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Chromosome Behavior in a Hypertriploid Plant of Ryegrass

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Summary. A hypertriploid ($2n = 22$) was detected in the progeny of a desynaptic diploid ($2n = 14$) plant of perennial ryegrass, *Lolium perenne* L. The hypertriploid did not differ in morphology from its maternal-sib diploids, but showed larger stomata and pollen. The microsporocytes showed a mean chromosome association of 4.3 I + 3.4 II + 3.2 III + 0.3 IV at metaphase I with a mean chiasma number of 14.4 per cell. The 33 types of observed chromosome configurations could be explained by assuming that the plant was trisomic for 6 chromosomes and tetrasomic for one chromosome. Karyotype analysis confirmed the above assumption and revealed tetrasomy of chromosome number VI. However, two chromosome associations, 1 I + 4 II + 3 III + 1 IV and 2 I + 5 II + 2 III – 1 IV suggested the presence of displaced duplications within the genome of ryegrass. The tetrasomic chromosome formed mostly a quadrivalent, which often broke down to form 2 II or 1 I + 1 III and rarely 2 I + 1 II or 4 univalents. Most of the univalents arose from the trisomic chromosomes and divided precociously at anaphase I, producing diads and tetrads with unequal chromosome numbers. The plant was highly sterile and set no seed on controlled crossing.

Introduction

Perennial ryegrass, *Lolium perenne* L. is a diploid (2n = 14) species. The inter-pollination of diploids with induced tetraploids seldom, if ever, produces viable triploids (Griffiths and Pegler, 1964). A rare case of triploidy was described by Myers (1944), who obtained a single triploid plant (2n = 21) by pollinating a tetraploid with diploids. The progeny of this triploid, obtained by pollination with diploids, included a large number of aneuploids such as trisomics, tetrasomics, etc., with chromosome numbers ranging from 2n = 14 to 18. The available information on chromosome behavior in triploid ryegrass is thus limited.

During investigations on desynapsis in diploid and tetraploid clones of ryegrass, a hypertriploid 2n = 22 was detected in the progeny of a desynaptic diploid plant. This paper reports the behavior of chromosomes during meiosis in pollen mother cells of this near-triploid plant.

Materials and Methods

The hypertriploid plant was obtained in the progeny of a diploid desynaptic seed parent which had been open-pollinated with normal diploids and desynaptic tetraploid clones. The hypertriploid was increased vegetatively into 15 clones and grown in a glass house along with its maternal-sib diploids. The somatic chromosome number was determined from root tip squashes stained with aceto-orcein, and meiosis was studied in microsporocytes.

The techniques for studying the somatic and meiotic chromosomes were the same as described earlier (Ahloowalia, 1969a). All photomicrographs were made from freshly prepared slides at an original magnification of ×1000, by using Kodak ortho-metallographic plates.

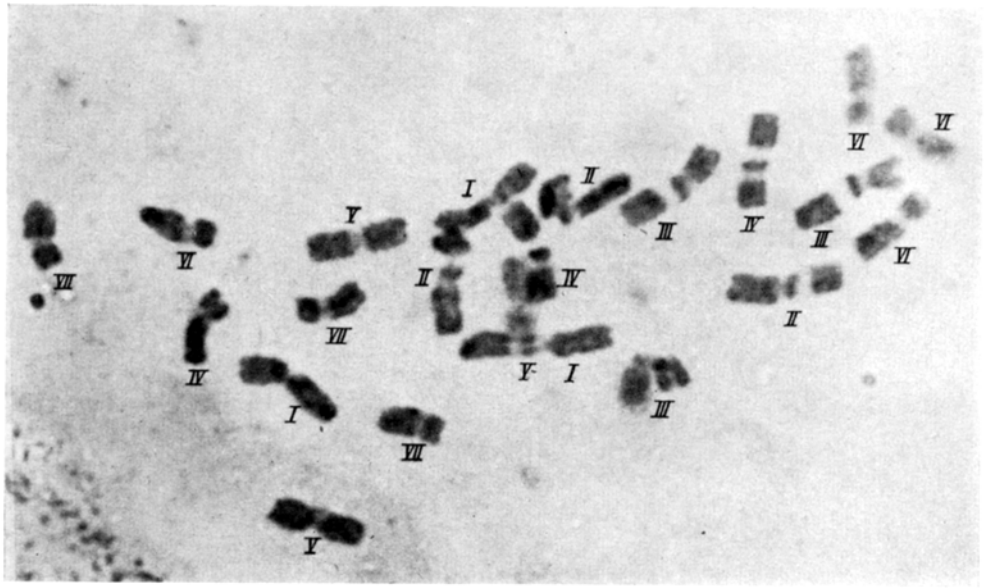

Fig. 1. Karyotype of hypertriploid, note tetrasomy for chromosome No. VI

Results

Morphology: The hypertriploid plant could not be distinguished from diploid maternal-sibs by any visible morphological character such as leaf shape and size, spike morphology, tillering capacity or growth vigor etc. The hypertriploid, however, had longer stomata (52.6 μ) than that of the diploids (42.1 μ).

Karyotype of somatic chromosomes: A study of the idiogram prepared from the root tip squashes showed that the plant under study had 22 chromosomes of which six (I—V and VII) were present 3 times each and one (No. VI) was present four times (Fig. 1). The latter is a sub-median chromosome and is slightly longer than the shortest chromosome (VII) in the genome.

Chromosome association at metaphase I: The microsporocytes at metaphase I showed a high frequency of univalents, ranging from zero to 12 per cell, although it was not possible to find unpaired univalents at pachytene. There was a wide variation in observed chromosome associations (Figs. 2—3) and 33 different types of configurations were found in one hundred cells (Table 1). The maximum and minimum associations observed were 6 III + 1 IV and 12 I + 5 II respectively; the mean association was 4.3 I + 3.4 II + + 3.2 III + 0.3 IV per cell. The observed chromosome configurations could be accounted for by assuming that six chromosomes were present three times each and one was present four times. In two cells, however, chromosome associations of 2 I + 5 II + + 2 III + 1 IV and 1 I + 4 II + 3 III + 1 IV were observed. Each of these associations included one achiasmatic bivalent, suggesting that at least two nonhomologous chromosomes within the genome shared some homology.

Table 1. *Variation of chromosome configurations in microsporocytes at metaphase I*

No. of cells	Chromosome association			
	I	II	III	IV
1	—	—	6	1
3	1	1	5	1
1	3	—	5	1
8	2	2	4	1
6	3	3	3	1
1	5	2	3	1
1*	1	4	3	1
1*	2	5	2	1
2	4	4	2	1
2	6	3	2	1
1	5	5	1	1
3	7	4	1	1
4	—	2	6	—
2	2	1	6	—
3	1	3	5	—
3	3	2	5	—
2	5	1	5	—
8	2	4	4	—
7	4	3	4	—
3	6	2	4	—
5	3	5	3	—
6	5	4	3	—
1	7	3	3	—
2	9	2	3	—
2	11	1	3	—
3	4	6	2	—
2	6	5	2	—
3	8	4	2	—
4	5	7	1	—
3	7	6	1	—
4	9	5	1	—
2	11	4	1	—
1	12	5	—	—

* Unexpected associations, include 1 achiasmatic bivalent each.

Chiasma number at metaphase I: The chiasma number in the hypertriploid ranged from 7 to 23 with a mean of 14.4 ± 3.4 per cell or 0.6 per chromosome. This number was lower than that of diploid maternal-sibs *Ds ds*, with 1 to 0.9 chiasmata per chromosome but nearly the same as of diploid maternal-sibs *ds ds*, with 0.5 to 0.8 chiasmata per chromosome (Table 3). The hypertriploid had a lower number of chiasmata per chromosome than that of the triploid (1.42) reported by Myers (1944).

An increase in number of chiasmata was strongly associated with an increased number of trivalents per cell (Fig. 4). The increase in number of univalents and bivalents reduced the chiasma number (Figs. 5—6).

Segregation at anaphase I and subsequent stages: Various kinds of segregations such as 10:10, 10:9, 9:9, 9:8, 8:12, 8:6 with 2, 3, 4, 5, 2 and 8 univalent laggards were observed. The 11:11 segregations

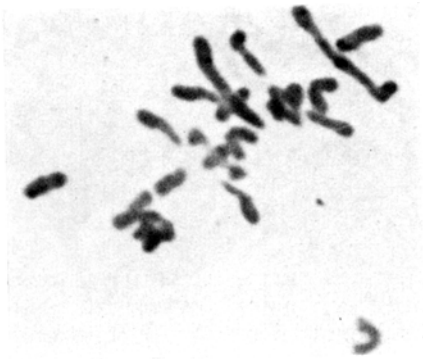

Fig. 2. 7 I + 6 II + 1 III

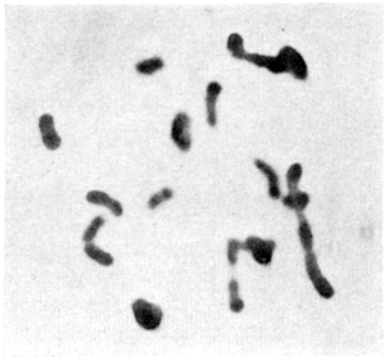

Fig. 3. 6 I + 3 II + 2 III + 1 IV

Fig. 2—3. Chromosome association at metaphase I

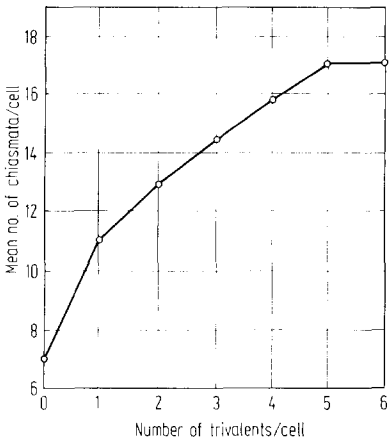

Fig. 4. Trivalents

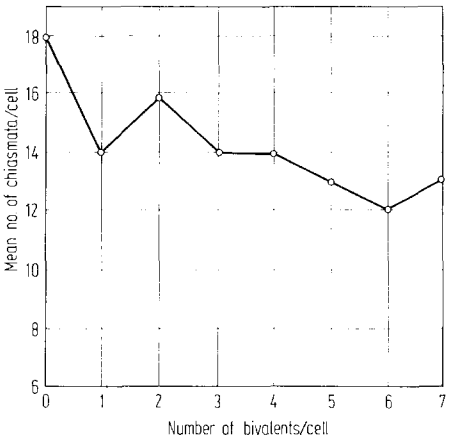

Fig. 5. Bivalents

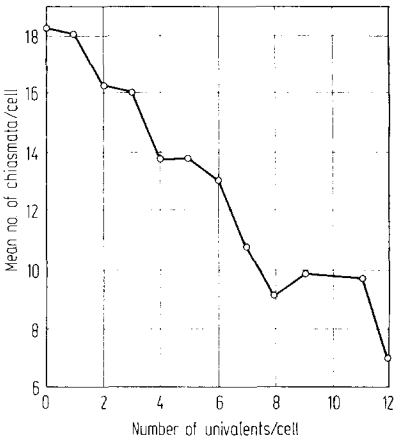

Fig. 6. Univalents

Figs. 4—6. Inter-relationship of chiasma number and chromosome associations at metaphase I

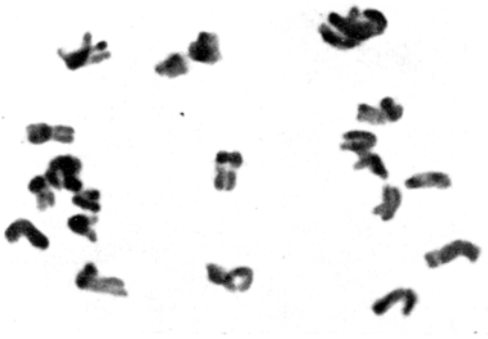

Fig. 7. Anaphase I, 9:9 segregation with 4 precociously dividing univalents

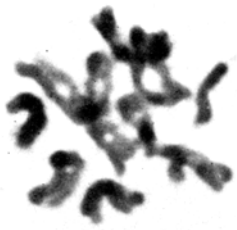

Fig. 9. A diad at prophase II with 9 chromosomes and one chromatid

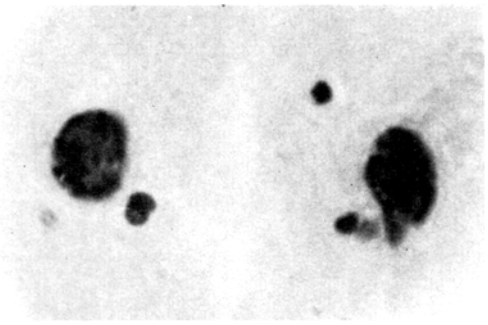

Fig. 8. Telophase I nuclei with chromatin masses and micronuclei

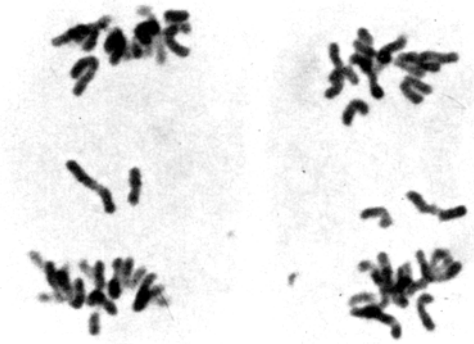

Fig. 10. Anaphase II showing unequal chromosome numbers in resulting tetrads

occurred in about 8% of the microsporocytes. The univalents nearly always divided precociously; the sister chromatids were mostly included in opposite nuclei at telophase I but sometimes formed micronuclei (Figs. 7—8). At telophase II, however, a number of chromatids appeared as 1—5 micronuclei or chromatin masses in the cytoplasm. Such segregations produced diads and tetrads with unequal chromosome numbers (Figs. 9—10).

Fertility: The hypertriploid plant showed a high degree of sterility. With alcoholic-safranin (Essad, 1962), about 54% of the pollen appeared to be normal but included pollen grains of varying sizes. However, when germinated on 20% sucrose solution with 1% boric acid, only 1—2% of these pollen produced pollen tubes. It is likely that the differences in pollen size were related to varying chromosome numbers in them. A comparison of the large-sized pollen of the

Table 2. *Percentage of microsporocytes with varying number of chromosome associations*

Type of association	Number of associations per cell								
	0	1	2	3	4	5	6	7	8—12
Univalents	5	7	19	15	12	14	7	7	14
Bivalents	1	9	21	19	25	14	6	—	—
Trivalents	1	17	13	24	26	12	7	—	—
Quadrivalents	70	30	—	—	—	—	—	—	—

Table 3. *Chiasma number at metaphase I in diploid and hypertriploid microsporocytes*

Suspected Genotype	2n	Mean Assoc. at MI	*Mean No. Chiasmata		
			Per cell	Per chromosome	Variance
Ds ds	14	7.00 II	13.3	0.9	3.1
Ds ds	14	7.00 II	14.4	1.0	1.8
Ds ds	14	7.00 II	13.3	0.9	3.3
Ds ds	14	7.00 II	14.0	1.0	1.4
Ds ds	14	7.00 II	12.9	0.9	2.6
ds ds	14	6.68 II	11.0	0.8	6.5
ds ds	14	5.80 II	7.0	0.5	2.4
ds ds ds	22	4.3 I + 3.4 II + 3.2 III + 0.3 IV	14.4	0.6	11.7

* Based on a minimum of 25 cells.

hypertriploid and diploids showed that pollen from the former measured on an average 36.4 μ × 31.9 μ and that of the latter 31.0 μ × 28.6 μ. The diploid pollen of a *Ds ds* maternal-sib showed 91.3% stainability. The hypertriploid set no seed on controlled pollination with *ds ds* diploids.

Discussion

The study of karyotype and chromosome behavior at metaphase I showed that the plant was a near-triploid (2n = 22) in which six chromosomes were present three times each and one was present four times. The natural survival of such a plant is a rare event. This plant could originate either when an aneuploid egg cell n = 8 was fertilized by n = 14 male gamete from a tetraploid plant or a haploid egg cell n = 7 was fertilized by an aneuploid n = 15 pollen. Although, it is usual to assume that transmission of aneuploidy is better through the female than male gametes, no other aneuploids were found in the progeny of the female parent. The female parent as well as the remaining progeny were all diploids. Since chromosome segregations of 13:15 at anaphase I have often been observed in microsporocytes of normal as well as desynaptic tetraploids (Ahloowalia, 1967, 1969a), it would appear that an aneuploid pollen (n = 15) had fertilized a haploid (n = 7) egg cell.

The hypertriploid plant showed a higher number of univalents per cell and a lower number of chiasmata per chromosome than that reported for the triploid by Myers (1944), even though most of the univalents originated from the trivalent forming chromosomes. The explanation for the difference between the two (triploid and hypertriploid) is in the nature of their origin. The hypertriploid originated from a desynaptic diploid (*ds ds*) and tetraploid (*ds ds ds ds*) and hence had the genotype *ds ds ds*, assuming that the extra chromosome (No. VI) did not carry the gene *ds*. Since the *ds* gene has been shown to be thermosensitive (Ahloowalia, 1969b), the wide variability in chromosome association and a high number of univalents perhaps resulted from temperature variations in glass house. This would also account for the absence of unpaired chromosomes at pachytene and their high frequency at metaphase I.

The chromosome configurations of 2 I + 5 II + + 2 III + 1 IV and 1 I + 4 II + 3 III + 1 IV were unexpected, since the number of bivalents plus trivalents should not exceed six when a quadrivalent was present. Each of these associations included an achiasmatic bivalent. Such "illegitimate" pairing of non-homologous chromosomes was also observed by Myers (1944). It is obvious that a very small intercalary translocation or a displaced duplication within the genome would result in a similar configuration. Since a pentavalent or two quadrivalents were not observed, it would appear that displaced duplication showed up as an achiasmatic bivalent, particularly when a synaptic gene mutation was also present within the genome. Such displaced duplications indeed may be common within the species *Lolium perenne*. Myers (1944), stated that, "among the seven chromosomes of the haploid set numerous duplications both within and between chromosomes" would be expected.

Since the hypertriploid did not differ in morphology from the diploid maternal-sibs, such plants would remain undetected even if they were formed more frequently than found. Such a behavior is different from that of the intergeneric allotriploids of ryegrass and meadow fescue (*Festuca pratensis*), where both the synthetic as well as natural hybrids can be distinguished from their diploid parents and hybrids (Ahloowalia, 1965; Essad, 1962; Wit, 1964).

The high sterility of the hypertriploid once again is different from the partial fertility of the triploid reported by Myers (1944). This can be explained again by desynaptic nature of the hypertriploid rather than to the presence of the extra chromosome. It, however, remains to be studied if fertility may be restored by growing the clones at a low temperature.

Literature

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