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Multiple Chromosomal Interchanges in Pearl Millet

D. S. Brar and J. L. Minocha
Department of Genetics, Punjab Agricultural University, Ludhiana (India)

Summary. Intercrossing and irradiation were successfully used in pearl millet (*Pennisetum typhoides*) to develop multiple interchanges involving up to the total complement of all the chromosomes in one complex. In interchange heterozygotes showing ⊙ 12 + 1 II and ⊙ 14, 90.9 and 87.8 per cent of the cells, respectively, had chromosome configurations other than that of 12 and 14 chromosomes. In general, the frequency of such cells resulting from breakdown of the expected complex configuration increased with the increase in the number of translocated chromosomes in the complex. The higher the number of chromosomes involved in the interchange ring, the higher were the pollen and ovule sterility. The results indicated that meiotic instability, deficiency-duplication gametes, and unequal distribution of chromosomes account for increased sterility of multiple interchange heterozygotes. Even though interchanges in pearl millet predominantly show the alternate type of segregation, sterility seems to be the major barrier for the exploitation of the multiple interchange method for gamete selection and the establishment of homozygous lines in this plant species.

Key words: *Pennisetum typhoides* – Chromosomal interchanges – Meiosis – Sterility

Introduction

Among the different cytogenetic techniques, chromosomal interchanges are important in establishing linkage groups and correlating cytological and genetical results (Burnham 1962). In diploid plant species, these interchanges form an important source of aneuploids (Khush 1973; Minocha et al. 1979). Multiple interchanges involving all the chromosomes of the haploid complement in one or two complexes are useful in such different plant improvement programmes as i) gamete selection and production of homozygous lines, ii) diploidization of autotetraploids, and iii) production of seedless fruits. Although Burnham in 1946 proposed the "Oenothera" or multiple interchange method for gametic selection and production of homozygous lines in plants where the chromosome number is relatively low and the interchanges show increased alternate segregation, the method could not be exploited successfully on account of the difficulty in producing such stocks and because of their increased sterility. Pearl millet, *Pennisetum typhoides* (Burm.) S. & H. is a suitable material to test this technique on account of its low chromosome number ($2n=14$) and the alternate type of segregation of the interchange complex (Singh and Tyagi 1973; Sidhu et al. 1977). In this laboratory, several interchanges involving up to all the chromosomes in one complex have been produced in pearl millet (Brar et al. 1973). This communication reports on the meiotic analysis of multiple interchange heterozygotes with a view to ascertaining the probable causes of sterility.

Materials and Methods

The material consisted of 15 interchange stocks in BIL-4, an inbred of pearl millet. In the first experiment, these interchange stocks were inter-crossed. The meiotic configurations in the F_1's were studied and those plants showing larger rings or new chromosome combinations were further crossed so as to involve additional chromosomes in the interchange complex. In the second experiment, seeds from the plants possessing larger rings were irradiated with gamma rays (20 kR) so as to synthesize interchanges involving all the chromosomes of the haploid complement in one or two complexes.

Newly synthesized multiple interchange stocks were analysed meiotically. Data on the chromosomal associations at diakinesis/metaphase I and distribution at anaphase I were recorded. Pollen sterility was determined by staining the pollen grains in acetocarmine. Pollen and seed fertility were recorded in interchange heterozygotes involving different number of chromosomes in the interchanges.

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Table 1. Synthesis of multiple interchanges through intercross and recurrent irradiation

Group	Parental cross	F_1 configuration	Nature of the translocated chromosomes
A	*Intercross Method*		
I	RT-1 × RT-3, -7, -16, -20 RT-2 × RT-3, -15, -16 RT-3 × RT-2/3, -9, -2 RT-7 × RT-3 RT-23 × RT-27 RT-26 × RT-16, -17, -23, -27	2 ⊙ 4 + 3II	Translocated chromosomes are different in the parental interchanges
II	RT-2 × RT-27 RT-9 × RT-1, -8, -14 RT-14 × RT-26 RT-15 × RT-19 RT-24 × RT-17, -26	⊙ 6 + 4II	One of the two translocated chromosomes is common
III	(RT-15 × RT-19) × RT-4 (RT-4 × RT-9) × RT-2	⊙ 8 + 3II ⊙ 10 + 2II	One or two of the translocated chromosomes are common

B	*Irradiation Method* Interchange stock	Meiotic configuration in the 1st cycle	2nd cycle
	⊙ 4 + 5II	2 ⊙ 4 + 3II	⊙ 14
	2 ⊙ 4 + 3II	⊙ 8 + 3II	⊙ 12 + 1II
	⊙ 6 + ⊙ 4 + 2II	⊙ 10 + 2II	–

Results

1 Synthesis of Multiple Interchanges

The results of the two methods (intercrossing and recurrent irradiation) followed to synthesize multiple interchanges are presented in Table 1. New chromosome combinations (2 ⊙ 4 + 3 II; ⊙ 6 + 4 II) were observed in inter-crosses of stocks heterozygous for a single interchange. Interchange heterozygotes with larger rings, ⊙ 10 + 2 II and ⊙ 8 + 3 II, for example, were obtained through the intercrossing. An interchange heterozygote showing a ring of 10 chromosomes was obtained in the cross of (RT-4 × –9) × RT-2. Similarly, a new chromosome combination (⊙ 6 + ⊙ 4 + 2 II) resulted in the cross of (RT-1 × –20) × (RT-15 × –19). Thus, through intercrossing interchanges involving up to 10 chromosomes, one or two separate rings were obtained.

In the second experiment, irradiation of interchange heterozygotes possessing ⊙ 4 + 5 II, 2 ⊙ 4 + 3 II, and ⊙ 6 + ⊙ 4 + 2 II resulted in the induction of new and larger chromosome rings. In the first cycle of irradiation, plants with ⊙ 10 + 2 II and ⊙ 8 + 3 II were produced. Seed irradiation of interchange heterozygotes possessing 2 ⊙ 4 + 3 II in the second cycle of irradiation resulted in both complex interchanges (⊙ 12 + 1 II) and those involving all the chromosomes of the complement in a single complex (⊙ 14).

2 Meiotic Behaviour

Observations on chromosomal associations at diakinesis/metaphase I and their distribution at anaphase I and pollen sterility were made on several multiple interchange heterozygotes obtained through intercrossing and recurrent irradiation (Table 2).

The interchange heterozygote (⊙ 6 + 4 II) showed an association of 6 chromosomes in 80.9 per cent cells. At AI, 31.4 per cent of the cells had an unequal distribution of chromosomes (other than 7-7). Interchange heterozygotes with 2 ⊙ 4 + 3 II had two configurations of four chromosomes in 86.3 per cent of the cells. Interchange heterozygotes with configurations of 8 and 10 chromosomes had unequal distribution of chromosomes in 45.7 and 46.3 per cent of the cells, respectively. Complex interchange heterozygotes (⊙ 12 + 1 II, ⊙ 14) had the expected maximum chromosome configurations in only 9.1 and 12.2 per cent of the cells. These interchange heterozygotes had as many as 52.9 per cent of the cells with unequal distribution of chromosomes. The multiple interchange heterozygotes were found to be meiotically

Table 2. Meiotic behaviour and pollen sterility of multiple interchanges in pearl millet

Multiple interchange stock	Diakinesis/metaphase I: No. of cells	Diakinesis/metaphase I: % cells showing the expected chromosome configuration	Anaphase I: No. of cells	Anaphase I: % cells showing unequal chromosome distribution	Pollen sterility %
⊙ 6 + 4II	209	80.9	102	31.4	53.9
2 ⊙ 4 + 3II	241	86.3	161	28.6	56.2
⊙ 8 + 3II	143	83.9	94	45.7	63.3
⊙ 10 + 2II	96	78.1	41	46.3	87.8
⊙ 12 + 1II	65	9.1	96	52.1	86.2
⊙ 14	49	12.2	54	52.9	98.0

unstable showing variable number of lower chromosome combinations. Pollen sterility increased with the increase in size of the interchange ring. Interchange heterozygotes with ⊙ 10, ⊙ 12, ⊙ 14 had 87.8, 86.2 and 98.0 per cent pollen sterility respectively. Besides increased pollen sterility these heterozygotes were highly seed sterile. No seed was obtained from plants with multiple interchanges involving either 12 or all of the 14 chromosomes of pearl millet.

Discussion

The occurrence of interchanges involving all the chromosomes of a haploid complement in one or two complexes has been reported in natural populations of different plant species. These interchanges have also been induced artificially (Burnham 1962). Burnham's proposal of 1946 initiated interest among other workers for gamete selection and production of homozygous lines using multiple interchanges. Since then, attempts have been made in many laboratories to produce such stocks (Yamashita 1951 in *Triticum monococcum;* Watanabe 1962, 1973 in *Tradescantia paludosa;* Burnham 1962 in corn; Sisodia and Shebeski 1965 in barley; Gottschalk and Milutinovic 1970 in peas; Brar et al. 1973 and Tyagi and Singh 1974 in pearl millet).

The results of the first experiment clearly demonstrate pearl millet to be a suitable material for the synthesis of multiple interchange stocks. The combination of intercrossing and recurrent irradiation was found to be highly effective in building up larger rings. The intercross method revealed that the interchanged chromosomes of two stocks could be combined in one complex resulting in larger rings. Synthesis of multiple interchanges (⊙ 8) through intercrossing, showed that crossing-over occurs in differential segments and the crossover products are recovered in the progeny. Recurrent irradiation resulted in the induction of many new interchanges. As a result of irradiation of seeds from plants possessing 2 ⊙ 4 + 3 II in the second cycle, plants with ⊙ 12 + 1 II and one involving all the chromosomes in a single complex (⊙ 14) were obtained.

The meiotic analysis of the complex interchange heterozygotes in pearl millet showed that they are meiotically unstable. In general, with the increase in size of the ring, the frequency of lower chromosome configurations increased due to breakdown of the interchange rings. In interchange heterozygotes showing ⊙ 12 + 1 II and ⊙ 14, as many as 90.9 and 87.8 per cent of the cells, respectively, had chromosome configurations other than the expected maximum configuration. The frequency of cells with unequal distribution of chromosomes was also high in the larger rings reported here (up to 52.9%) compared to an average of 13.8 per cent of the stocks heterozygous for a single interchange (Brar 1973). Data on pollen sterility indicated that the sterility is associated with the size of the ring; increased size of the ring is followed by increased sterility. Both pollen and ovule sterility increased in complex interchange heterozygotes (⊙ 12 + 1 II, ⊙ 14).

The multiple interchange method of establishing homozygous lines can be used only if methods are found to improve the fertility of complex interchange heterozygotes. One way would be to develop complex interchanges from intercross of only those interchanges of which the heterozygotes have high fertility. Variation in pollen sterility (26–43%) has been recorded in different interchange heterozygotes of pearl millet (Brar 1973).

The results reported here demonstrate that intercrossing and irradiation used singly or in combination can lead to the development of multiple interchanges in crop plants. In pearl millet, the crossover products in the differential segments are easily recovered after intercrossing. This seems to be promising for the synthesis of multiple interchanges. The meiotic behaviour indicated that the interchange heterozygotes with larger rings are meiotically unstable, resulting in increased frequency of lower chromosome combinations (90.9% cells). These lower chromosome combinations account for increased sterility resulting from deficiency-duplication gametes. In such situations, random orientation of lower chromosome configurations could account for sterility even if there is alternate segregation. Burnham's suggestion that this technique is suitable for species with low chromosome numbers and showing the alternate type of segregation needs further consideration of another factor: intact ring formation at metaphase I. The utilization of such stocks in pearl millet for establishing homozygous lines is limited on account of increased sterility. Increased sterility as reported here may be due to (i) high frequency of lower chromosome combinations, (ii) unequal distribution of chromosomes and (iii) deficiency-duplication gametes resulting from adjacent segregation. Even though alternate segregation is predominant in barley, *Datura* and pearl millet, the multiple interchange method could not be successfully used for gamete selection because of sterility. Keeping in mind the studies on multiple interchanges in different crops, its use in gamete selection remains to be a difficult proposition.

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Dr. D.S. Brar
Department of Plant Breeding
Punjab Agricultural University
Ludhiana (India)

Dr. J.L. Minocha
Department of Genetics
Punjab Agricultural University
Ludhiana (India)