

## INDUCED PARTIALLY STERILE INVERSION HETEROZYGOTE IN *ORNITHOGALUM VIRENS* L.

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### Abstract

Normal looking plants of *Ornithogalum virens* L. (Liliaceae) raised from  $\gamma$ -ray irradiated seeds were partially sterile. Meiotic studies carried out on this plant for two consecutive years revealed the frequent occurrence of a dicentric chromosome bridge accompanied by an acentric fragment at anaphase I (AI). Paracentric inversion followed by crossing over in the inverted region might have resulted in this type of consequence at AI. Normal plants of *O. virens* ( $2n = 6$ ) showed regular presence of 3 bivalents at metaphase I (MI) and equal 3 : 3 separation of chromosomes at AI. Presence of 2 to 6 univalents in the pollen mother cells (PMCs) of the treated plant indicated the occurrence of occasional asynapsis possibly due to induced inversion. Early movement, lagging and unequal separations of chromosomes were encountered rather infrequently at AI. Pollen sterility of this inversion heterozygote plant was 44.80 and 54.64% in the first and second year, respectively compared to only 1.12 to 3.90% in the control plants.

### Introduction

Occurrence of structural modifications involving changes in chromosomal parts rather than in a set of chromosomes may be spontaneous but unusual. Frequency of such variations can be augmented artificially by the treatment with mutagenic agents. *Ornithogalum virens* L. being a good material for karyological observation (Jauhar and Storey 1982), offers adequate scope for study of structural aberrations because of its larger size and low number of chromosomes ( $2n = 6$ ) with good stainability. Since this species reproduces both sexually and asexually, cytogenetic consequences of induced chromosomal aberrations could be maintained through vegetative propagation. Noda (1960) reported an interchanged heterozygote of *Lilium maximowiczii* and could maintain this aberration by vegetative propagation.

With these prospects in mind, meiotic consequences of post- $\gamma$ -irradiated *O. virens* plants were observed. Among the irradiated plants two have been identified as structural heterozygotes (Biswas and Biswas 2006). By regular investigations in the rest of the irradiated materials, characterization of a partially sterile plant has also been accomplished subsequently. A precise account of its cytogenetic peculiarities due to irradiation has been embodied in the present communication.

### Materials and Methods

Treatment with different doses of  $\gamma$ -rays (10Kr-1hr gap-10Kr, 15 Kr-1hr gap-15Kr and 20Kr-1hr-20Kr) were made from the Co<sup>60</sup> source at Central Research Institute of Jute and Allied Fibre, Barrackpore, West Bengal, India to irradiate well-dried fresh seeds of *Ornithogalum virens* L. (Liliaceae). LD<sub>50</sub> dose was determined earlier as 20Kr-1hr-20Kr. Seedlings were raised by sowing the irradiated and control (untreated) seeds in experimental pots. Usual flowering occurred normally in two years old plants and suitable-sized flower buds were collected for successive two

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years from the treated as well as untreated plants between 9.00 and 10.00 a.m. for meiotic investigations. The flower buds were fixed immediately in Carnoy's fluid (6: 3: 1 v/v ethyl alcohol, chloroform and acetic acid). After 24 hrs the fixed buds were preserved in 70% alcohol at 4° C following repeated washing with the same. Anthers from the fixed buds were smeared in 1% aceto-carmin solution for analyzing PMCs. Pollen fertility was scored by staining pollen grains collected from freshly opened flowers in 1% aceto-carmin solution. The pollen grains showing deformity and lack of stainability were considered as sterile. Photomicrographs were taken from suitable preparations.

## Results and Discussion

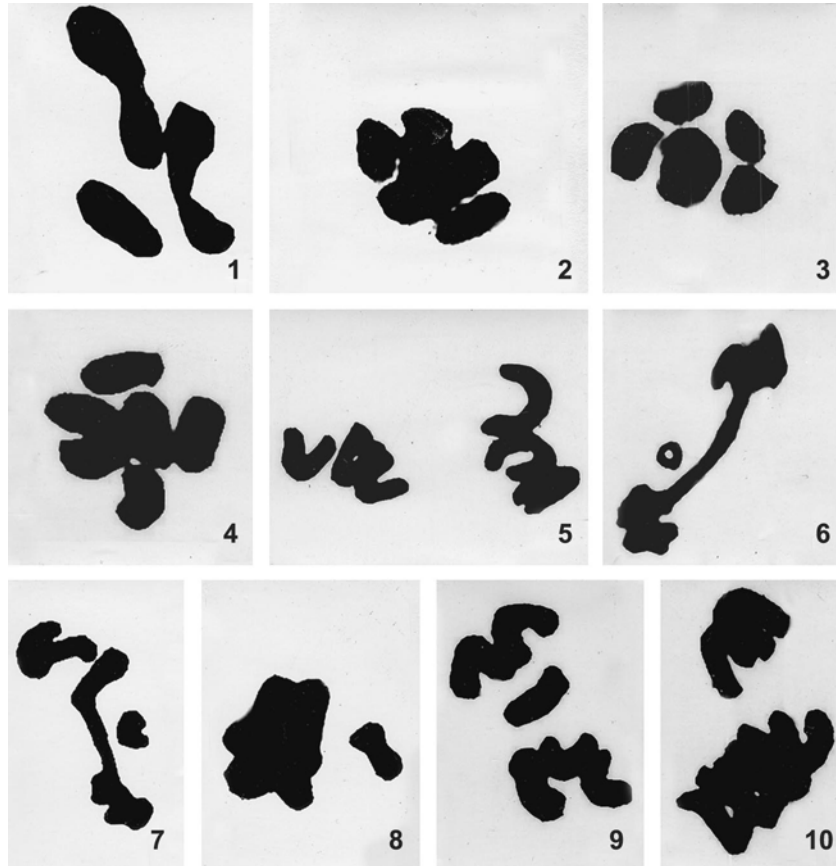
Cytogenetic investigations were carried out for two successive years in the control and post irradiated plants of *O. virens*. The irradiated plants were phenotypically indistinguishable from the control plants barring remarkably reduced fertility manifested in one of the treated plants raised through irradiation with the LD<sub>50</sub> dose 20Kr-1hr gap-20Kr gamma ray. Meiotic studies revealed regular occurrence of three bivalents in PMCs of control plants at MI (Fig. 1). On the contrary, presence of 2 and 4 univalents were met with in adequate number of PMCs at MI of the partially sterile plant during both the years of observation barring the occurrence of 6 free univalents in few PMCs in the second year (Table 1). More frequently, two univalents appeared with two bivalents, sometimes, conspicuous presence of 4 univalents with one bivalent and clear 6 univalent (none of

**Table 1. Frequency of different types of chromosomal configurations and chiasma in meiotic chromosomes of *O. virens*.**

| Plants  | Year of observation | No. of PMCs observed | Chromosome configuration at MI    | Frequency of PMCs (%) | Chiasma frequency at diakinesis |                  |
|---------|---------------------|----------------------|-----------------------------------|-----------------------|---------------------------------|------------------|
|         |                     |                      |                                   |                       | Chiasma/cell                    | Chiasma/bivalent |
| Control | First               | 472                  | Bivalent association in all cases |                       | 8.87                            | 2.94             |
|         | Second              | 534                  | Bivalent association in all cases |                       | 8.37                            | 2.61             |
| IH      | First               | 336                  | 2 II + 2 I                        | 5.54                  | 6.51                            | 2.17             |
|         |                     |                      | 1 II + 4 I                        | 1.19                  |                                 |                  |
|         | Second              | 240                  | 2 II + 2 I                        | 6.41                  | 5.49                            | 1.83             |
|         |                     |                      | 1 II + 4 I                        | 0.83                  |                                 |                  |
|         |                     | 6I                   | 1.25                              |                       |                                 |                  |

the chromosomes showing any pairing tendency) were also come across (Figs. 2-4). Intrachromosomal effect of inversion heterozygote (IH) has been studied earlier and in the present inversion heterozygote of *Ornithogalum virens* the occurrence of inversion due at least in part has reduced the pairing affinity to the other chromosomes which has resulted the formation of univalents (Gupta 1997, Canady *et al.* 2006, Pertuze *et al.* 2002). It has also been opined that cryptic structural differences might influence chromosome pairing, crossing over and segregation (Palmer *et al.* 2000). Asynapsis as well as a high degree of non homologous pairing in the inversion heterozygote has also been reported earlier (Nur 1968). Chiasma frequency was found to range from 6.51 to 5.49 per cell and 2.17 to 1.83 per bivalent in the post-irradiated plant; while in the control plants, it varied from 8.87 to 8.37 per cell and 2.94 to 2.61 per bivalent (Table 1). Equal separation of 3 : 3 chromosomes at AI was the usual feature in the control plants but in the treated plant a chromosome bridge was found to be accompanied with a loop or a small fragment in 18.75 and 18.06% male meiocytes at AI during the first and second years of observations, respectively (Figs. 5 - 7). Early movement, lagging and unequal separation of chromosomes were also encountered in a few microsporocytes (Figs. 8 - 10, Table 2). Presence of a dicentric bridge at AI

accompanied with an acentric fragment in a post-irradiated phenotypically normal looking but partially sterile plant of *O. virens* suggested it to be an inversion heterozygote. The dicentric bridge observed in the partially sterile plant has appeared possibly as an outcome of induced paracentric inversion followed by crossing over in the inverted region. Consequences of paracentric inversion have been studied in *Zea mays* (McClintock 1938), *Vicia faba* (Sjodin 1971), *Hordeum vulgare* (Ekberg 1969, Prasad 1975, Yu and Hockett 1979), *Tridax procumbens* (Cequea *et al.* 2003) and *Withania somnifera* (Iqbal and Datta 2006).



Figs. 1 - 4. Pairing association of meiotic chromosomes at MI in control and IH of *O. virens*. 1. 3II in control. 2. 2II with 2 univalents in IH. 3. 3II with 4 univalents in IH. 4. 6 univalents in IH. 5-10. Chromosomal separation in control and IH. 5. Equal separation of AI chromosomes in control. 6. AI with a dicentric bridge and a loop in IH. 7. A dicentric bridge with a small fragment at AI in IH. 8. Early separation at MI in IH. 9. AI with a lagging chromosome in IH. 10. Unequal separation at AI in IH. (Bars = 10  $\mu$ m). Magnification: Figs. 1, 5 - 6, 9 and 10 = 2100  $\times$ ; Figs. 2 - 4, 7 and 8 = 1800  $\times$ .

Comparatively reduced number of chiasma in the present induced inversion heterozygote provides circumstantial evidence in favour of univalent formation owing to pairing disturbances. This may be attributed to partial asynapsis. Low frequency of chiasma has been ascribed for the occurrence of univalents (Aguiar-Perecin *et al.* 1984). An inversion in a chromosomal part often reduces recombination immediately outside the inverted segment as well as within it (Srb *et al.* 1970). It has also been opined that presence of inversion heterozygosity in certain region of a

particular chromosome pair has been shown to influence crossing over in other chromosomes and also in the same chromosome outside the inverted segment (Gupta 1997).

**Table 2. Irregularities at AI and pollen sterility of *O. virens*.**

| Plants  | Year of observation | Total No. of PMCs | PMCs showing anomalies (%) at AI |                                |                    |                    | Pollen sterility |
|---------|---------------------|-------------------|----------------------------------|--------------------------------|--------------------|--------------------|------------------|
|         |                     |                   | Early movement                   | Dicentric bridge with fragment | Unequal separation | Lagging chromosome |                  |
| Control | First               | 255               | -                                | -                              | -                  | -                  | 1.12             |
|         | Second              | 246               | -                                | -                              | -                  | -                  | 3.90             |
| IH      | First               | 264               | 5.00                             | 18.75                          | 0.89               | 1.49               | 44.80            |
|         | Second              | 193               | 0.89                             | 18.06                          | -                  | 1.04               | 54.64            |

Only 1.12 to 3.90% pollen sterility could be recorded in the control plants, whereas the treated plants were found to be partially sterile showing 44.80 to 54.64% pollen sterility during the two consecutive years of observations. In paracentric inversion heterozygote, expected frequency of pollen sterility would be 50% when single bridge appears with a fragment or a loop at AI (Gupta 1997). Pollen sterility recorded (44.80 to 54.64%) in the present partially sterile paracentric inversion heterozygote was close to the expected frequency. In barley Yu and Hockett (1979) reported partial sterility with paracentric inversion. Ekberg (1969), however, induced different types of sterility accompanied with this type of inversion. Crossing over in the inverted chromosomes leads to production of deficiency-duplication chromatids, which would impair the ability of the gametes to function in inversion heterozygote (Gupta 1997, Cequea *et al.* 2003).

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