 2 I; 12. 1 0 X + III; 13. 1 XII.





Figs. 14 - 15: Non-disjunctional separation of chromosomes at A I. 14. 5:7; 15. 4:8.

separation was positive but not significant ($r = 0.16$, $p<0.05$).

The P_2 showed higher frequencies of II (1.94) and I (2.23) per cell as compared to III (0.19), IV (0.59), V (0.20), VI (0.17), VII (0.01), VIII (0.09), IX (0.002), X (0.02), and XII (0.01) estimated from 604 PMCs. PMCs with varying multivalent (rings - 23.17%; Chains - 77.48%) chromosomes associations were recorded in 74.1% M I cells of P_2 (Figs. 2-13) and the modal chromosomal association has been I IV + 4 II noted in 5.96 percent cells. The frequency of disjunctional separations (6/6) at A I was more in P_2 (55.55%, scored from 387 cells) than that of the non-disjuntional type (Figs. 14,15). The marked plant revealed 30.44% anaphase I abnormalities (1015 cells scored), which included unequal chromosome separations (5/7 to 3/9), bridges with or without fragments and laggards (1 to > 2). The A II cells showed one (7.03%) or two (0.74%) micronuclei (668 cells analyzed). The percentage of stainable

pollen grains in P_2 was 19.51 (4.3% - 30.5%). Correlation of pollen stainability with 6/6 A I separation was positive but not significant ($r = 0.29$, $P < 0.05$). The marked plant produced 18-24 seeds / inflorescence and a total of 185 seeds. Out of forty three progenies raised from 100 P_2 seeds, 39 were multiple translocated plants and the rest were non translocated.

Discussion

Ring and chain multivalents are the indication of heterozygous translocation; which may appear due to unequal lenght of the translocated segments and the number and position of chiasmata[2]. The standard normal plants and the treated plants growing under similar environmental conditions demonstrated the occurrence of complex ring and chain multivalents only in a gamma-irradiated plant which clearly indicated that irradiation has induced heterozygous translocation. The marked P_2 plant revealed multivalent configuration (III to XII) with association to bivalents and univalents at metaphase I. Such type of chromosomal pairing can be explained if multiple translocation is assumed involving all the chromosomes of the haploid complement. In nature multiple translocations in *R. spathacea* var. *bicolor* have originated due to the presence of genetic flux which contributed to chromosomal instability[3]. However, in nature the accumulation of multiple translocation takes quite a long time and possibly this existing natural tendency in *Rhoeo* might have been accelerated due to gamma - irradiation, which has resulted in occurrence of multiple translocations in a single step in the present study.

Translocated heterozygotes in most cases are concomitantly associated with high pollen sterility as was also noted in the present case. The marked P_2 plant revealed higher frequency of unstainable pollen grains than the frequency of disjunctional separation of multivalents indicating that all numerically balanced cells were surely not genetically balanced. The standard normal plants showed 96.7 percent balanced AI cells, although average pollen sterility assessed in the plants was high as 25.67% and furthermore fluctuation of pollen sterility in P_2 over the seasons was quite prominent and thus it may be predicted that observed pollen sterility in the plant types may not entirely be the outcome of cytogenetical consequences. Zimmerman[4] suggested environmental influence coupled with cytogenetical cause as the possible outcome of pollen abortion in *Rhoeo*.

Seed setting in the translocated plant was higher as compared to standard normal plants. Such incidence of enhanced seed setting has also been reported in a translocated line of *Pisum* which has been attributed to possible genetic repair mechanism[5]. The induced fertile translocated plant described in the text may further be exploited for cytogenetical studies in *Rhoeo*, which is rather meagre in the species[6-7]

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