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# Induction of Multiple Chromosome Interchanges in Pearl Millet, *Pennisetum typhoides*

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**Summary.** With a view to building up a series of translocation stocks and also, eventually, a complex heterozygote involving all the 14 chromosomes of pearl millet, its seeds were exposed to gamma rays from a $^{60}$Co Gamma Source. Seeds from the meiotically established interchanges were given several cycles of gamma irradiation. Frequency of the incidence of interchange heterozygotes in different generations was scored. As well as receiving recurrent irradiation, different translocation stocks were intercrossed to build up higher interchange configurations and also to identify the translocated chromosomes. Interchanges involving more than 12 of the 14 chromosomes were not obtained, presumably because the somatic and gametic sieves operate more rigorously when more chromosomes are involved in interchanges. It is nevertheless suggested that the karyomorphological features of pearl millet, including its symmetrical karyotype, are favourable for the induction, and also perhaps the maintenance, of translocation heterozygosity. Possible use of translocation heterozygotes in building aneuploids and certain duplications of value in breeding is suggested.

## Introduction

Chromosome interchanges, being good cytogenetic markers, are useful in genetic investigations and basic studies in cytology (Burnham, 1962; Ramage, 1964, 1970). They are also good tools for creating new variation and for directed duplications, so may be useful in plant breeding (Hagberg, 1964; Hagberg and Hagberg, 1969); they offer considerable potential for chromosome engineering studies (Ramage, 1964, 1969). Chromosomal re-arrangements are also known to confer some adaptive advantage on certain natural populations (Rees, 1961; Rees and Sun, 1965; Rana and Jain, 1965). They may also result in repatterning of karyotypes (Jain, Vasudevan and Basak, 1963) and the alteration of basic chromosome number (see Jauhar and Joshi 1969), so they are important from the evolutionary standpoint. Thus, the study of the course and consequences of structural changes in chromosomes is fundamental to our understanding of many biological phenomena. Although series of translocation stocks have been developed in several important plant species, including maize (Burnham, 1956) and barley (Hagberg and Hagberg, 1969), no systematic attempt has been made to produce such translocations in pearl millet (*Pennisetum typhoides*), an important grain and fodder crop of the tropics. Preliminary investigations on induced chromosomal interchanges in pearl millet have been briefly reported by the author (Jauhar, 1972). The objective of the present study was to build up a set of translocation stocks in this cross-fertilizer and eventually to obtain a complex interchange heterozygote involving all 14 chromosomes of its complement. Details of the progress made in this direction are reported in this paper.

## Material and Methods

Seeds of an established variety of pearl millet, viz. T 55, were irradiated with 20 kR and 30 kR gamma rays from a $^{60}$Co Gamma Source at this Institute. Initially 200 seeds were used for each treatment. The treated and control seeds were sown in pots. Meiosis in many of the $M_1$ plants (individually labelled tillers) was studied and those having translocations identified. Spikelets from individual tillers were fixed in acetic alcohol (1:3) to which a few drops of ferric chloride had been added. Anthers were squashed in 1.5 per cent aceto-carmine. Analysis of different stages of meiosis was done and photomicrographs taken from temporary mounts of PMC's.

Seeds from the cytologically identified plants having interchanges were given another cycle of gamma irradiation (20 kR). Seeds from the semi-sterile plants (most of which had been cytologically analysed) were collected for further treatment with gamma rays. In this way, third, fourth and subsequent cycles were repeated in order to obtain larger translocation rings. In this paper the first generation of plants arising from the irradiated seeds will be termed the $M_1$ and the subsequent generations following recurrent cycles of irradiation will be designated $M_2$, $M_3$, $M_4$, etc.

## Observations

### 1. Control Plants

Analysis of ten randomly selected control plants showed regular meiosis with seven bivalents at diplotene, diakinesis and metaphase I; the smallest bivalent, generally a rod, was invariably associated with the nucleolus (Fig. 1). Anaphase I and subsequent stages were also normal showing 7:7 disjunction. Pollen fertility, as judged by stainability with aceto-carmine, was found to be 97.5 per cent.

### 2. Identification of Interchange Stocks

The interchange stocks had generally a distinctive panicle morphology and preliminary identification could be done from their semi-sterile pollen. Such plants were studied meiotically and most were found to have a ring or chain configuration of four chromosomes at metaphase I. In this way translocation stocks were established.

### *3. Studies on $M_1$ Plants and Recurrent Irradiation*

Meiosis in the $M_1$ population revealed a large number of plants having translocations. Of the 165 plants screened under both the treatments, as many as 32 showed signs of chromosome interchanges and these plants were then subjected to critical analysis. While 31 of these plants showed heterozygosity for one translocation leading to the formation of a multiple of four chromosomes, one plant had a configuration of six chromosomes. A $\odot^4$ was generally observed in these plants (Figs. 2, 3). Sometimes the translocated chromosomes formed chains which were either long rods, or J- or U-shaped configurations (Figs. 4, 5). Seeds from these plants with translocations were exposed to 20 kR $\gamma$-rays, because this dose gave better survival of plants.

### *4. $M_2$ and $M_3$ Populations*

In $M_2$ only 15 plants could be cytologically analysed, but seeds from 150 morphologically aberrant plants were collected for treatment with $\gamma$-rays and for raising the $M_3$ population. Of the 15 $M_2$ plants analysed, 12 had interchange configurations, of four chromosomes and three had interchange multiples of six chromosomes. The frequency of translocation heterozygotes in the $M_3$ population is summarised in Table 1.

Table 1. *The frequency of incidence of interchange hetero zygotes in $M_3$ population at early diakinesis-metaphase 1*

| Type of multiple configuration | Number of interchanges involved | Number of plants scored | Pollen fertility per cent |
|---|---|---|---|
| $1\ C^4 + {}^5II$ | 1 | 18 | 76.5 |
| $1\ \odot^4 + {}^5II$ | 1 | 25 | 72.4 |
| $2\ C^4 + {}^3II$ | 2 | 3 | 56.5 |
| $2\ \odot^4 + {}^3II$ | 2 | 6 | 59.2 |
| $1\ \odot^4 + I\ C^4 + {}^3II$ | 2 | 4 | 54.8 |
| $1\ \odot^6 + {}^4II$ | 2 | 12 | 55.0 |
| $1\ C^6 + {}^4II$ | 2 | 3 | 59.5 |
| $1\ \odot^6 + 1\ \odot^4 + {}^2II$ | 3 | 3 | 42.5 |
| $1\ C^6 + 1\ \odot^4 + {}^2II$ | 3 | 2 | 35.6 |
| $1\ \odot^8 + {}^3II$ | 3 | 2 | 37.5 |

Note 1: C = Chain $\odot$ = Ring

Note 2: In this table even somewhat incomplete rings (which might have been broken by pressure during squashing) have been classified as rings: this is particularly true for the complex rings. 8-shaped figures have also been classified as rings.

As seen in Table 1, plants heterozygous for 1 to 3 interchanges formed various types of chromosome configuration. Rings, or chains or combinations of both rings and chains were noticed. Figure 5, for example, shows a ring multiple of four chromosomes and a chain of four chromosomes also involving the nucleolar chromosomes. Similarly, figure 8 shows a chain of six chromosomes and a ring of four chromosomes. When the nucleolar chromosome was involved in an interchange, the resulting multiple was generally a chain (Figs. 5, 6).

### *5. Complex Heterozygotes in $M_4$, $M_5$, and $M_6$ Populations*

Meiotic screening of the $M_4$ population revealed one complex heterozygote having a ring of 10 chromosomes also involving the nucleolar chromosomes (Fig. 9). In addition to this interchange heterozygote, others having shorter rings and chains (listed in Table 1) were also observed. No plant with a configuration of more than 10 chromosomes (i.e. heterozygous for four interchanges) was found in the $M_4$. Attempts to induce more complex interchange multiples in the $M_5$ and $M_6$ generations did not meet with success. However, an incomplete ring of 12 chromosomes was noticed in one meiocyte of an $M_7$ plant.

Some meiocytes having an almost completely disintegrated chromatin mass were also observed in the $M_4$ and $M_5$ populations. Figure 10, for example, shows a PMC with two bivalents intact and the remaining ones, including the nucleolar bivalent, disintegrated into a complex chromatin mass. Such cells mostly occurred in the complex heterozygotes with interchange multiples of six to ten chromosomes.

### *6. Inter-Crossing and Tentative Classification of Translocation Stocks*

The translocation stocks isolated in this study were tentatively classified into 31 classes and initially designated as tr-1 to tr-31, all heterozygous for one interchange. Of these, only 16 could be used effectively for inter-crossing. When the two stocks carried an interchange for the same chromosome, homozygotes with bivalents only were recovered; when they had a chromosome in common, interchange multiples of six chromosomes (Fig. 6 and 7) were obtained; when the two interchange stocks involved different chromosomes, heterozygotes with two interchange multiples of 4 chromosomes (Fig. 5) were obtained. Thus, the initial 31 translocation stocks were later classified as follows:

Table 2. *Tentative classification of translocation heterozygotes*

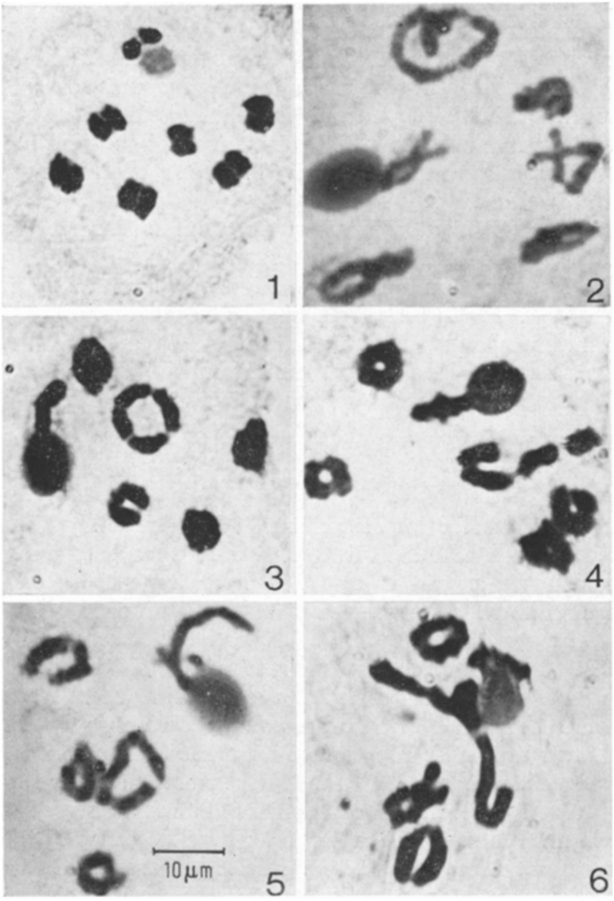
| Class No. | Translocation stocks | Chromosomes involved in interchange | Classified as |
|---|---|---|---|
| 1 | tr-1, tr-7, tr-11, tr-17, tr-19 | 1 and 2 | Tr-1 : 2 |
| 2 | tr-6, tr-27, tr-29 | 1 and 6 | Tr-1 : 6 |
| 3 | tr-9, tr-30 | 1 or 2 and 7 | Tr-1 or 2: 7 |
| 4 | tr-14, tr-15, tr-18 | 3 and 4 or 5 | Tr-3 : 4 or 5 |
| 5 | tr-22, tr-25 | 3 or 4 and 7 | Tr-3 or 4 : 7 |

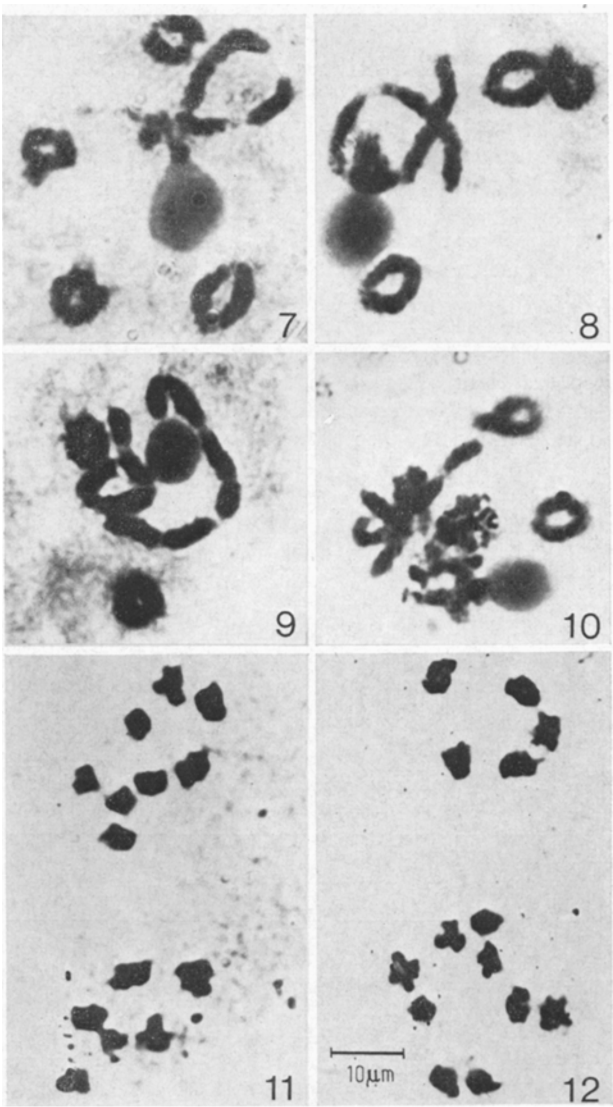
Classes 1, 3, and 5 were almost confirmed because they were cytologically identifiable.

### 7. *Anaphase Disjunction and Pollen Fertility*

Anaphase I and later stages were accompanied by various types of irregularity. Anaphase I and telophase I showed 8:6 distribution (Fig. 11) in several cases, 9:5 (Fig. 12) and, rarely, 10:4 distribution of chromosomes to the poles. Remarkably, in some complex heterozygotes chromosome distribution was 7:7, possibly because of the disjunctional orientation of interchange multiples at metaphase I.

Pollen sterility increased roughly with the increase in number and complexity of interchange configuration (Table 1). There was considerable variation in pollen size, presumably because of varying chromatin content. Such apparently imbalanced gametes appear to be functional in some cases (Jauhar, 1970) and are being taken advantage of in the production of





Figs. 1—12. Meiosis in control plants and of interchange heterozygotes of pearl millet

Fig. 1. Diakinesis in a control plant showing $7_{\text{II}}$; all bivalents are loosely associated having their chiasmata completely terminalized; the smallest bivalent, a rod, is associated with the nucleolus

Figs. 2—12 show meiosis in interchange heterozygotes

Fig. 2. Early diakinesis showing a ring of four chromosomes and $5_{\text{II}}$

Fig. 3. Diakinesis with $1 \odot^4 + 5_{\text{II}}$

Fig. 4. Diakinesis with 1 chain of 4 chromosomes (J-shaped configuration) $+ 5_{\text{II}}$

Fig. 5. Diakinesis in a plant heterozygous for two interchanges showing $1\,\text{C}^4 + 1 \odot^4 + 3_{\text{II}}$

Figs. 6 and 7. PMC's of a hybrid between two translocation heterozygotes having one translocated chromosome in common. Note one interchange configuration of 6 chromosomes and 4 bivalents; the nucleolar bivalent is also involved in interchanges

Fig. 8. Diakinesis in a multiple heterozygote showing a $\text{C}^8 + \odot^4 + 2_{\text{II}}$

Fig. 9. A PMC of a complex interchange heterozygote showing only $2_{\text{II}}$ with the rest of chromosomes including the nucleolar ones involved in a multiple interchange of 10 chromosomes (partly broken)

Fig. 10. Diakinesis with only two bivalents intact and the rest involved in a sort of disintegrated, entangled mass

Figs. 11 and 12. Anaphase 1 showing unequal disjunction of 8:6 and 9:5 chromosomes in translocation heterozygotes aneuploids, including trisomics and monosomics. Anaphase disjunction of chromosomes in some randomly chosen plants is given below.

Table 3. *Frequency of anaphase distribution of chromosomes*

| Plant No. | PMC's with distribution of chromosomes at anaphase I | | | |
|---|---|---|---|---|
| | 7:7 | 8:6 | 9:5 | 10:4 |
| 1 | 11 | 21 | 8 | 2 |
| 2 | 15 | 16 | 4 | 0 |
| 3 | 10 | 19 | 3 | 0 |
| 4 | 32 | 28 | 14 | 1 |
| 5 | 12 | 12 | 2 | 0 |

## Discussion

### *1. Building up a Complete Interchange Ring*

Among the large number of chromosome aberrations induced by ionizing radiations, translocations or interchanges are by far the most common. Complete ring formation involving all the 14 chromosomes has been achieved, through X-irradiation, in diploid wheats (Yamashita, 1951), *Tradescantia paludosa* (Watanabe, 1962) and barley (Sisodia and Shebeski, 1964), among others. Another effective means of obtaining large interchange multiples is to inter-cross different stocks, every time selecting plants with larger configurations for further inter-crossing (Darlington and Gairdner, 1938; Rana, 1965; Schertz, 1970). In the present study recurrent irradiation coupled with inter-crossing of translocation stocks was undertaken to produce higher interchange multiples. Results of meiotic analysis up to the $M_4$ generation were quite encouraging because a complex interchange involving 10 of the 14 chromosomes was obtained. Complex interchanges involving more than 10 chromosomes were not recovered in the $M_5$ or even in the $M_6$ generations. In the $M_7$, however, one plant having a complex interchange of 12 chromosomes was scored. Interchanges involving more than 12 chromosomes could not be obtained even by further cycles of irradiation coupled with inter-crossing of heterozygous stocks. This could be because of the limitations imposed on viability. The somatic and gametic sieves seem to operate more rigorously when more chromosomes are involved in interchanges. Another possibility is that sometimes instead of inducing larger interchanges, subsequent irradiations may break down new ones, as in barley (Chang, 1959; Wang, 1959) where this could result from differential sensitivity of certain parts of the chromosomes to irradiation (Wang, 1959).

Since the nucleolar bivalent is the shortest in the complement of pearl millet, it is not surprising that more chains are formed when the nucleolar chromosomes are involved in translocations (Figs. 5 & 6). This may be because a chiasma is formed in the non-nucleolar arm only and the presence of the nucleolus interferes with chiasma formation in the other arm.

### *2. Identification of Translocated Chromosomes*

Once different translocation stocks were meiotically established, they were crossed in different combinations with a view to identifying the translocated chromosomes. When two interchange stocks (classified tentatively as tr-1 to tr-31) were crossed, study of diakinesis or metaphase I in the $F_1$ revealed whether the translocated chromosomes of the parental stocks were identical or different; configurations of $3_{II} + 2_{IV}$ (Fig. 5) indicated that the translocated chromosomes of the parents were different, while $4_{II} + 1_{VI}$ (Figs. 6, 7) showed that the parental stocks had one chromosome in common. In this fashion a tentative classification of the translocation stocks was made (Table 2). This method of chromosome recognition was preferred to that based on karyotypic analysis because each of the somatic chromosomes is not easily distinguishable. These translocation stocks, when fully recognized, may facilitate the establishment of linkage groups in this important crop plant.

### *3. Building up of Aneuploids and Possible Duplications*

In an interchange heterozygote having a configuration of four chromosomes, a 3:1 disjunction may occasionally produce an $n + 1$ gamete which, on fusion with a normal gamete, may give rise to a trisomic plant (Hagberg, 1954; Ramage, 1960). In this way, at anaphase I of interchange heterozygotes having a multiple of four chromosomes, 8:6 disjunction was obtained (Fig. 11); similarly, in some plants heterozygous for two interchanges, 3:1 disjunction of both configurations occasionally gave rise to a 9:5 distribution of chromosomes (Fig. 12). If the imbalanced gametes are functional in pearl millet (see Jauhar, 1970), it may be possible to build up an array of aneuploids, including trisomics and possibly monosomics, which might be helpful in the genetic study of this commercial crop plant. Some interchange trisomics have been isolated and are under study. It may be possible also to isolate certain plants with duplications of desired segments of chromosomes, as has been attempted in maize (Gopinath and Burnham, 1956) and barley (Hagberg, Persson and Wiberg, 1963).

### *4. Karyomorphology and the Incidence of Interchanges*

Complex hybridity is a regular feature of certain groups of plants, such as the Euoenotheras of North America, and it is now clear that the chromosomal organization has important functional and evolutionary implications. It has been inferred that the response of a species to radiation-induced chromosomal interchanges is conditioned by certain chromosomal attributes (James, 1965; Rana, 1965). The karyomorphology of pearl millet was critically examined to find certain features which might be conducive to the induction of translocations. The chromosomes of pearl millet are mostly isobrachial with median or sub-median centromeres. Chiasma frequency is 1.84 ± 0.085 per bivalent so that most of the chromosomes associate predominantly as ring bivalents (Fig. 1). The chiasmata are mostly terminally located, and at diakinesis their terminalization is complete so that the bivalents look somewhat loose and desynapsed (Fig. 1). The favourable response of pearl millet to initial induction of chromosome interchanges appears to be, at least partly, a function of its symmetrical karyotype, as has been indicated in *Oenothera* (Cleland, 1962), *Isotoma petraea* (James, 1965) and *Chrysanthemum carinatum* (Rana, 1965).

The natural occurrence of such translocations in several populations of pearl millet (Powell and Burton, 1969) substantiates this view. Chromosomal heterozygosity is a characteristic and adaptive feature of many natural populations. Whether any adaptive advantage is associated with the naturally occurring interchange heterozygosity in pearl millet is not yet known. However, the fact that some interchanges tend to be maintained in the natural populations of *Pennisetum typhoides* suggests that they have adaptive value and may have breeding implications.

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