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FREQUENCY, ORIGIN, AND SURVIVAL OF ANEUPLOIDS IN TETRAPLOID RYEGRASS

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Aneuploids with 26 to 30 chromosomes occurred in progenies of induced tetraploids of *Lolium perenne* in a frequency of 6 to 23% in C_1 families, 12% in C_2 and 6% in advanced generations. The most frequent types had 27 and 29 chromosomes. Most were apparently recovered through female transmission, which varied for aneuploid gametes with different chromosome numbers and which probably depended on the chromosome involved. In general, aneuploids showed a reduction in fertility. Even with the same chromosome number, fertility varied perhaps as a result of differences between the chromosomes involved. Aneuploids could not be distinguished from eu-tetraploids on a morphological basis. Tetraploids late and early in respect to flowering did not differ in aneuploid frequency. No reversion to diploidy was observed in successive generations.

Introduction

Aneuploids have been isolated and identified in several autotetraploid crop plants either from natural populations, or from induced tetraploid progenies as in rye, *Secale cereale* (HAGBERG & ELLERSTRÖM, 1959; AASTVEIT, 1963), barley, *Hordeum vulgare* and sugar beet, *Beta vulgaris* (ROMMEL, 1963), red clover, *Trifolium pratense* and several other plants (Ref. ELLERSTRÖM & SJÖDIN, 1966).

Perennial ryegrass *Lolium perenne* L. is a natural diploid ($2n = 14$) species. Although a number of workers have induced tetraploids of perennial ryegrass (MYERS, 1939, 1945; WIT, 1959; SCHUMANN, 1967; ROO, 1968), the detection of aneuploids in tetraploid progenies has been documented by only a few of them. Aneuploids with 15 to 18 chromosomes were reported by MYERS (1944) in the progeny of a triploid plant of perennial ryegrass. These aneuploids had a reduced viability, narrow leaves, shorter stature, poorly developed roots, reduced spikelet number and abnormal floral development. Two trisomic plants have also been listed by ESSAD et al. (1966). In the related species of *L. multiflorum* var. *westerwoldicum*, the progenies of

the induced tetraploids included aneuploids with 27, 29 and 30 chromosomes (BARCLAY & ARMSTRONG, 1966).

During the course of tetraploid perennial ryegrass breeding, aneuploids were found in the C_0 (first), C_1 (second), C_2 (third), and advanced generation polyploids. This report presents data on the type, frequency survival and fertility of these aneuploids and discusses their possible origin in the light of the meiotic behaviour.

Materials and Methods

Most of the aneuploids were found in the C_1 families of C_0 tetraploids. Each family was the progeny of a single C_0 tetraploid plant inter-pollinated with other tetraploids in isolation. Fifty such C_1 families were grown in a field for two years along with an advanced generation (perhaps C_8 or C_9) imported tetraploid variety Petra (D. J. VAN DER HAVE, Kapelle, Netherlands). Two hundred plants per family were raised; however, cytological study was confined to the selected plants from 29 families and the variety Petra only. The parental C_0 tetraploids of these C_1 families had been obtained by doubling seven diploid varieties, namely Irish Commercial (I.C.), S. 23, Viris, New Zealand Section, Barenza, Mommersteegs' hay type and Øtofte (AHLOOWALIA, 1967a).

The C_1 progeny of a C_0 aneuploid plant with 25 chromosomes (AHLOOWALIA, 1966) also included several aneuploids.

The C_2 generation was obtained by inter-pollination of selected C_1 tetraploids and the bulk-seed was sown as single spaced plants.

The selected polyploid plants were analysed for chromosome number by root tip squash technique (AHLOOWALIA, 1965). The chromosome number was rechecked in pollen mother cells at anaphase I and chromosome associations were studied at metaphase I. The techniques of fixation, staining and smear preparation were the same as described for tetraploids (AHLOOWALIA, 1967b).

Seed set was determined by inter-pollination of aneuploids and tetraploids.

Results

TYPE AND FREQUENCY OF ANEUPLOIDS

Of the 341 C_1 plants examined for chromosome number about 17% were aneuploids, the remaining being eu-tetraploids. The types of aneuploids and their frequencies within groups of families are given in Table 1. The frequencies of aneuploids varied considerably among the families as well as between the varietal groups. The aneuploids with 27 and 29 chromosomes were by far the most frequent. The aneuploids with 26 and 30 chromosomes were restricted to two families each. The mean eu-tetraploid recovery was 83% in C_1, 88% in C_2 and 94% in the advanced generation polyploids, indicating a progressive decrease in aneuploid frequency with succeeding generations (Table 1).

TABLE 1

TYPE AND FREQUENCY OF ANEUPLOIDS IN C_1, C_2 AND ADVANCED GENERATION POLYPLOIDS

Variety	No. of families	No. of plants	Aneuploid percentage				
			$2n = 26$	27	29	30	Total %
I.C.	8	75	3	11	4	5	23
S. 23	7	161	–	9	7	1	17
Viris	3	14	–	–	–	–	0
NZ Selec.	2	18	6	–	11	–	17
Barenza	3	18	–	–	6	–	6
Mommer.	3	51	–	9	2	–	11
Øtofte	3	6	–	33	17	–	50
C_2	1	32	–	–	12	–	12
Petra*)	1	27	–	3	3	–	6

*) advanced generation tetraploid.

The aneuploids with 27 and 29 chromosomes did not occur in equal frequencies, either in the individual C_1 families, or within the varietal groups or for the total population of 341 plants (Table 2). Similarly, the aneuploids with 26 and 30 chromosomes did not occur in equal ratios. This suggested a differential transmission of the parental gametes with varying chromosome numbers. In the advanced polyploids, however, 27 and 29 types occurred in an equal ratio.

TABLE 2

FREQUENCY OF ANEUPLOID ZYGOTES AND THEIR PARENTAL GAMETES IN C_1 POLYPLOIDS

Chromosome number of zygotes ($2n$)	parental gametes (n) × (n)	Observed zygote number	frequency
26	12 × 14	3	0.009
	13 × 13		
27	13 × 14	30	0.088
	12 × 15		
28	14 × 14		
	13 × 15	284	0.833
	12 × 16		
29	15 × 14	18	0.053
	16 × 13		
30	15 × 15	6	0.018
	16 × 14		

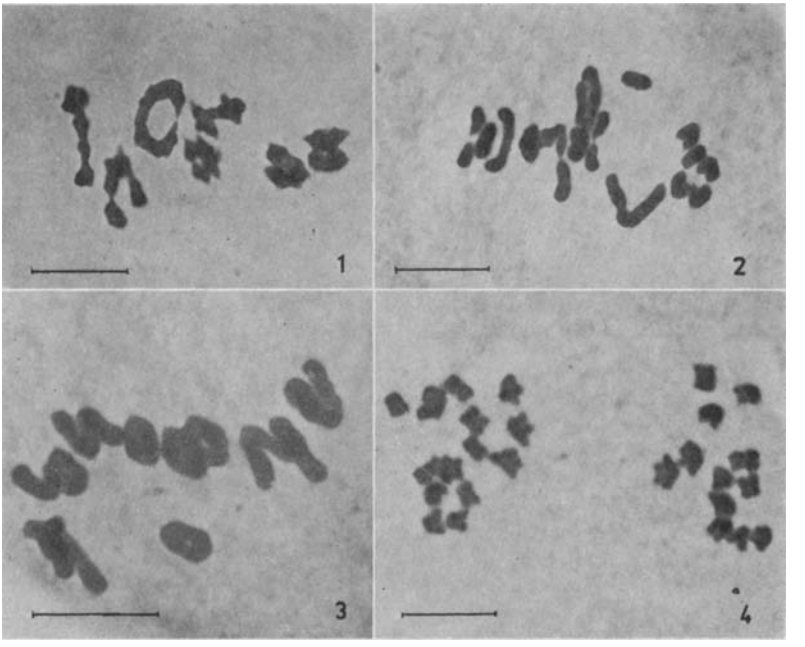
CHROMOSOME ASSOCIATION AT METAPHASE I

The mean and maximum observed chromosome associations in the pollen mother cells at metaphase I are given in Table 3. The C_1 tetraploids showed 6 II + 4 IV and 4 II + 5 IV per cell very frequently and cells with 7 IV (Figure 1) were rare. Aneuploids with 27 chromosomes often showed a trivalent along with other associations (Figure 2); a univalent plus a bivalent sometimes resulted from the breakdown of the trivalent. Aneuploids with 29 chromosomes were characterised by the presence of a pentavalent. These associations in 27 and 29 chromosome aneuploids suggested that only one of the parental gametes was either deficient for one chromosome or had one extra; thus aneuploids with 27 chromosomes were trisomics and those with 29 pentasomics. The aneuploid with 30 chromosomes showed up to two pentavalents (Figure 3), indicating that the two extra chromosomes were non-homologous, and the plant was a double pentasomic. This plant had a relatively high mean number of univalents and bivalents per cell.

TABLE 3

MEAN AND MAXIMUM ASSOCIATION IN POLLEN MOTHER CELLS AT METAPHASE I IN C_1 POLYPLOIDS

2n	No. of plants	No. of cells	Mean number per cell I	II	III	IV	V	Maximum assoc.
27	5	139	0.8	4.4	0.7	3.8	—	1 III + 6 IV
28	6	111	0.3	4.8	0.2	4.7	—	7 IV
29	2	40	0.3	2.1	0.4	5.2	0.5	6 IV + 1 V
30	1	20	3.1	6.1	0.9	2.6	0.4	2 II + 4 IV + 2 V



Figures 1–3. First metaphase in aneuploid ryegrass pollen mother cells
Figure 1. 7 IV in a tetraploid.
Figure .2 1 I + 5 II + 4 IV in an aneuploid with 27 chromosomes.
Figure 3. 2 II + 4 IV + 2 V in an aneuploid with 30 chromosomes.
Figure 4. Anaphase I segregation of 12 : 15 in aneuploid with 27 chromosomes.
Horizontal line: 10 μ.

ANAPHASE I SEGREGATION

The tetraploids and aneuploids showed imbalanced chromosome segregations at anaphase I. Segregations such as 13:15 and 12:16 were common in tetraploids, 13:14 and 12:15 in 27 chromosome aneuploids (Figure 4), 14:15 and 13:15 + univalent laggard in 29 chromosome types, and 15:15, 12:13 + 5 univalent laggards in the single plant with 30 chromosomes studied. The lagging univalents usually divided precociously and the resulting sister chromatids were usually included in telophase I nuclei.

SURVIVAL AND FERTILITY

The C_1 aneuploids survived for two years in the field and appeared to be as viable as their maternal-sib eu-tetraploids.

TABLE 4

SEED SET ON ANEUPLOID AND TETRAPLOID PLANTS ON OPEN POLLINATION

	Chromosome number ($2n$)	No. of spikes	No. of seeds per plant	$\bar{x}$ per spike
Aneuploids	25*)	27	73	3
	27	24	13	1
	27	24	72	3
	27	41	118	3
	27	65	231	4
	27	32	308	10
	27	74	416	6
	27	31	489	16
	27	52	571	11
	27	84	716	9
	29	31	353	11
	29	65	402	6
	29	63	531	8
	30	88	885	10
Eutetraploids	28	27	129	5
	28	57	539	9
	28	72	682	10
	28	81	1069	13
	28	85	1591	19

*) Obtained in C_0 following colchicine treatment (AHLOOWALIA, 1966).

The aneuploids showed a wide variation in seed set per plant as well as per spike (Table 4). In general, aneuploids were less fertile than the eu-tetraploids, although some with 27 and the one with 30 chromosomes approached seed set of tetraploids. The differences in spike number and seed set between aneuploids with the same chromosome number perhaps reflects the presence or absence of different chromosomes of the genome. The anaphase I segregations and seed set of aneuploids suggested that aneuploids could survive well in the succeeding generations.

MORPHOLOGY

Most of the aneuploids did not differ markedly from their tetraploid maternal sibs, and could not be distinguished visually from the latter. Some of the 27 chromosome types, however, showed slightly narrower leaves. The C_1 progeny of the selfed C_0 25 chromosome aneuploid, which included aneuploids with 27, 29 and 30 chromosomes, varied in spike shape.

Discussion

The occurrence of aneuploids in the progenies of induced tetraploids of ryegrass is a parallel example to that reported in induced tetraploids of rye (HAGBERG & ELLERSTRÖM, 1959), barley and sugar beet (ROMMEL, 1963) and several other crops (See ELLERSTRÖM & SJÖDIN, 1966). The presence of aneuploid zygotes with, 26, 27, 29 and 30 chromosomes confirms the functioning and viability of aneuploid gametes. Such gametes originate from chromosome segregations of 13:15 and 12:16 at anaphase I in the C_0 tetraploids (AHLOOWALIA, 1967b). On this basis, at least three different models could be proposed to account for the origin of observed 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 (Table 2). 2. That 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 chromosomes either did not function or were eliminated in competition, since zygotes with $2n = 24, 25, 31$ and 32 resulting from 12×12, 12×13, 15×16 and 16×16 combinations were not detected in C_1. 3. That aneuploid gametes

functioned only through one side (male or female) and the reciprocal gamete was always the normal ($n = 14$) type; thus no reconstituted 28 type (13×15 and 12×16) would be expected. In this model, since the transmission of the normal gamete will equal to one, the aneuploid zygote frequency would also represent the transmission of the aneuploid gametes.

The first two hypotheses did not fit the observed data, since the observed number of eu-tetraploids was far greater than expected. Further, the last of the three hypotheses seemed to fit the observed meiotic behaviour of 27 and 29 chromosome aneuploids. All the observed metaphase I configurations in these aneuploids agreed with the model that the aneuploid zygotes originated from aneuploid $\times$ normal matings (13×14 and 15×14) rather than from two aneuploid gametes (12×15 and 13×16). Perhaps, most of the aneuploid gametes were transmitted through the female side.

The two classes of aneuploids with $2n = 27$ and 29 did not occur in equal ratios except in case of the advanced generation polyploids. This could result from unequal transmission of $n = 13$ and 15 gametes as well as from intragenomal differences with regard to individual chromosome transmission. With subsequent breeding generations and selection for tetraploid stability, one would expect that aneuploid gametes would carry the most transmissible chromosome, when the ratio of the deficient and extra chromosome carrying zygotes would approach 1:1. The latter indeed appeared to be so in the polyploid variety Petra.

The aneuploids with 26 and 30 chromosomes were restricted to only two families each, suggesting that their occurrence was not only rare but also that certain genotypes favoured the survival of more than one chromosome imbalance. The frequencies of these types, however, were too low to permit reliable conclusions. The origin of these two types was likely from 12×14 and 16×14 gametes. The zygote frequency of these aneuploids is so low, that even if $n = 12$ and 16 gametes did function equally well on both male and female side, the frequency of zygotes with 24 and 32 chromosomes would be 8×10^{-5} and 16×10^{-6}.

The C_1 aneuploids did not differ morphologically from their tetraploid sibs. This is in contrast to the observations on a 25 chromosome aneuploid detected in C_0 (AHLOOWALIA, 1966) and its progeny which

showed plants with abnormal leaf and spike shapes. The aneuploids obtained by MYERS (1944) were also abnormal. The extreme heterozygosity of the aneuploids may explain their unchanged morphology, since the plants had originated from a polycross. Moreover, most of the leaf and spike characters appear to be under a polygenic control (COOPER, 1960). Inbreeding of these aneuploids, however, may reveal morphological differences associated with the presence or absence of certain chromosome.

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