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FREQUENCY OF ANEUPLOIDS IN C_1 AND C_2 GENERATIONS OF TETRAPLOID RYEGRASS (*Lolium perenne* L.)

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ABSTRACT

In this study we used 24 C_1 and 85 C_2 plants to determine frequency of aneuploids in the C_1 and C_2 generations of tetraploid ryegrass. In the C_1 generation, there were 33.30% diploids, 41.84 eutetraploids and 24.86 aneuploids. In the C_2 generation, there were 32.94 diploids, 45.88% eutetraploids and 21.18% aneuploids.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is a diploid plant with a low chromosome number (n=7). It is a cross pollinating crop and the fodder production which can be increased by autotetraploidy is more important than seed yield. The first autotetraploid plant of perennial ryegrass was obtained by Myers (1939) using colchicine treatment. After Myers a number of workers have induced tetraploidy (Wit, 1959, Roo, 1968; Ahloowalia, 1967a; Sağsoz, 1974). But the aneuploids in tetraploid progenies have been reported by only a few of them. Aneuploids with 15 to 18 chromosomes were reported by Myers (1944) in the progeny of a triploid plant. Myers (1945) was also the first to study meiotic behavior in induced autotetraploids. Later Ahloowalia (1967b) published some results of cytological studies on autotetraploids. He also studied aneuploids (1966, 1970, 1971, 1972). After this a study was made by Simonsen (1973) on aneuploid perennial ryegrass. Reduced fertility of artificial autotetraploids as compared with the diploids is a common feature.

The aim of this study was to determine the type and frequency of aneuploids in the C_1 and C_2 generations of induced tetraploid ryegrass.

MATERIAL AND METHODS

Twenty-six tetraploid C_0 plants were obtained after colchicine treatment (Sağsöz, 1974). Ten out of the twenty-six plants survived in the field. The seeds were harvested from each of those plants. Thirty C_1 seeds from each plant were germinated in pots. After two months they were transplanted to the fleld and visual selection was made based on morphological characters, winter hardiness and seed setting. The C_2 seeds were harvested from these plants. The C_2 generation was obtained by interpollination of C_1 tetraploids.

In this research, chromosome number was determined in 24 C_1 plants and 85 C_2 plants. The polyploid plants in C_1 and C_2 were analyzed for chromosome number by root-tip squash technique, a modification of Abloowalias' (1965) method. The root-tip samples were taken and were put in a solution of α-monobromonaphtalene for three hours, then fixed in a solution of 95 percent ethanol, glacial acetic acid and chloroform (6:3:1). They were hydrolyzed with 1 N HCl for ten minutes at 60°C and stained with Feulgen.

RESULTS AND DISCUSSION

We examined root tips from 24 C_1 plants and 85 C_2 plants to determine their chromosome number The frequencies of diploids, tetraploids and various types of aneuploids in C_1 and C_2 generations are given in Table 1 and 2, respectively. In the C_1 generation there were 33.30% diploids, 41.84% eutetraploids and 24.86 aneuploids [20.76% hypoploid (2n=25, 26 and 27), 4.10% hyperploid (2n=29)]. In the C_2 generation, there were 32.94% diploids, 45.88% eutetraploids, and 21.18% aneuploids [9.41% hypoploid (2n=26 and 27), 11.77% hyperploid (2n=29 and 30)]. The frequency of diploids is equal in both the C_1 and C_2 generations (Table 1 and 2; Fig. 1).

While in the C_1 generation, chromosome number of aneuploids varied from 25 to 29, in the C_2 generation varied from 26 to 30. The percentages of aneuploids with 26 and 27 chromosomes in the C_1 generation were higher than others. Whereas, in the C_2 generation, the percentages of aneuploids with 27 and 29 chromosomes were higher than others (Fig 1).

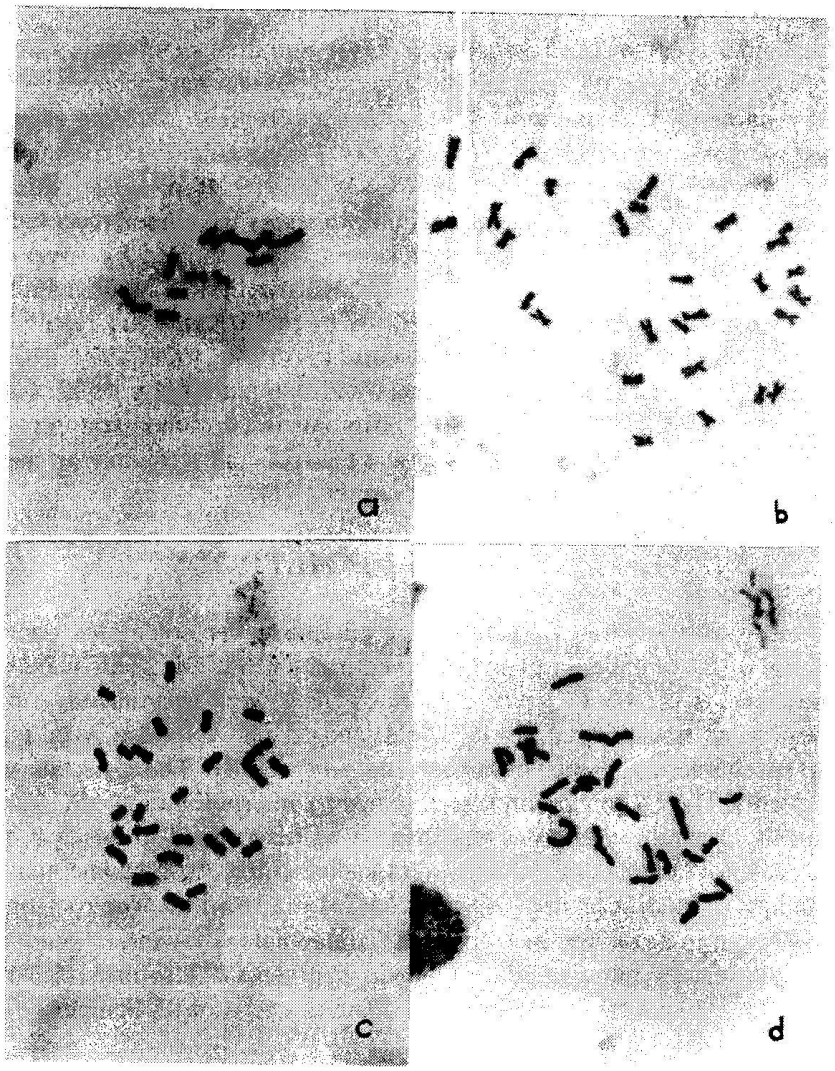

Figure 1. Metaphase chromosomes in root-tip cells of *Lolium perenne* L. **a:** diploid (2n=14), **b:** tetraploid (2n=28), **c:** hypoploid (2n=27), **d:** hyperploid (2n=29) (1000X).

If the C_1 progenies had been cytologically analyzed and selected initially, either a very low percentage of diploids or none would have been found in the C_2 generation. Most probably the diploid plants have come from mixoploid C_0 plants. This fact has been shown by Ahloowalia (1967a,b).

Table 1. The frequency and type of aneuploids in the C_1 generation of tetraploid ryegrass.

	Diploid	Tetraploid	Aneuploids				
	(2n=14)	(2n=28)	2n=25	2n=26	2n=27	2n=29	Total
Number	8	10	1	2	2	1	6
%	33.30	41.84	4.10	8.33	8.33	4.10	24.86

Table 2. The frequency and type of aneuploids in the C_2 generation of tetraploid ryegrass.

	Diploid	Tetraploid	Aneuploids				
	(2n=14)	(2n=28)	2n=26	2n=27	2n=29	2n=30	Total
Number	28	39	1	7	9	1	18
%	32.94	45.88	1.18	8.23	10.59	1.18	21.18

Diploids are not expected to be present in C_2 progenies. The presence of aneuploidy, which affects the stability of tetraploid plants, is very important to tetraploid ryegrass breeding programs. The percentage of aneuploids in the C_1 and the C_2 generations in this study is a parallel example to those reported by Abloowalia (1971) in ryegrass and by Hagberg and Ellerstrom (1959) in rye.

The presence of aneuploids with 26, 27, 29, and 30 chromosomes in the C_2 generation has shown that aneuploids are viable and functional. A similar observation was also reported by Ahloowalia (1971). He suggested that aneuploid gametes originate from chromosome segregation of 13:15 and 12:16 at anaphase I in the C_0 tetraploids. He proposed that on this basis at least three different models could account for the origin of the observed aneuploids: (1) That the gametes with n=12, 13, 14, 15, and 16 combined in all possible ways to produce the observed zygotes, (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 case, since the transmission of the normal gamete would be equal to one, the aneuploid zygote frequency would also represent the transmission of the aneuploid gametes. According to same research worker the first two hypotheses were not supported by his experimental data.

Many researchers mentioned that aneuploids arise because of anomalies in chromosome replication, separation and movement after colchicine treatment. The occurrence of multivalents at metaphase I and irregular chromosome distribution at anaphase I results in abnormal gametes. These abnormal gametes also cause aneuploids to be formed in progenies with very high frequencies (Hagberg and Ellerstrom, 1959; Ahloowalia, 1966, 1970, 1971, 1972).

It is likely that aneuploids frequently occur in ryegrass but escape detection. Myers (1939) obtained a single triploid seed by open pollination of a tetraploid by diploids in isolation. In our observation on C_2 progenies, the percentages of aneuploids with 27 and 29 chromosomes were higher than the aneuploids with 26 and 30 chromosomes. This suggested that the aneuploids which have one chromosome extra or one chromosome less had a better chance at survival than the aneuploids which have 26 or 30 chromosomes. The same result was reported by Ahloowalia (1971) in aneuploid ryegrass.

Morphologically the aneuploids did not show any difference from the tetraploids. Likewise, the same observation was reported by Ahloowalia (1971). Also, Ellerstrom and Sjodin (1966) reported that morphologically there was no difference between aneuploid and tetraploid red clover. This is contrast to the observation on an aneuploid with 25 chromosomes detected in the C_0 and its progeny which showed some abnormal leaf and spike shapes (Ahloowalia, 1966). Also, Klinga (1986) found some morphological differences in aneuploid ryegrass.

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