Cytomorphological Behaviour and Origin of Aneuploids in Tetraploid Pearl Millet (*Pennisetum americanum* (L.) K. Schum)

R. Sai Kumar, U. P. Singh, R. B. Singh¹ and R. M. Singh

Department of Genetics and Plant Breeding,
Institute of Agricultural Sciences,
Banaras Hindu University,
Varanasi 221 005, India

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Aneuploids have been isolated and identified in several induced tetraploid crops as in rye (Hagberg and Ellerstrom 1959, Aastveit 1963), barley and sugarbeet (Rommel 1963) and ryegrass (Ahloowalia 1966, 1971) and red clover (Ellerstrom and Sjodin 1966).

Pearl millet (*Pennisetum americanum*) is a natural diploid ($2n=14$) species. Although a number of workers have induced tetraploid of pearl millet (Hagberg and Ellerstrom 1959, Jauhar 1970), the detailed cytological study of aneuploids observed in tetraploid progenies has not been reported so far.

During the course of tetraploid pearl millet breeding, some aneuploids were found in C_2 generation of polyploids. This report presents data on type, meiotic behaviour and fertility of three aneuploids and discusses about their possible origin in the light of the meiotic behaviour.

Materials and methods

Some aneuploids were found in the C_2 families of tetraploids. Each family of C_2 descended from a single C_1 tetraploid plant. One hundred such families were grown in a field. The number of plants in each family varied from 10 to 20. The parental C_0 tetraploid of these C_2 families had been obtained by doubling the chromosomes of one inbred (H8) of pearl millet. The C_2 generation was obtained by interpollination and selfing of selected fertile tetraploids of C_1 generation and bulk-seed was sown in the field.

The selected aneuploid plants were analysed in PMCs for their chromosome association and distribution in meiosis. Important meiotic configurations were photographed.

Results

Three chromosome variants viz., 27-chromosome type (hypotetraploid) and

¹ Present address: Dr. R. B. Singh, Regional IBPGR Officer, FAO, Phra Atit Road, Bangkok, Thailand.

29- and 30-chromosome types (hypertetraploid) were isolated in a population of C_2 generation consisting of more than one thousand tetraploid plants of pearl millet. Besides a high degree of pollen and ovule sterility noted in the variants, morphologically they were considerably reduced in plant height, number of tillers, leaf length, panicle length and width compared to their parental tetraploids.

Cytological observations

A detailed cytological behaviour of these chromosome variants are shown in Tables 1 and 2.

27-chromosome variant (hypotetraploid)

The frequency of cells with varying chromosome configurations at diakinesis stage are given in Table 1 (Figs. 1-9). In this case the mean number of univalents, bivalents, trivalents, quadrivalents and pentavalent/cell at diakinesis stage was 7.15, 6.21, 1.27, 0.86 and 0.03, respectively. This variant showed a higher frequency of PMC's either with $10^I + 7^{II} + 1^{III}$ /cell or with $11^I + 4^{II} + 2^{IV}$ followed by $12^I + 6^{II} + 1^{III}$ /cell. The univalent/cell varied from 0 to 12 while the number of bivalents and trivalents/cell ranged from 4 to 10 and 1 to 4 respectively. As compared to other configurations, the frequency of quadrivalents/cell was considerably low which ranged between 1 to 3 per cell. Since this variant being a hypotetraploid in nature is supposed to be a tetrasomic for 6 chromosomes and trisomic for one chromosome in its genome. Furthermore, as a rule, this should form an association of $6^{IV} + 1^{III}$ in most of the cells. But a high frequency of univalents and bivalents/cell at the expense of low frequency of quadrivalents and trivalents/cell in this variant may be on account of frequent breakdown of quadrivalent and trivalent forming chromosomes into univalents and bivalents in most of the cells. The parental tetraploid of this variant was typified by the frequent distribution of 13-15 and 14-14 at AI. The occurrence of such gametes ($n=13$ and 14) upon fertilization may produce such aneuploids in the progeny generation. As compared to others, the chiasma frequency per cell in this case was minimum (15.13 ± 0.65 /cell) and which ranged from 11 to 20/cell. This variant was unusually associated with frequent splitting of univalent laggards [(3 to 8) (Figs. 6 to 8)] alongwith unequal separation of chromosomes at AI. Approximately 36% of PMC's were observed with 10-8-9 distributions (Fig. 7) followed by 13-14 [(24%) (Fig. 9)] 12-4-11 [(20%) (Fig. 8)] and 13-3-11 (20%) distributions in descending order of magnitude.

29- and 30-chromosome variants (hypertetraploids)

The Figs. 10-18 and Figs. 19-27 depict the chromosome association and distribution in 29- and 30-chromosome variants respectively. In case of 29-chromosome variant the average frequency of univalents, bivalent, trivalent and quadrivalent/cell was 1.17, 8.83, 0.83 and 1.92 respectively. Similarly, the average frequency of univalent, bivalent, trivalent, quadrivalent, pentavalent and hexavalent/cell in 30-chromosome variant was 2.98, 7.37, 1.48, 0.82, 0.54 and 0.30 respectively (Table 1). Thus, the maximum percentage of chromosomes have formed bivalents in both cases.

Figs. 1-9. Variation in chromosome configurations at diakinesis, metaphase I and different chromosomes distribution at anaphase I in 27-chromosomes variant. 1, diakinesis, $3^{IV} + 1^{III} + 5^{II} + 2^I$. 2, diakinesis, $1^{IV} + 3^{III} + 5^{II} + 4^I$. 3, diakinesis, $1^V + 1^{IV} + 2^{III} + 5^{II} + 2^I$. 4, metaphase I, $7^{II} + 13^I$. 5, metaphase I, $9^{II} + 9^I$. 6, anaphase I, 11-3 (splitting) + 1^{II} (late disjunction)-11. 7, anaphase I, 10-8 (splitting)-9. 8, anaphase I, 12-4 (splitting)-11. 9, anaphase I, 14-13.

Figs. 10-18. Variation in chromosome configurations at diakinesis, metaphase I and different chromosomes distribution at anaphase I in 29-chromosomes variant. 10, diakinesis, $2^{IV} + 2^{III} + 7^{II} + 1^I$. 11, diakinesis, $3^{IV} + 8^{II} + 1^I$. 12, diakinesis, $3^{IV} + 1^{III} + 6^{II} + 2^I$. 13, metaphase I, $1^{III} + 10^{II} + 6^I$. 14, metaphase I, $1^{IV} + 10^{II} + 5^I$. 15, metaphase I, $1^{IV} + 2^{III} + 7^{II} + 5^I$. 16, anaphase I, 16-13. 17, anaphase I, 12-3 (1 bivalent late disjunction + 1^I)-14. 18, anaphase I, 15-14.

In case of 30-chromosome type, the frequency of pentavalent association was relatively higher as compared to those of other variants. But a rare occurrence of one hexavalent in certain cells (Fig. 19) also indicates the hexasomic nature of one chromosome as two pentavalents were never observed in a single cell.

Figs. 19-27. Variation in chromosome configurations at diakinesis, metaphase I and different chromosomes distribution at anaphase I in 30-chromosome variant. 19, diakinesis, $1^{VI} + 1^{IV} + 4^{III} + 3^{II} + 2^I$. 20, diakinesis, $1^{IV} + 3^{III} + 7^{II} + 3^I$. 21, diakinesis, $1^V + 2^{IV} + 1^{III} + 6^{II} + 2^I$. 22, metaphase I, $1^{IV} + 10^{II} + 6^I$. 23, metaphase I, $1^{IV} + 1^{III} + 8^{II} + 7^I$. 24, metaphase I, $1^{IV} + 3^{III} + 5^{II} + 7^I$. 25, anaphase I, 15-2-13. 26, anaphase I, 15-15. 27, anaphase I, 14-3-13.

A higher frequency of bivalents in both cases also increases the number of chiasmata per cell as mean number of chiasmata in 29- and 30-chromosome variants was 20.80 ± 0.45 and 18.46 ± 0.44 respectively. The parental tetraploid (C_1) of 29-chromosome variant also showed the maximum distribution of 14-14 followed by 13-15 at AI. But tetraploid, producing 30-chromosome type on selfing, was noticed with maximum disjunction of 14-14 followed by 12-16 at AI. Thus, these hypertetraploids were originated due to high percentage of foresaid distribution of chromosomes noticed in their corresponding tetraploid parents.

At AI 13-16 distribution was maximum [(44%) (Fig. 16)] followed by 13-1-15 [(40%) (Fig. 17)] and 14-15 (16%) in the 29-chromosome variant while 30-chromosome type may be seen with varying kinds of unequal disjunction at AI (Table 2). The maximum frequency of cells (44%) showed equal distribution (15-15) (Fig. 26) followed by some unequal distributions like 16-14 (24%) and 17-13 (10%). But other distributions like 15-1-14, 15-2-13 (Fig. 25) and 14-3-13 (Fig. 27) were relatively less frequent.

Fertility

The pattern of AI distribution has a strong bearing on the fertility of pollen and ovules in different variants. Mostly irregular distributions and frequent precocious splitting of univalent laggards in the hypotetraploid has led to the high inviability of gametes. Likewise, in case of 30-chromosome variant, often occurrence of aneuploid gamete may result into the complete sterility of both pollen and ovules. However, in 29-chromosome type, gametes with $n=14, 15$ were produced in considerable amount which probably led to some fertility of ovules and seed set rarely.

Discussion

The occurrence of aneuploids in the progenies of induced tetraploids of pearl millet is a parallel example to that reported in induced tetraploids of rye (Hagberg and Ellerstrom 1959), barley and sugarbeet (Rommel 1963) and ryegrass (Ahloowalia 1966, 1971). Although tetraploids in *Pennisetum* have been induced and isolated with colchicine treatment by other workers (Jauhar 1970, Nameeta 1973), the detection of aneuploids in C_2 generation has not been documented so far. It is likely that aneuploids frequently occur in this grass and they escaped detection. The presence of aneuploid zygotes with 27-, 29- and 30-chromosome confirms the functioning and viability of aneuploid gametes. Such gametes may have their origin from chromosome segregation of 13:15 and 12:16 observed in the parental tetraploids of C_1 generation. On this basis, at least three different models could be proposed to account for the origin of aneuploid zygotes: 1. That the gametes with $n=12, 13, 14, 15, 16$ combined in all possible ways to produce the observed zygotes as listed. 2. The gametes with $n=13, 14$ and 15 were the only ones which combined to produce the observed zygotes, and those with $n=12$ and 16 either did not function or were eliminated in competition, since zygotes with $2n=24, 25, 31$ and 32 resulting from $12 \times 12, 12 \times 13, 15 \times 16$ and 16×16 could not be detected in C_2 generation. 3. That aneuploid gametes functioned only through one side (male or female) and the reciprocal

gametes were always the normal ($n=14$) types, thus no reconstituted 28-chromosome types (13×15 and 12×16) would be expected. In this model, since the transmission of normal gametes will equal to one, the aneuploid zygote frequency would represent the transmission of aneuploid gametes.

The first two hypotheses do not seem to be correct one, since the observed number of eu-tetraploids was for greater than expected. Further, the last of three hypotheses seem to fit the observed meiotic behaviour of 27- and 29-chromosome aneuploids. All the observed MI configurations in the aneuploids agreed with the model that the aneuploid zygotes originated from aneuploid $\times$ normal matings (13×14 and 15×14) rather than from mating of two aneuploid gametes (12×15 and 13×16). Perhaps most of the aneuploid gametes were transmitted through female side. This case of aneuploid production in pearl millet is parallel to that of tetraploid ryegrass (Ahloowalia 1971). The origin of specific aneuploid depends upon the frequent occurrence of specific type of aneuploid gametes found in the corresponding parental tetraploids. For example, 13-15 and 14-14 distributions were mostly frequent in parental tetraploids (C_1) of 27-chromosome variant. Similarly, two parental tetraploids of 29- and 30-chromosome variants were highly associated with 14-14; 13-15 and 12-16, 14-14 distributions respectively. Occasionally, genotypes favour the survival of more than one chromosome imbalance and hence the occurrence of one 30-chromosome variant in the tetraploid progeny may be a case of rare event.

With progressive increase in the chromosome dosage of aneuploids varying from 27- to 30, an associated decrease in the morphology and fertility of these variants could not be observed as the fertility of 29-chromosome type was relatively higher than those of other variants. This observation, therefore, suggests that different chromosomes of the genome were possibly involved in these aneuploids. In both hypertetraploids, the quadrivalent forming chromosomes are mostly broken down into bivalents. Thus, the presence of $14^{II}+1^I$ in a number of PMC's of 29-chromosome variant as well as a high frequency of bivalents observed in other hypertetraploid suggest for "preferential pairing". Moreover, it appears that diploidization in these variants has been induced probably due to accumulation of certain modifiers in the advanced generation of selected tetraploids. A case of "strong" preferential pairing has also been noted in an aneuploid ($2n=25$) of ryegrass (Ahloowalia 1966).

Summary

Aneuploids with 27- to 30-chromosomes were isolated in the progenies of induced tetraploids of pearl millet in a lower frequency in C_2 generation. These aneuploids were apparently recovered through female transmission, which varied for aneuploid gametes with different chromosome numbers. Aneuploids could be distinguished from eu-tetraploids on a morphological basis. Cytological study showed that 27-chromosomes variant was characterized by a high frequency of univalents and their precocious splitting at AI followed by the formation of bivalents in addition to rare presence of trivalents and quadrivalents/cell. But bivalents were highly frequent in both hypertetraploids. Thus, a frequency of bivalent is suggestive of

“preferential pairing”. Moreover, the induced bivalentization noted in both eu- and aneuploids of advanced generation tetraploids of pearl millet may be accounted to high selection pressure.

References

- Aastveit, K. 1963. The relation between fertility and cytological properties in autotetraploid rye. *Genetics Today, Proc., XI Intern. Cong. Genet.* **1**: 214.
- Ahloowalia, B. S. 1966. Chromosome pairing in aneuploid perennial ryegrass. *Heredity* **57**: 129-134.
- 1971. Frequency, origin and survival of aneuploids in tetraploid ryegrass. *Genetica* **42**: 129-138.
- Ellerstrom, S. and Sjodin, J. 1966. Frequency and vitality of aneuploids in a population of tetraploid red clover. *Hereditas* **55**: 166-182.
- Hagberg, A. and Ellerstrom, S. 1959. The competition between diploid, tetraploid and aneuploid rye. *Hereditas* **45**: 369-416.
- Jauhar, P. P. 1970. Chromosome behaviour and fertility of the raw and evolved synthetic tetraploids of pearl millet (*Pennisetum typhoides*) Stapf et Hubb. *Genetica* **41**: 407-424.
- Nameeta, N. 1973. Establishment of aneuploids and their use in pearl millet (*Pennisetum typhoides* (Burm) S. and H.), Improvement. Ph. D. Thesis, Meerut University, Meerut, India.
- Rommel, M. 1963. Aneuploids in some artificially induced polyploids of cultivated plant. *Genetics Today, Proc. XI Intern. Cong. Genet.* **1**: 213-214.
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