DETERMINATION OF SOME MEIOTIC CHARACTERISTICS AND SEED SET IN AUTOTETRAPLOID PERENNIAL RYE (*Secale montanum* Guss.)

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ABSTRACT

In this study, the generations C_1, C_2 and C_3 of autotetraploid perennial rye (*Secale montanum* Guss.) were compared in terms of seed set and some cytological characteristics including irregularities in metaphase I (univalents), anaphase I and II (lagging chromosomes and/or chromatids and bridge) and micronuclei in tetrads. According to generations (C_1, C_2 and C_3) average cell rate with univalent was 18.51, 14.56 and 9.38%, rate of irregular anaphase I cells was 3.44, 15.93 and 7.86%, rate of irregular anaphase II cells was 20.93, 16.48 and 7.16%, rate of tetrads with micronuclei was 18.17, 14.06 and 6.17% and seed set rate was 27.72, 43.50 and 59.59% respectively. Furthermore, a negative correlation was found between seed set and univalents in metaphase I and abnormal anaphase I and II cells, and tetrads with micronuclei. The correlations among the characters investigated during meiotic division were statically significant. The result of this study showed that selection of vigorous plants in autotetraploid perennial rye stimulated seed setting by increasing cytological balance. On the other hand, the abnormalities in early stage of meiotic division were determined to be reflected to the next stages of meiosis.

INTRODUCTION

Although induced autopolyploids have many superior characteristics, they have important problems such as low fertility. This problem originates from cytological instability and physiological inharmony due to unbalanced gene combination (Elgersma, 1990). It was speculated that low seed number per plant resulted from decreasing number of flower per plant and percentage of fertile flower (Aastveit, 1968). A number of previous studies determined that the reasons for low fertility in different species was univalents and trivalents (Hazarika and Rees, 1967; Hossain and Moore, 1975; Narasinga and Pantulu, 1982; Evans and Rahman, 1990; Sağsöz and Akgün, 1994). Moreover, it was noted that increasing number of univalents in pollen mother cells decreased pollen viability (Roseweir and Rees, 1962) and chromosomes at anaphase I should segregate even to poles for high fertility in induced autotetraploids (Fedak, 1975; Arundhati et al., 1983). On the other hand, many physiological characteristics in addition to cytological factors affect on the seed set or fertility in autotetraploid populations (Aastveit, 1968; Hossain and Moore, 1975).

Induced autotetraploid perennial rye is superior to its diploid in respect to many morphological characteristics examined, while percentage of seed set of autotetraploid is highly lower than that of diploid (Özer and Sağsöz, 1991). Many researchers reported that decreasing in seed setting can be overcome in advanced generations, by using a suitable selection method. (Bremer and Bremer-Reinders, 1954; Hagberg and Ellerstrom, 1959; Moore and Ellerstrom, 1987).

The objectives of this study were to determine some cytological characteristics and fertility in advanced generations of autotetraploid perennial rye and to determine relationships among these characteristics.

MATERIALS AND METHODS

The seeds of diploid (2n=14) perennial rye (*Secale montanum* Guss.) were collected from the plants naturally grown at Erzurum, Turkey. The plants were selected according to morphological characteristics such as morphological vigour and high tillering capacity. The seeds were germinated and a week after germination, seedlings were treated with 0.1% of colchicine solution to obtain autotetraploid perennial rye (C_0) plants (Özer and Sağsöz, 1991). C_1 plants were produced from the C_0 plants were grown in 10 cm-diameter plastic pots containing a mixture of soil:farm manure:sand (1:1:1) under the greenhouse condition. The vigorous and high tillering plants were selected from the C_1 population and the plants were transplanted into the plots by spacing 15 x 30 cm on and between rows, respectively, in the field. To obtain the following generations (C_2 and C_3), vigorous plants with large spikes plants were selected and used for seed production. Spike samples were taken from 25 plants from each of the C_1, C_2 and C_3 generations (total 75 spike) and meiosis were investigated in these spikes. Seed setting of the same plants was also determined on the two spikes (each generation had 2x25= 50 spikes) from each plant, and seed setting was calculated for each plant. The plant samples that were used for both meiotic investigation and to estimate seed setting were the cross-pollinated progenies of the plants within the same population. Approximately 1000 m distance among populations were kept due to cross-pollination among plants of different generations. Thus, cross-pollination should be occurred among plants in the same generation.

The spike samples used for meiotic studies were fixed with Carnoy's solution (6:3:1; ethanol: glacial acetic acid: chloroform) and stored in 75% alcohol in refrigerator. Squash preparations from 2 or 3 anthers were stained with 2% Aceto-orcein (Moore, 1963). To determine meiotic irregularity univalents at 100 metaphase I (MI) cells, irregular segregations (lagging chromosomes or chromatids and bridges) at 50 anaphase I (AI) cells and 50 anaphase II (AII) cells (Arundhati et al., 1983) and the number of micronuclei in 150 tetrads were studied in each plant. Relationships between chromosomal behavior in different stages of meiosis and seed set was investigated. The means of the data obtained from three populations were compared by using the "t" test for significance.

RESULTS

Metaphase I: At the metaphase I of meiotic division, univalents in relation to seed setting were studied although complete determining the numbers and the pairing form of the chromosomes were difficult (Fig. 1). The ratio of cells with univalents at metaphase I among the generations were significant (C_1/C_2 t=2.926**, C_1/C_3 t=7.426**, C_2/C_3 t=3.549**). As it was shown in Table 1, the cell rates with univalents at metaphase I were determined as 18.51% in the C_1, 14.56% in the C_2 and 9.38% in the C_3 generations. Simultaneously, the number of

Table 1. The Numbers and Percentages of Metaphase I Cells with Univalents in Different Generations of Autotetraploid Perennial Rye.

Gener-ations	Num. Plant	Total Num. Cell	Numbers of Univalent in Metaphase I				Num. Univa. Per Cell	Rates of Cells with Univalent		
				1	2	3	4		Mean	Min.	Max.
C_1	25	3571	Num.	480	157	23	1	0.24	661	21	44
			%	13.44	4.40	0.64	0.03		18.51	12.88	25.14
C_2	25	4391	Num.	534	91	12	2	0.17	639	16	33
			%	12.16	2.07	0.27	0.05		14.56	6.06	23.08
C_3	25	2611	Num.	180	56	8	1	0.12	245	3	23
			%	6.89	2.14	0.31	0.04		9.38	2.52	20.18

univalent per cell was the highest in the C_1 generation (0.24), whereas the C_3 generation had the lowest value (0.12). The ratio of pollen mother cells with univalents in the C_1, C_2 and C_3 populations were 13.44%, 12.16% and 6.89%, respectively. The pollen mother cells with four univalents were rarely observed in all populations.

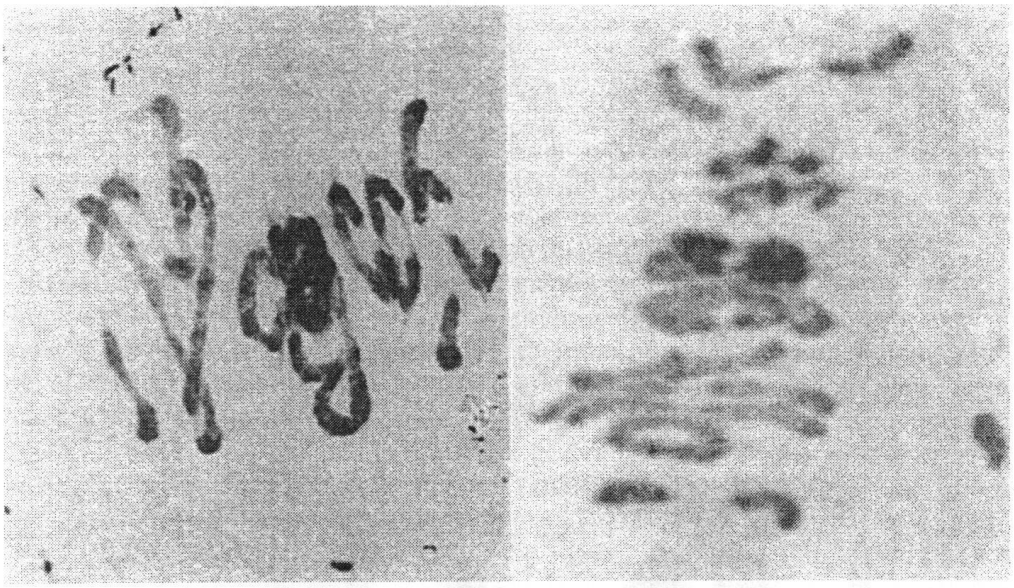

Figure 1. Cells of metaphase I in the autotetraploid perennial rye (1000X).

Anaphase I: Regular and irregular segregations, lagging chromosomes and/or chromatids and cells with bridges were observed at anaphase I (Fig. 2). Generally, lagging chromatids or fragments were defined in the cells containing bridges and these cells were considered as together cells with bridges. The rates of irregular segregated cells at anaphase I was significantly different (C_1/C_2 t=4.806**, C_1/C_3 t=10.360**, C_2/C_3 t=5.064**) with respect to the generations. The rate of irregular segregating cells were determined as 23.44% in the C_1, 15.93% in the C_2 and 7.86% in the C_3 populations (Table 2). In all populations, the greater part of laggings was chromosomes and in the C_1, C_2 and C_3 populations, the rates of cells with lagging chromosome were 17.07%, 9.90% and 5.52%, respectively. The rate of cells with bridges at anaphase I were similar in the C_1 (3.22%) and the C_2 (3.35%) generations, while the rate of cells with bridges was significantly reduced in the C_3 population (0.87%).

Table 2. The Numbers and Percentages of Irregular Anaphase I (AI) in Different Generations of Autotetraploid Perennial Rye.

Gener-ations	Num.Plant	Total Num. Cell		Irregular Anaphase I Segregations			Rates of Irregular A I Cells		
				Lagging Chromosome	Lagging Chromatid	Bridge	Mean	Min.	Max.
C_1	25	2390	Num.	408	75	77	560	13	27
			%	17.07	3.14	3.22	23.44	15.96	42.11
C_2	25	3132	Num.	310	84	105	499	1	17
			%	9.90	2.68	3.35	15.93	3.57	36.91
C_3	25	2191	Num.	121	32	19	172	2	10
			%	5.52	1.46	0.87	7.86	3.51	25.74

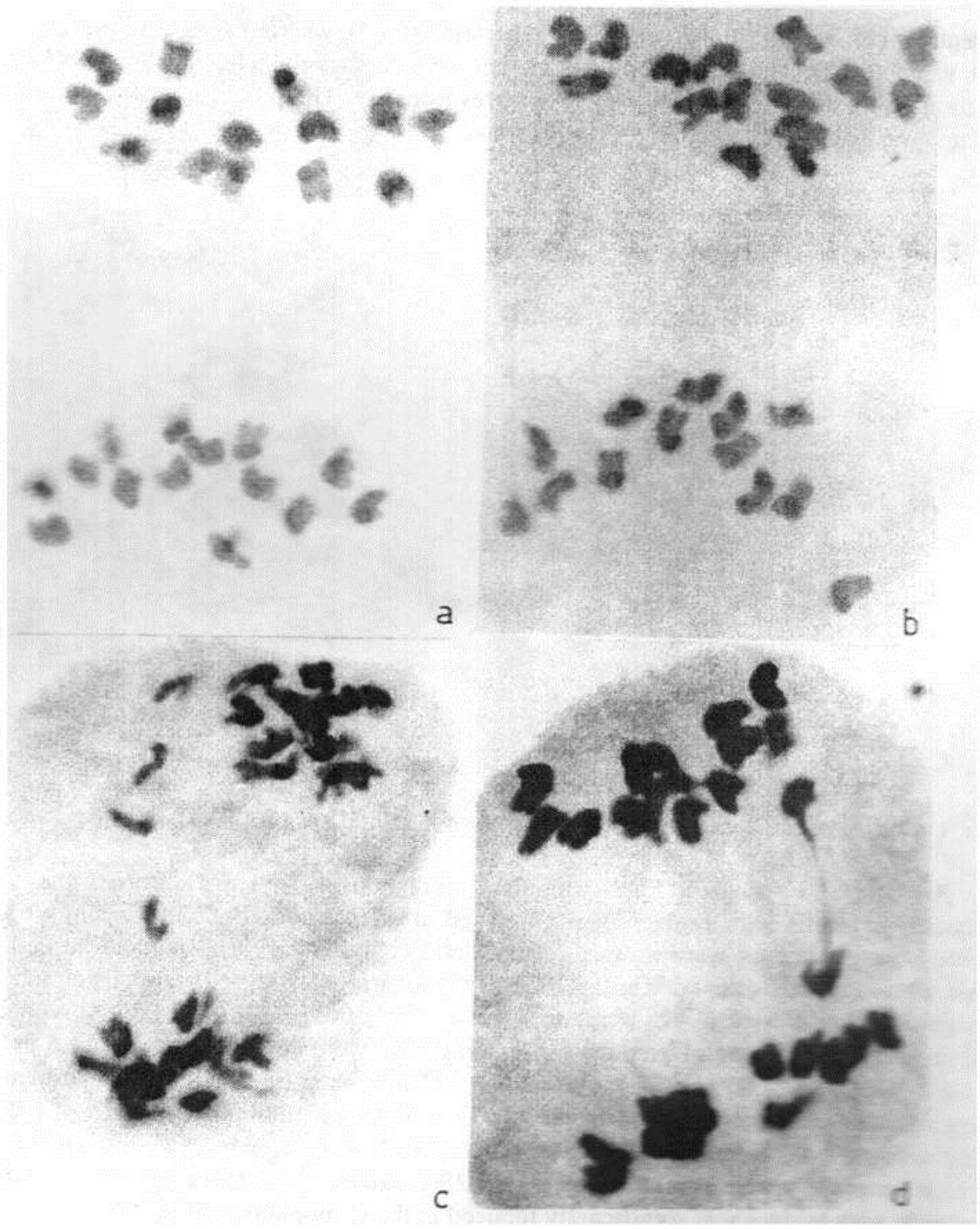

Figure 2. The anaphase I cells of autotetraploid perennial rye: **a.** balance (14:14), **b.** unbalance (13:15), **c.** lagging chromatids, **d.** bridge (1000X).

Anaphase II: The cell number with lagging chromosomes and/or bridges were determined at anaphase II in the C_1, C_2 and C_3 generations of autotetraploid perennial rye. Similar to other cytological characters examined in anaphase II, the ratio of cells showing irregular segregation varied from generation to generation (C_1/C_2 t=2.463*, C_1/C_3 t=11.099**, C_2/C_3 t=5.782**). In the C_1, C_2 and C_3 populations, the ratio of cells with irregular segregation were 20.93%, 16.48% and 7.16% respectively. The most common of abnormal anaphase II segregation was cells with lagging chromosome (Table 3).

Table 3. The Numbers and Percentages of Anaphase II (AII) Cells with Lagging Chromosome and/or Bridges in Different Generations of Autotetraploid Perennial Rye.

Generations	Num. Plant	Total Num. Cell	Irregular A II Segregations			Rates of Irregular A II Cells		
				Lag. Chromo.	Bridge	Mean	Min.	Max.
C_1	25	1869	Num.	346	45	391	14	29
			%	18.51	2.41	20.93	15.38	40.85
C_2	25	2113	Num.	293	55	348	6	24
			%	13.87	2.60	16.48	4.92	35.82
C_3	25	2081	Num.	136	13	149	1	13
			%	6.54	0.62	7.16	1.25	18.31

Tetrads: The number of micronuclei in tetrads that is a criteria for meiotic stability were different among generations examined in the perennial rye (C_1/C_2 t=2.6767*, C_1/C_3 t=14.3423**, C_2/C_3 t=6.3447**). According to our data summarized in Table 4, the ratio of tetrads with micronuclei were 18.17% in the C_1, 14.06% in the C_2 and 6.17% in the C_3 generations. The numbers of micronuclei per tetrad were 0.30 in the C_1, 0.24 in the C_2 and 0.10 in the C_3. The percentage of tetrads without micronuclei increased in advanced generations or in other words, the number of micronuclei per tetrad decreased. In all populations, the percentage of tetrads with one micronucleus was higher than those of other tetrads with two, three and four micronuclei. In the C_1, C_2 and C_3 populations, the ratio of tetrads with one micronucleus were 8.64%, 6.78% and 3.26%, respectively (Table 4). On the other hand, tetrads with four or more micronuclei were highly rare.

Table 4. The Numbers of Micronuclei in Tetrads and the Percentages of Tetrads with Micronuclei in Different Generations of Autotetraploid Perennial Rye.

Generations	Num. Plant	Total Num. Tet.	Numbers of Micronuclei in Tetrads					Micronuclei Per Tetrad	Rates of Tetrads with Micronuclei		
				1	2	3	4		Mean	Min.	Max.
C_1	25	4435	Num.	383	330	60	33	0.30	806	17	44
			%	8.64	7.44	1.35	0.74		18.17	12.59	29.30
C_2	25	5014	Num.	340	267	58	40	0.24	705	13	39
			%	6.78	5.33	1.16	0.80		14.06	7.29	27.46
C_3	25	5222	Num.	170	112	32	8	0.10	322	3	15
			%	3.26	2.14	0.61	0.15		6.17	1.57	11.72

Percentage of Seed Set: The percentages of seed set of autotetraploid perennial rye were given in Table 5. As it was seen in Table 5, the highest seed set was determined in C_3 (59.59%), while the lowest seed set was obtained from the C_1 (27.72%). The differences among generations were statically significant. In autotetraploid perennial rye, selection for regular meiosis increased seed set. It could said that, both establishment of cytological balance and vigor growth of plants have affected on seed set.

Table 5. Percentages of Seed Set in Different Generations of Autotetraploid Perennial Rye.

Gener-ations	Number Plant	Percentages of Seed Set (%)			
		Mean	Min.	Max.	C.V.
C_1	25	27.72	12.96	39.20	26.91
C_2	25	43.51	21.05	61.11	24.56
C_3	25	59.59	47.61	70.46	12.21

C_1/C_2 t= -6.0928**; C_1/C_3 t= -15.2506**; C_2/C_3 t= -5.7942**

** P< 0.01

Correlations Between Meiotic Behavior and Seed Set: Meiotic irregularities is one of the reasons of low seed set in induced autotetraploid populations. The correlation coefficients among seed set and percentage of metaphase I cells with univalents, percentage of irregular anaphase I, percentage of irregular anaphase II and percentage of quartets with micronuclei were calculated from the averages of C_1, C_2 and C_3 populations (Table 6). Seed set was negatively correlated with the four meiotic characteristics. On the other hand, the relationships among meiotic characteristics were positively significant. It shows that abnormal chromosomal behavior in the first stage of meiosis and continued during later stages of meiosis.

Table 6. The Correlation Coefficients (r) Among Characters Examined in Different Generations of Autotetraploid Perennial Rye.

Characters	2	3	4	Seed Set
1. Metaphase I with univalent	0.783**	0.732**	0.573**	-0.841**
2. Abnormal anaphase I		0.853**	0.822**	-0.792**
3. Abnormal anaphase II			0.824**	-0.733**
4. Tetrads with micronuclei				-0.744**

** Significant at level P< 0.01

DISCUSSION

In this study, there has been an important progress in seed setting and improvements of meiotic stability of autotetraploid perennial rye by selecting the plants with well developed and big ears. Hossain and Moore (1975) supported our result by stating that the selection to be directed towards plants grow up well is just as important as cytological factors related to increasing the fertility in the autotetraploid rye. Furthermore, they have put forward that the significant positive correlation between seed set and spike characters in the population may indicate that spike length and spike number represent physiological vigour during the initiation of the reproductive phase and may be exploited in practical plant breeding.

In this work, metaphase I univalents, the rate of cells showing irregular deviation and the number of seeds have been dwelled upon in order to determine the meiotic regularity of the examined populations.

In an autotetraploid, four homolog chromosomes can be paired as 1 quadrivalent, 1 trivalent + 1 univalent, 2 bivalent or 1 bivalent + 2 univalent in metaphase I or they can remain as univalent as well. According to this, cells with one univalent detected in our research indicated that there was absolutely one trivalent. Other univalent numbers can result from other possible pairings. Hilpert (1957) has put forward that pairing in the form of trivalent + univalent will cause the irregular segregation of chromosomes to the sister cells and those remaining lagging in the anaphase I might be lost in the second division.

In the previous studies on the autotetraploid rye under greenhouse conditions it has been determined that the average univalent number per cell is 0.68 (Sağsöz and Akgün 1994) in the C_0 generation whereas it was 0.55 in the C_1 plants growing up without any selection being practiced. Especially, after selection of the well-grown plants with many tillers, the univalent

frequency per cell has decreased to 0.24 in the meiotic analysis on these plants. In the following generations, the continuity of this pressure from the selection has considerably decreased the univalent number per cell. It was determined that the chromosomes have been paired frequently as bivalent and this has been followed by quadrivalent in autotetraploid perennial rye (Sağsöz and Akgün, 1994; Akgün, 1994). Similar results have been reported by the other workers on autotetraploid rye with respect to pairing configurations of the chromosomes (Hilpert, 1957; Aastveit, 1968; Aslım, 1994). In a study using the C_2 populations of the different two tetraploid rye, it has been determined that the univalent number per cell varied between 0.055-0.510 in metaphase I and, the rate of pollen mother cells with one, two and three univalents was 91.7, 7.7 and 0.6%, respectively (Popova et al., 1978). Evans and Rahman (1990) have reported that the segregation of chromosomes in anaphase I has been regular in the pollen mother cells which do not contain trivalent and univalent in autotetraploid barley. Besides, they have detected that some quadrivalents may not be segregate balanced (2:2). Again, unbalanced segregation of quadrivalents has been noticed by many researchers studying different species (Hilpert, 1957; Simonsen, 1975; Arundhati et al., 1983) in the C_3 generation, the average rate of cells with univalent has been found to be higher than the rate of cells showing irregular segregation in the anaphase I. It has been thought that the source of this case that segregation of some univalents to poles are balance or as the result of the segregation of bivalents in the early phase (desinapsis), especially in the cells containing more than one univalents emerge showing balanced segregation of chromosomes. On the other hand, it should not be forgotten that some quadrivalents configurations may irregularly segregate and those segregation of AI may be unbalanced.

In this study, positive and important correlations were determined among cytological characters in meiosis. Similar results were detected by the researchers working on different plants (Sağsöz, 1976; Akgün, 1994; Tosun, 1995). This shows that any abnormality starting in the early stage of meiosis (especially, in the stage of chiasmata forms) were carried to the following stages and thus aneuploids or nonfunctional microspores occur. During the meiosis analysis the cells with diploid (2n=14) chromosomes or lacking chromosomes have observed in autotetraploid perennial rye. Certainly, this aneuploid gametes will contribute to inhibit the fertilization.

Meiotic analyses are usually made in the pollen mother cells. This kind of irregularities detected pollen mother cells also emerge in the mother cells of embryo sack. Evans and Rahman (1990) reported that embryo and endosperm abnormalities determined in the earlier periods of development were influential on seed set, in the autotetraploid barley. Again, Bennett et al. (1975), reported that antipot cells had influence on providing the "coenocytic endosperm" with its protein requirements and the number and behavior of these cells differ considerably in abnormal ovaries. This is the evidence reveal that microspors are functional is just as influential as that embryo sack develops normally, on going to seed, in autotetraploid plants.

The rate of seed set determined in the plants belonging to the various generations of autotetraploid perennial rye have shown differences. In a study conducted under greenhouse conditions by Özer and Sağsöz (1991) in which has been noticed that the rate of seed set in the C_0 (19.36%) was considerably low as compared to the rate of its diploids (42.38%) in autotetraploid perennial rye. It is known that after artificial tetraploids being obtained seed set will increase depending on the selection. Vitvitskii (1982) demonstrated that there was not remained any important difference between diploids and tetraploids in relation to seed set after selection in the rye during six years. In another study conducted by Bremer and Bremer-Reinders (1954) after a selection along seven generations seed set in tetraploid plants has risen from 60% to 75%. Similarly in our study, an important progress was found in the rate of seed set and average seed set increased from 27.72% to 59.59%.

Due to a close relationships between regular meiosis and high seed set in autotetraploid plants, selection to be made in order to improve one of this features will positively influence on

the other. On the other hand, it is known that genetic factors as well as environmental factors have influence on seed set (Aastveit, 1968; Hebblethwaite and McLaren, 1979; Elgersma, 1990).

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