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Induction of Segmental Interchanges in Pearl Millet (*Pennisetum typhides*)

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Summary. Dry seeds of two varieties of *Pennisetum typhoides* (2n=14), 'Tift 23-B' and 'Bil-3B', were treated with gamma rays, diethyl sulphate (DES) and ethylene imine (EI) at their approximate LD_{50} dosages and the pollen mother cells of the M_1 (first generation immediately after the seed treatment) plants were analysed at diakinesis for multivalent configurations resulting from segmental interchanges. While quadrivalents and trivalents were commonly found in all the mutagenic treatments, hexavalents were seen in the gamma-ray treatment only. Ring quadrivalents were common in all the treatments and their frequency was higher in gamma-ray treatment than in the treatments with the chemical mutagens of which EI produced more quadrivalents than DES. The variety 'BIL-3B' was more responsive to all the mutagens used than 'Tift-23B' in which, excepting in gamma-ray treatment, no multivalents were observed in EI and DES treatments.

The quadrivalents induced by different mutagens were of different types involving different chromosomes, indicating some kind of specificity of the mutagens in causing chromosome breaks. Thus, in EI-induced quadrivalents the nucleolar chromosome, the shortest chromosome of the complement, was involved, whereas in the case of DES and gamma rays it was the longest chromosome of the complement that was involved in the quadrivalent. Apparently the breaks must have been produced in different chromosomes preferentially.

Self-pollinated seeds of two heterozygotes whose interchanges were induced by EI and gamma rays were given a second cycle treatment with gamma rays, again at the LD_{50} dosage (35 kR), and interchange stocks involving different chromosomes, up to a maximum of eight chromosomes were realized. Alternate use of EI and gamma rays offered better possibilities of obtaining inter-change heterozygotes involving more, if not all, chromosomes in a ring than two successive treatments with gamma rays alone.

Keywords: Mutagens – Specificity – Interchange – Second cycle-Octovalent

Introduction

Reciprocal translocations or segmental interchanges, among the various types of chromosomal aberrations, are of much importance in genetics and plant breeding and have been artificially induced in several crop species (Burnham 1956; Hagberg, Lehman and Hagberg 1975). Different types of interchanges, with break points at different regions of the chromosomes, are very useful as cytogenetic markers for creating new karyotypes, for directed duplications, for linkage tests and for producing trisomics.

Chemical mutagens, from the early days of their discovery, gave some evidence of more specificity or non-random action as compared to ionizing radiations. A number of chemical mutagens are said to produce breaks in the heterochromatic (McLeish 1953), centromeric (Swaminathan et al. 1962) and satellite (Kihlman and Levan 1951) regions of the chromosomes. Gamma rays and X-rays also have been reported to induce non-random chromosome breaks (Evans and Bigger 1961; Lozanyi 1969).

Diethyl sulphate (DES) and ethylene imine (EI), like other alkylating agents, induce chromosomal changes as shown by DES in barley (Heiner et al. 1960) and *Sorghum* (Sree Ramulu 1971) and by EI in *Triticum durum* (Edward and Williums 1966) and in barley (Nawar et al. 1971). The frequency of interchanges induced by chemical mutagens is generally less than that induced by ionizing radiations. However, Akhund-Zade (1968) observed that EI and gamma rays induced equal frequency of chromosomal aberrations in peas but the types of aberrations produced differed while according to Ghatnekar (1964) EI produced more multivalents than X-rays.

Pearl millet, an often cross-pollinated, important grain and forage crop and a favourable material for cytogenetical studies as it has a small number (2n=14) of individually identifiable, fairly long chromosomes, was chosen for these studies. It was treated with the chemical mutagens DES and EI and also with the physical mutagen gamma rays with the following objectives: (i) to constitute differ-

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ent translocation stocks (ii) to assess the efficiency and specificity of the mutagens used and (iii) to find out the varietal response to these mutagens.

Since structural heterozygosity, where all the chromosomes of a complement are involved in a ring, offers the possibility of maintaining heterosis in crop plants, an attempt was made to bring all the chromosomes of pearl millet into a single ring as has been done in barley (Sisodia and Shebeski 1965), in *Aegelopoides monococcum* (Yamashita 1951) and in *Pennisetum typhoides* (Brar et al. 1973; Tyagi 1976).

Materials and methods

Dry seeds of two inbred varieties, 'Tift-23B' and 'BIL-3B', of pearl millet were treated with a physical mutagen, gamma rays, and two chemical mutagens, DES and EI, at their approximate LD_{50} dosages as assessed previously on the basis of seedling growth. At this level the effect of the different mutagens become comparable. The LD_{50} dosages given were 35 kR gamma rays, 0.5 percent DES and 0.04 percent EI. The moisture content of the seeds at the time of treatment was 10.2 percent for 'Tift-23B' and 10.8 percent for 'Bil-3B'. Dry seeds were irradiated with gamma rays from a ^{60}CO source with an exposure rate of 2.5 kR/min. at 30 ± 1°C. The appropriate concentration of DES was prepared in distilled water by constantly stirring the mixture in an agitator (DES does not easily dissolve in water) while the EI solution was prepared by merely hand-shaking the mixture. Seeds of both varieties, enclosed in separate muslin cloth bags, were immersed together in a freshly prepared 100 ml solution of each mutagen and the vials were vigorously shaken at frequent intervals. The treatment duration was one hour at 30°C. After the completion of the treatment the seed lots were throughly washed with distilled water. The treated seeds, along with their controls, were first sown in shallow pans and the 12-day old seedlings were transplanted into earthern pots and maintained in them until the experiment was completed. About 60 plants were grown for each treatment.

Self-pollinated seeds of two M_1 (first generation after the seed treatment) plants of 'BIL-3B', each with different interchange rings of four chromosomes, induced by gamma rays and EI, were irradiated again with 35 kR gamma rays alone as a second cycle of treatment and the pollen mother cells of the progeny were subsequently examined for multivalent associations.

For cytological analysis, flower buds from the main tillers were fixed in 1:3 acetic-alcohol for 24 hours and then stored in 70 percent alcohol in a refrigerator until used. Slides were prepared by the usual acetocarmine technique. From each plant 50 well spread out pollen mother cells (PMCs), selected at random at diakinesis, were studied. Photomicrographs were taken from temporary mounts using a Zeiss microscope with an oil-immersion objective 90X and an occular 7X.

Results

Cytological analysis of ten randomly selected control plants showed normal meiotic divisions with seven regular bivalents, the smallest bivalent being associated with the nucleolus. The treated plants in the M_1 generation (first generation after the seed treatment) exhibited multivalent associations including hexavalents, quadrivalents and trivalents resulting from segmental interchanges, the quadrivalents being more predominant.

Hexavalents

In both varieties, hexavalents which resulted from reciprocal translocations involving segments of three non-homologous chromosomes were observed only after the gamma-ray treatments. They always appeared in a cell as an open ring or a chain of six chromosomes along with four normal bivalents (Fig. 1). Their frequency was higher in 'Tift-

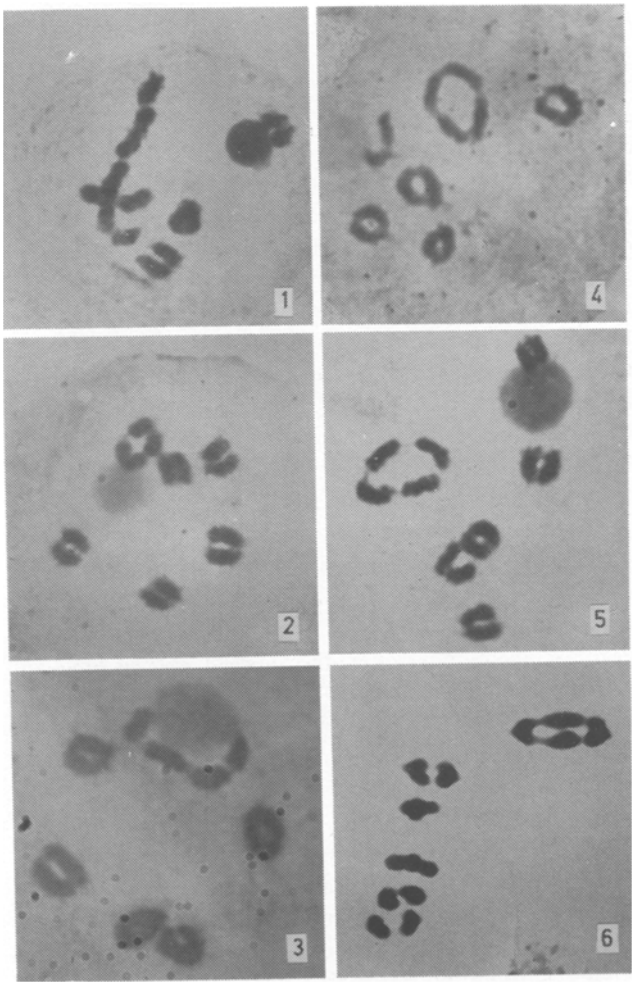

Fig. 1. 1 VI(chain) + 4 II in gamma-ray treated plant of 'Tift-23B'

Fig. 2 and 3. EI-induced closed ring and open ring quadrivalents + 5 II in 'BIL-3B'. Quadrivalents are associated with nucleolar chromosomes

Fig. 4. DES-induced quadrivalent involving the longest chromosome in 'BIL-3B'

Fig. 5 and 6. Gamma-ray induced ring quadrivalents in 'BIL-3B' and 'Tift-23B' respectively

Table 1. Comparative chromosome configurations at diakinesis for different mutagens at LD_{50}

Variety	Treatments dose/con.	No. of PMCs analysed	Percentage frequency				% of total abnormal cells
			1 VI 4 II	1 IV 5 II	1 III 1 I 5 II	7 II	
BIL-3B	35 kR γ-rays	867	0.34	44.29	3.69	51.67	48.32
	0.5% DES	693	0	20.05	1.15	78.78	21.21
	0.04% EI	1080	0	21.01	2.03	76.94	23.05
	Control	787	0	0	0	100.00	0
Tift-23B	35 kR γ-rays	976	0.40	28.99	8.09	62.50	37.50
	0.5% DES	775	0	0	0	100.00	0
	0.04% EI	755	0	0	0	100.00	0
	Control	871	0	0	0	100.00	0

23B' (0.40 percent) than in 'BIL-3B' (0.34 percent). Hexavalents were not observed in the plants treated with either of the chemical mutagens.

Quadrivalents

Among the various types of multivalent associations, quadrivalents arising as a result of reciprocal translocations between two non-homologous chromosomes were found more frequently at diakinesis than the other two types of configurations (Table 1). They were either closed or open rings and comprised of two translocated and two normal chromosomes. The hexavalents always appeared in the form of chains or open rings and the quadrivalents predominantly as closed rings and rarely as chains, the latter being due to early terminalization of chiasma. As no cell with two or more quadrivalents was observed, the percentage of cells with a quadrivalent denotes the frequency of quadrivalents in a treatment. The quadrivalents were observed more frequently in 'BIL-3B', the frequency being more (44.29 percent) in gamma-ray treatment than in either DES or EI treatments, where their frequencies were 20.05 and 21.01 percent, respectively. Quadrivalents were less frequent in 'Tift-23B', their frequency being 28.99 percent in gamma-ray treatment only.

The quadrivalents induced by different mutagens were of different types and involved different chromosomes, thus indicating some kind of specificity of the mutagens in inducing chromosome breaks. This was more apparent in the EI-treatment in 'BIL-3B' where the nucleolar chromosome (chromosome No. 7), the shortest of the complement, was involved in the translocation ring (Figs. 2 and 3). Although it is difficult to identify the individual meiotic chromosomes, a careful examination of the size differences among the chromosomes showed that the longest chromosome of the complement (chromosome No. 1) was involved in the translocation ring in DES treatment (Fig. 4). Similarly, in the gamma-ray treatment chromosome No. 1 was also identified to be involved in the quadrivalent formation (Fig. 5 and 6). It was not possible to identify precisely the segments of the non-homologous chromosomes involved in the translocations, but the predominant occurrence of ring interchange complexes in gamma-ray and DES treatments indicated that the sites of breaks may be in the vicinity of the centromere with the interchange segments relatively long and more or less equal in length. In the EI-induced quadrivalents, one of the interchange segments appeared to be relatively short with a break point in the short arm of the nucleolar chromosome. Open ring-quadrivalents occurred with a high frequency.

Trivalents

In addition to quadrivalents and hexavalents, trivalents were also found with a high frequency and they always appeared at diakinesis as a chain of three chromosomes accompanied by a univalent (Fig. 7). As in the case of quadrivalents no cell with more than one trivalent plus a univalent was seen. The trivalents were always found in plants in which quadrivalents also occurred in all the mutagenic treatments (Table 1). Obviously, the trivalents might have arisen due to an early failure of chiasma or its terminalization resulting in the dissociation of a quadrivalent into a trivalent and a univalent, as also reported by Pantulu (1967). The trivalents in EI-treated plants were associated with the nucleolus. As in the case of quadrivalents, the frequency of trivalents was also high in the gamma-ray treatment as compared to the chemical treatments. They were also more frequent in 'Tift-23B' than in 'BIL-3B'.

The pollen mother cells with different multivalent asso-

ciations in both varieties treated with various mutagens (Gamma-ray >> EI > DES – when compared at the comparable LD_{50} dosage) was in the sequence of magnitude. The two inbreds differed markedly in their ability to produce multivalent associations: 'Bil-3B' responded to all three mutagens used better than 'Tift-23B'.

Second Cycle Treatment

Two plants in 'BIL-3B', selected from the selfed progeny of the plants derived from the seeds that had received two cycles of treatment with 35 kR gamma rays, were selected and their pollen mother cells analysed for multivalent associations at diakinesis. The results are tabulated in Table 2.

Plant 1: The originator of this plant in the M_1 generation with 0.04 percent EI treatment had a quadrivalent frequency of 47.29 percent and a trivalent plus a univalent frequency of 4.58 percent. The translocation mainly involved the nucleolar chromosomes. After the second cycle of treatment with gamma rays, plant 1 showed complex interchanges involving hexavalents, octovalents and a hexavalent plus a quadrivalent with a frequency of 10.41, 8.33 and 77.08 percent, respectively. In addition, a few cells (4.13 percent) with only a quadrivalent were also observed. The hexavalents and octovalents were always in chain configurations. The nucleolar chromosomes were found regularly associated only with the hexavalents and octovalents (Figs. 8 and 9) and not with the quadrivalents, which apparently resulted from the second cycle of treatment with gamma rays alone. The hexavalents and octovalents involving the nucleolar chromosomes also resulted from the second cycle of treatment. Neither two quadrivalents nor cells with only bivalents were found in the plants that had received a second cycle of treatment.

Plant 2: This was a descendent of a M_1 plant with a quadrivalent frequency of 88.14 percent and a trivalent plus a univalent frequency of 7.90 percent induced by gamma rays. The chromosomes involved in the multivalent associations were different from those of the EI treatment. In the second cycle of treatment with 35 kR gamma rays in plant 2, different types of multivalent associations – two rings of four chromosomes each, one hexavalent and one hexavalent and a quadrivalent with a frequency of 33.33, 25.49 and 41.17 percent, respectively – were found. In the pollen mother cells where a hexavalent

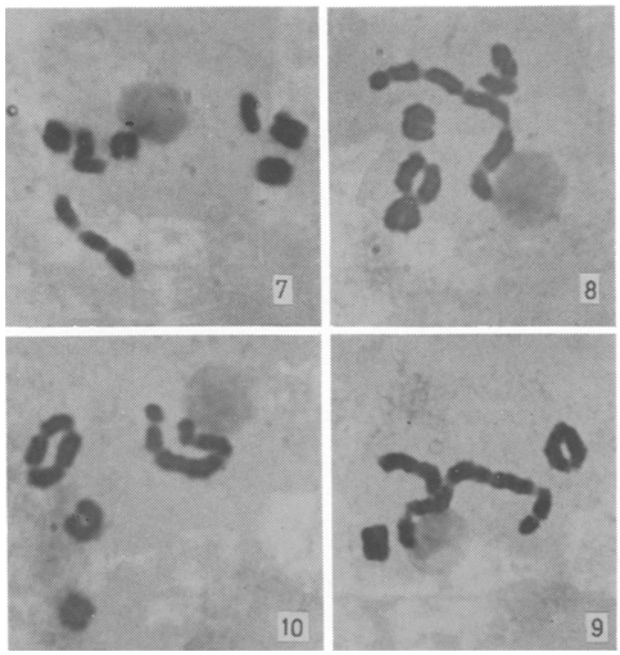

Fig. 7. 1 III + 1 I + 5 II
Fig. 8 and 9. 1 VI +4 II and 1 VIII + 3 II respectively, involving nucleolar chromosomes in second cycle treatment, EI followed by gamma ray
Fig. 10. 1 VI including the nucleolar chromosomes + 1 IV + 2 II in second cycle treatment, gamma ray followed by gamma ray

Table 2. Comparative multivalent associations at diakinesis in the first and second cycles of treatment in the variety 'BIL-3B'

Treatment	Plant No.	No. of PMCs analysed	Percentage freqeuncy						
			1 IV 5 II	1 III 1 I 5 II	2 IV 3 II	1 VI 4 II	1 IV 1 VI 2 II	1 VIII 3 II	7 II
I Cycle									
0.04% EI	1	480	47.29	4.58	0	0	0	0	48.12
35 kR γ-ray	2	253	88.14	7.90	0	0	0	0	3.95
II Cycle									
0.04% EI + 35 kR γ-ray	1	96	4.13	0	0	10.41	77.08	8.33	0
35 kR γ-ray + 35 kR γ-ray	2	51	0	0	33.33	25.49	41.17	0	0

and a quadrivalent were present, the nucleolar chromosome was involved in the hexavalent (Fig. 10), as in plant 1. It is obvious that the hexavalents involving the nucleolar chromosomes were induced by two successive cycles of gamma-ray treatment. In this plant neither an octovalent nor only bivalent were observed.

From the second cycle of treatment it is obvious that the alternate use of chemical and physical mutagens offers more possibilities of obtaining interchange heterozygotes involving all the chromosomes of the complement in a ring than cyclic treatment with a single mutagen.

Discussion

Among the mutagens used in these experiments gamma rays induced multivalent formations to a greater extent than the chemical mutagens. This is similar to the results obtained by Heiner et al. (1960) in barley and by Sree Ramulu (1971) in *Sorghum*. Burton and Powell (1966) observed that ethylmethane sulphonate induced a lesser frequency of multivalents than did thermal neutrons in *Pennisetum typhoides*. Similar results were obtained with EI by Edwards and Williams (1966) in *Triticum durum*, by Akhund-Zade and Khvostova (1966) in peas and by Nawar et al. (1971) in barley, as compared with gamma rays.

The translocation rings of four chromosomes induced by DES, EI and gamma rays in the present study were of different types involving different chromosomes indicating some kind of specificity of the mutagens in causing chromosome breaks. In 'BIL-3B', EI-induced quadrivalents involved the nucleolar chromosomes (chromosome No. 7), the shortest of the complement. It is therefor evident that EI breaks this chromosome preferentially. Similarly, DES and gamma rays were found to break the longest chromosome of the complement (chromosome No. 1) preferentially in the variety 'BIL-3B'. Evans and Bigger (1961) correlated the localization of break points in radiation-induced interchanges with the heterochromatic regions of the chromosomes. This could be the reason for the break in chromosome No. 1. According to Venkateswaralu and Pantulu (1968) it has a larger amount of heterochromatin on either side of the centromere. However, the selective action of hydrogen peroxide (H_2O_2) generated in the cell during irradiation at specific regions of the chromosomes cannot be ruled out.

The frequent occurrence of translocation rings in gamma-ray and DES treatments indicate that the break points might be located close to the centromere as the exchange segments of the chromosomes involved appeared to be more or less of equal length. The break point in EI-induced quadrivalents may be in the short arm of one of the nucleolar chromosomes as indicated by the frequent occurrence of open ring or chain configurations. Hagberg et al. (1975) and Kaul (1977) made similar observations in barley and *Allium* respectively.

Krishnaswamy and Rangaswamy Iyengar (1941) with X-rays and Pantulu (1967) and Jauhar (1974) with gamma rays found induced quadrivalents in *Pennisetum typhoides* with and without involving the nucleolar chromosome, indicating random breakage of chromosomes involved in interchanges. Similar observations were made by Burton and Powell (1966) and Srinivasachar and Mohan Das (1971) in *Pennisetum typhoides* treated with either physical or chemical utagens. In the present study it was observed that gamma rays in 'Tift-23B' and 'BIL-3B' and DES and EI in 'BIL-3B' at LD_{50} dosages of the mutagens produced non-random breakage of chromosomes. Since DES and EI are known to attack preferentially the guanine base of DNA it is likely that the regions of the chromosomes broken by these chemicals are rich in G-C base-pairs. Shevchenko (1969) has reported that EI induced specific breakage of chromosomes in *Crepis capillaris*. Radiation-induced non-random distribution of chromosome breaks are also found described in the literature. Thus, Evans and Bigger (1961) found distribution of chromosome breaks in the mid-region of the acrocentric *S* chromosome. Similarly, Lozanyi (1969) noted preferential occurrence of chromatid and isochromatid gaps in the *M* chromosome in X-ray and gamma-ray irradiated *Vicia faba*. Sjodin (1971) observed that ionizing radiations were nearly five times more effective in inducing translocations than chemical mutagens in *Vicia faba* and caused non-random breaks within and among the chromosomes.

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