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Trisomics and Aneuploids of Ryegrass

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Summary. Primary trisomics ($2n + 1 = 15$), double trisomics ($2n + 1 + 1 = 16$) and aneuploids with 24 to 30 chromosomes, as well as a diploid and tetraploids, were found in the progeny of a hypertriploid ($2n = 22$) plant of perennial ryegrass, *Lolium perenne* L. Trisomics and double trisomics differed in their mean chromosome association, chiasma number and spike morphology. A few aneuploids and tetraploids had reciprocal translocations. The diploid, primary trisomics and tetraploids were more fertile than the double trisomics and aneuploids. Most trisomics and aneuploids were probably produced through female transmission. One double trisomic had a high univalent number, a low chiasma number and loose chromosome coiling. Both the extra chromosomes carried secondary constrictions. The gene for desynapsis might be located on one of these chromosomes.

Introduction

Trisomics have been isolated and produced in a number of plant species, e.g. barley, Datura, maize, rye, sugar beet, tobacco, tomato etc., and their value in gene location and chromosome mapping has been well documented (Hermsen, 1970). However, very little is known about trisomy and its effects in ryegrasses.

Perennial ryegrass, *Lolium perenne* L. is a diploid species (2n = 14). In this species, the occurrence of trisomics, tetrasomics and aneuploids with chromosome numbers from 15 to 18 has been reported in the progeny of a triploid plant (Myers, 1944). The trisomics were, in general, weak and had reduced viability. Two trisomics were also listed by Essad *et al.* (1966), without any reference to the phenotypic effects of the extra chromosomes.

In the present study, trisomics, double trisomics and aneuploids were obtained in the progeny of a hypertriploid plant. This report presents data on their frequency, meiotic behaviour and fertility and describes the effects of the extra chromosomes on plant morphology.

Materials and Methods

A hypertriploid plant ($2n = 22$) was open-pollinated with a diploid, tetraploid and several aneuploids ($2n = 25$ to 30). The hypertriploid plant was tetrasomic for chromosome VI and trisomic for each of the remaining six chromosomes, and perhaps carried the desynaptic gene *ds* in a triplicate condition (Ahloowalia, 1970).

One hundred seeds were germinated, giving sixty-one seedlings. Eight of these died soon after transplanting. Of the surviving plants, 46 plants were examined for chromosome number from the pollen mother cells (p.m. c.'s) at first metaphase and anaphase.

Two spikes per plant were fixed in a solution of ethanol, chloroform and acetic acid (6:3:1 v/v) mordanted with a saturated solution of ferric chloride in 45% acetic acid (5 ml/100 of the fixative). Smears of p.m.c.'s were stained with 0.5% acetic carmine. Pollen from freshly dehiscing anthers was stained with a solution of 1% alcoholic safranin, glycerol and water (1:2:1 v/v) and no less than one hundred pollen grains per plant were counted to determine pollen fertility. Seed set was obtained by mutual open-pollination of the progeny.

Results

The chromosome number of the progeny ranged from $2n = 14$ to 30. Plants with $2n = 16$, 25 and 26 were more frequent than the other types (Table 1). If the functional pollen from the surrounding plants was either $n = 7$ or $n = 14$, then the zygote frequency represents the frequency of female gamete formation and transmission in the mother plant. Thus, egg cells $n = 7$ to 16 were produced, and those with $n = 9$, 11 and 12 were formed and transmitted more frequently than the other types.

Table 1. *Frequency of zygotes, their chromosome number and likely mode of formation in ryegrass*

Chromosome number ($2n$)	Zygote frequency	Parental gametes (n) × (n) ♀ ♂
14	0.02	7 × 7
15	0.07	8 × 7
16	0.20	9 × 7
24	0.02	10 × 14
25	0.20	11 × 14
26	0.22	12 × 14
		13 × 13
27	0.11	13 × 14
28	0.13	14 × 14
		13 × 15
29	0.02	15 × 14
30	0.02	16 × 14

Meiotic Behaviour of the Diploid and Trisomics

The diploid plant showed a mean association of 6.9 bivalents per cell and occasional cells with two univalents. One of the bivalents was often present off the metaphase I plate, and stained very deeply.

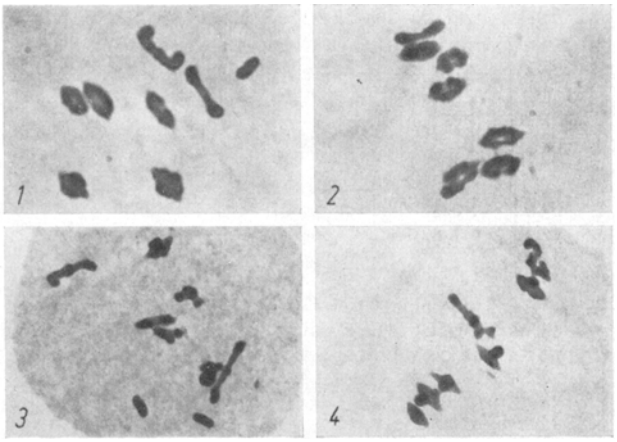

Fig. 1. 1 I + 7 II; Fig. 2. 6 II + 1 III; Fig. 3. 2 I + 7 II; Fig. 4. 5 II + 2 III and one pair of B-chromosomes

All the plants with 15 chromosomes were primary trisomics ($2n + 1$) (Figs. 1 and 2) and those with $2n = 16$ double trisomics ($2n + 1 + 1$) (Table 2). The three primary trisomics differed in their mean univalent and trivalent number per cell (Table 2), indicating that the extra chromosomes were different. In the double trisomics, the extra chromosomes either formed the expected two trivalents or stayed as univalents (Figs. 3 and 4), and varied in frequency of univalent and trivalent formation (Table 2). One trisomic (No. 40) had one chromosome with a large deletion in one arm, which either formed a heteromorphic trivalent or remained as univalent. This extra chromosome may be a tertiary trisomic; the expected pentavalent was prevented by its small size.

Table. 2. *Mean chromosome association and chiasma number in diploid and trisomics of ryegrass*

Plant No.	Mean No. per cell			Mean number of chiasmata	
	I	II	III	per cell	per bivalent*
$2n=14$					
17	0.02	6.90	—	13.6	1.98
$2n=15$					
47	0.60	6.60	0.50	14.4	2.04
1	0.75	6.60	0.35	13.4	1.91
15	0.97	6.27	0.50	14.0	2.05
$2n=16$					
39	0.50	5.35	1.60	15.3	2.12
32	0.95	5.35	1.45	11.3	1.48
19	1.05	6.05	0.95	16.9	2.43
11	1.13	6.03	0.93	16.8	2.40
7	1.20	6.05	0.90	16.3	2.35
30	1.30	5.70	1.10	13.1	1.87
40	1.44	5.96	0.88	12.6	1.75
13	2.73	5.00	1.09	11.6	1.78
56	4.75	4.65	0.65	8.9	1.60

* contributing bivalents only.

Another double trisomic had a pair of B chromosomes (Fig. 3) which did not synapse with other chromosomes. One double trisomic (No. 56) had up to 10 univalents per cell, with uncoiled and elongated bivalents. Both the extra chromosomes were nucleolar, and from the size of the trivalents, they were perhaps chromosomes II and IV. This plant showed a reduced chiasma number of 8.9 per cell (see below). Either one or both of the chromosomes may carry the gene *ds* which determines desynapsis (Ahloowalia, 1969).

Chiasma Number at Metaphase I

The mean chiasma number of the diploid, primary trisomics, and double trisomics is given in Table 2. Some of the trisomics apparently affected chiasma number per cell, as shown by the chiasma number (chiasma number of bivalents/no. of bivalents) of the diploid, primary trisomics and double trisomics. This ranged in double trisomics from 1.48 to 2.43 per bivalent (Table 2).

Segregation at First Anaphase

The primary trisomics showed segregations of 7:8 and 7:7 + 1 univalent laggard. In the double trisomics ($2n = 16$), however, segregations of 7:9, 8:8, 7:7 + 2 lagging univalents, 6:6 + 4 univalent laggards, and 4:4 plus 8 univalent laggards were observed.

Obviously, the extra chromosomes affected the synapsis and segregation of non-homologous chromosomes.

Meiotic Behaviour of Aneuploids and Tetraploids

The mean chromosome associations in the aneuploids and tetraploids are given in Table 3. The aneuploid with 24 chromosomes showed a maximum association of 4 III + 3 IV. Other observed associations could be explained on the assumption that the plant was tetrasomic for 3 chromosomes and trisomic for four chromosomes. Most univalents at metaphase I resulted from the trivalent forming chromosomes. In segregations such as 9:10, 10:11 with 5 and 3 univalents, they lagged and divided precociously.

The plants with 25 chromosomes were tetrasomic for four chromosomes and trisomic for 3 chromosomes each, since a few cells showed 3 III + 4 IV. However, a pentavalent association in one of them, and others such as 5 II + 1 III + 3 IV, 1 I + 6, II + 3 IV, and 1 I + 4 II + 4 IV, indicated at least one reciprocal translocation. These plants showed a relatively higher mean bivalent- and a reduced trivalent-number per cell than the other two plants without a translocation. It is obvious that a large reciprocal translocation in two chromosomes present 3 times each produced a univalent and a pentavalent, or three bivalents, instead of the expected two trivalents. In a few cells, heteromorphic bivalents, tri-

Table 3. *Mean chromosome association at first metaphase in aneuploid and tetraploids of ryegrass*

Plant No.	(2 *n*)	No. of pmc's	Mean association at M I					Others
			I	II	III	IV	V	
10	24	20	2.5	4.3	2.1	1.7	—	—
3	25*	21	1.9	5.0	1.7	1.5	0.4	—
22	25	15	1.7	3.5	2.1	2.5	—	—
31	25*	21	1.3	3.9	1.8	2.7	—	—
45	25	20	1.2	3.1	2.1	2.9	—	—
53	25*	20	1.2	4.2	1.9	2.4	—	—
4	26	23	1.1	4.7	1.3	2.9	—	—
14	26	10	0.8	3.3	1.4	3.6	—	—
21	26*	11	1.7	4.7	1.6	2.4	0.1	—
23	26	20	0.8	4.2	1.5	3.1	—	—
42	26	10	1.4	2.6	2.2	3.2	—	—
46	26*	13	7.2	4.2	1.3	1.3	0.2	—
57	26	14	1.4	5.2	1.2	2.6	—	—
58	26	20	0.9	4.3	1.7	2.9	—	—
36	27	10	—	1.8	1.0	5.1	—	—
38	27*	20	1.7	5.0	1.6	2.7	0.2	—
48	27*	20	1.0	4.9	0.8	3.4	—	0.05 VII
59	29	20	0.6	3.7	0.5	4.6	0.3	—
41	30*	20	1.1	3.5	1.3	3.2	1.1	0.05 VI
24	28	12	—	4.7	—	4.6	—	—
33	28*	20	1.4	4.0	0.8	3.6	0.2	0.1 VI
34	28	20	0.6	5.6	0.3	3.8	—	—
61	28	22	0.3	7.1	0.2	3.2	—	—

* likely presence of a reciprocal translocation in the genome.

valents and quadrivalents confirmed the presence of a reciprocal translocation. Perhaps heteromorphic bivalents and multivalents involved different translocations in plants with the same chromosome number. In one plant, one chromosome of the heteromorphic bivalent was acrocentric, while in the others such chromosomes had median to submedian centromeres.

Plants with 25 chromosomes showed 12:13 segregation at anaphase I in about 30% of the cells. Other segregations, such as 11:14 (13%), 12:12 + 1 laggard (13%) and 11:12 + 2 laggards (20%), were also observed. Types such as 10:15, 10:14, 10:13 and 10:10 with 0, 1, 2, and 5 univalent laggards were rarely seen. The lagging chromosomes always divided precociously and sister chromatids were usually included in the telophase I nuclei.

Most plants with 26 chromosomes showed association of 2 III + 5 IV, indicating that two chromosomes were present 3 times. However, two plants (No. 21 and 46) showed an occasional pentavalent. Such associations suggest a reciprocal translocation. Alternatively, one chromosome was present five times, four chromosomes present four times each, one present three times and one present twice. One of these plants showed up to 15 precociously divided univalent laggards with 5:6 segregation at anaphase I. Other plants showed segregations such as 11:15, 13:13 and 12:13 + 1 univalent laggard.

Of the three plants with 27 chromosomes, one (No. 38) showed an occasional pentavalent while another (No. 48) showed a configuration of 7 chromosomes. Such associations involve chromosome translocations, and could be explained if six chromosomes were present 4 times each and one was present three times. Anaphase I segregations such as 13:14, 12:14, 12:13, 12:12, 11:12 with 0 to 4 univalent laggards were common in such plants.

The single plant with $2n = 29$ showed the expected association of 6 IV + I V and all the other associations suggested that one chromosome (VI or VII) was present five times. This plant showed anaphase I segregations of 14:15, 14:14 + 1 laggard and 13:15 + 1 laggard.

The plant with 30 chromosomes showed the expected 2 pentavalents occasionally; however, a hexavalent together with 2 IV + 7 II + 2 I, or associations such as 1 I + 1 III + 4 IV + 2 V or 2 I + + 2 II + 6 IV etc., were also observed and could not be accounted for unless one heterozygous reciprocal translocation was also present. This plant showed segregations of 13:17, 14:16, 15:15, 14:15+1 laggard, 13:15 + 2 laggards etc.

Seven quadrivalents were seen in a few cells of the tetraploids. One tetraploid (No. 33), however, had a pentavalent and a hexavalent, suggesting the presence of two reciprocal translocations. The anticipated association of 7 quadrivalents was not seen; instead, 6 IV + 1 III + 2 I were observed. Yet another tetraploid (No. 61) had a high bivalent number per cell and the spikes were highly abnormal.

Fertility

Among the characters of pollen fertility, spike number, and seed set, only the first character was associated with chromosome number (Table 4). The mean pollen fertility of diploid, primary trisomics and tetraploids was higher than of any other type of aneuploid. Spike number per plant did not show any specific pattern, but seed set per spike was the highest in $2n = 28$ plants and declined in plants with either higher or lower chromosome number.

Table 4. *Fertility of diploid, trisomics, aneuploids and tetraploids of perennial ryegrass*

Chromosome No. (2 *n*)	Pollen fertility (%)		No. of spikes per plant		Seed No./spike	
	Range	Mean	Range	Mean	Range	Mean
14	—	90	—	6	—	1
15	76—87	80	14—28	21	1—3	2
16	13—78	46	2—45	20	1—5	2
24	—	68	—	3	—	0
25	35—86	58	7—30	17	1—6	3
26	10—86	65	3—35	19	1—16	7
27	48—89	75	2—24	9	3—9	6
28	49—96	81	4—30	22	1—18	8
29	—	66	—	19	—	6
30	—	51	—	25	—	4

Morphology

The trisomics, double trisomics and aneuploids showed marked differences in their morphology, i.e. leaves and spikes. Most of the plants were highly vigorous and tillered profusely. In leaf shape, trisomics and double trisomics approached the diploid plant, while aneuploids with 24 to 30 chromosomes resembled the tetraploids. The diploid, trisomics and double trisomics could be distinguished from the other aneuploids and tetraploids by their narrow leaves, thinner and slightly smaller spikes, and fewer florets in their spikelets. Some plants with $2n = 26$ had compact spikes. In one double trisomic and one 25-chromosome aneuploid, the rachis was very thin and elongated. One tetraploid, a double trisomic and a 25-chromosome plant showed viviparous development. The compaction, crinkling and in some cases branching of the rachis at the internodes, gave most of the trisomics and aneuploids a very distinctive appearance compared with the normal diploid and tetraploid plants (Figs. 5–9).

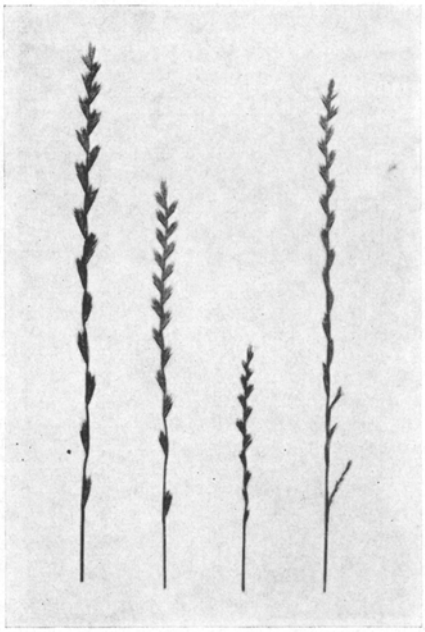

Fig. 5. Extreme left: Diploid. Right: Primary trisomics

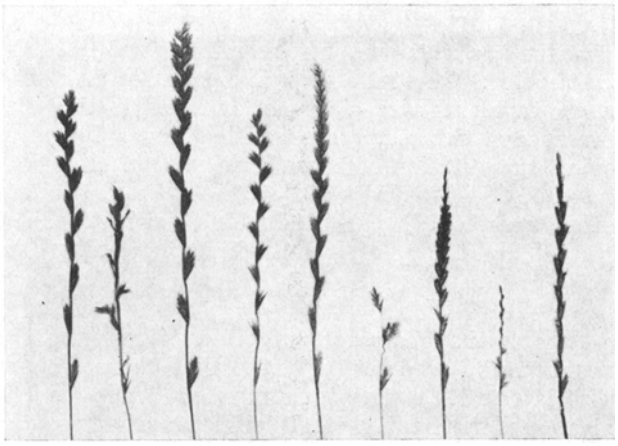

Fig. 6. Extreme left: Diploid. Right: Double trisomics

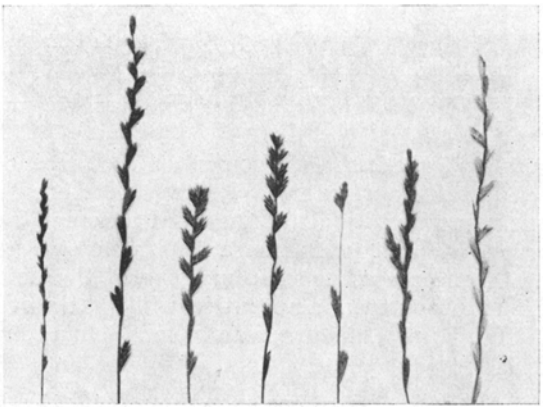

Fig. 7. Extreme left: Diploid. Extreme right: Tetraploid. Middle: Aneuploids with 25 chromosomes

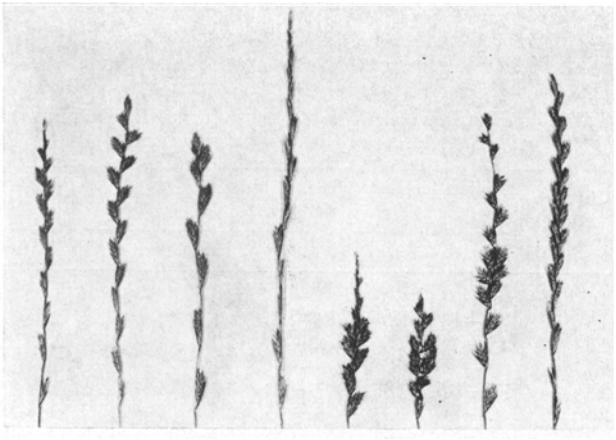

Fig. 8. Extreme left: Diploid. Extreme right: Tetraploid. Middle: Aneuploids with 26 chromosomes

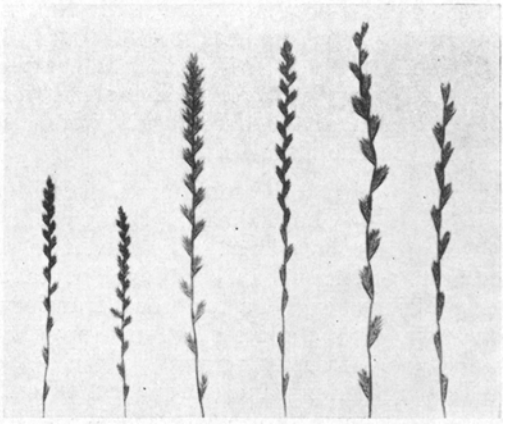

Fig. 9. Extreme left: Diploid. Extreme right: Tetraploid. Middle: Aneuploids with 27 chromosomes

Figs. 5–9. Spikes of diploid, trisomics, aneuploids and tetraploids

Discussion

The survival and partial fertility of trisomics and double trisomics with highly vigorous growth habit opens up the possibility of chromosome mapping, gene location and chromosome transfer, hitherto lacking for ryegrasses in general and *L. perenne* in particular. Although the extra chromosomes have not been identified yet, the variation in meiotic behaviour, fertility, and morphology of trisomics strongly suggests that they represent the seven different chromosomes of the genome. Hence, the seven primary trisomics in perennial ryegrass could be isolated and are likely to show the characteristic phenotypes known for the trisomics of *Datura*, tomato and tobacco (Ref. Hermsen, 1970). The range in chromosome number in the present study is parallel with that of the *pale-sterile* asynaptic genotype in *Nicotiana tabacum* L. used for obtaining monosomics and trisomics (Clausen and Cameron, 1944). The mother plant in the present study was, however, desynaptic. Thus the value of asynaptic and desynaptic genotypes in obtaining new variants, an essential step in the improvement of any crop, can be fully appreciated.

Some of the trisomics and double trisomics obtained are highly vigorous compared with the weak types reported by Myers (1944). Since no further reports appeared on trisomics, it is assumed that most of the material developed by Myers was lost.

Some of the chromosome associations in the aneuploids and tetraploids, and the presence of a deleted chromosome in a double trisomic, suggest that chromosome breakages in the mother plant produced reciprocal translocations and deficiencies. This suggests the possibility of obtaining more tertiary trisomics and establishing new linkage groups.

The meiotic behaviour in the aneuploids and trisomics suggests that most of the aneuploids are produced by transmission through the egg cell, as proposed for aneuploids in an earlier report (Ahloowalia, 1971). If the zygote frequency represents the frequency of egg cell transmission (since pollen transmission will be equal to one), then egg cells with $n = 7$ to 16 chromosomes are viable, but are selectively fertilized, i.e. egg cells with 7 to 9 chromosomes combine with 7-chromosome pollen, while the egg cells with 10 to 16 chromosomes are fertilized with pollen with 14 chromosomes (Table 1). Absence of the expected zygotes with 17 to 23 chromosomes would also emphasize a mechanism of selective fertilization. Further, it appears that the egg cells with $n = 9$, 11 and 12 are more viable than the other types. It is possible, however, that the increased viability also reflects the formation of egg cells with certain chromosome numbers. The mother plant had shown chromosome segregations of 8:9, 8:12, 9:9, 9:10, 9:12, 10:12, 11:11 with varying numbers of univalent laggards at the first anaphase in the pollen mother cells (Ahloowalia, 1970). A similar pattern of segregation might occur in the formation of the egg cells. Thus, egg cells with 9, 11, and 12 chromosomes both form and function more frequently than the other types, so that zygotes with 16, 25 and 26 chromosomes are more frequent than the other types.

The variation in univalent and chiasma number of primary and double trisomic warrants a comment here. Extra chromosomes, when present as bivalents, contributed to chiasmata in varying numbers, suggesting intragenomal differences in chiasma control. Since the female parent carried the desynaptic gene in triplicate, some of the double trisomics could carry the same gene in a heterozygous (*Ds ds ds*) condition and perhaps show partial expression. One such double trisomic had a reduced chiasma number, and the two extra chromosomes were associated with the nucleolus, which in turn is the main organelle of RNA synthesis. It is thus tempting to correlate these observations and surmize that defective RNA synthesis in a genotype of *Ds ds ds* may be involved in post-chromosomal synaptic stages — e.g. condensation, coiling, chiasma formation and terminalization. However, such a hypothesis needs critical experimental evidence. Perhaps the cistrion for this control is located on one of the two nucleolar chromosomes (II or III).

The trisomics and aneuploids showed marked differences in their leaf and spike morphology. In a previous report, it was stated that aneuploids with 26 to 30 chromosomes could not be distinguished from the tetraploids (Ahloowalia, 1971). It appears that the highly heterozygous nature of the aneuploids masks the effects of extra chromosomes. The mother plant in the present study is of inbred origin. Further inbreeding by sib-mating should produce still more marked differences between the different trisomics.

Literature

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