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Seed set and some cytological characters in different generations of autotetraploid perennial rye (*Secale montanum* Guss)

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Abstract Artificial autotetraploids of diploid perennial rye (*Secale montanum* Guss) plants were obtained by colchicine treatment. C_1, C_2 and C_3 generations of autotetraploid perennial rye were compared for seed set and cytological characteristics such as irregularities in metaphase I (univalents) and anaphase I and II (lagging chromosomes and/or chromatids and bridge formation) and micronuclei in tetrads. In the three generations (C_1, C_2 and C_3) the percentage of cells with univalents was 18.51, 14.56 and 9.38% respectively. The percentage of cells with irregular anaphase I with univalents was 23.43, 15.93 and 7.85%, and for anaphase II cells it was 20.93, 16.47 and 7.16. The percentage of tetrads with micronuclei was 18.17, 14.07 and 6.17% and seed set was 27.72, 43.51 and 59.59% respectively. A negative correlation was found between seed set and the cytological characteristics investigated. The correlations among the characters investigated during meiosis division were statistically significant. Selection of vigorous plants of autotetraploid perennial rye increased seed setting by increasing the cytological balance. However, abnormalities in the early stage of meiosis division were reflected in the later stages of meiosis.

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INTRODUCTION

Wild plant species are increasingly important in the development of new crops. The wild perennial rye (*Secale montanum* Guss.) is believed to be the ancestor of the cultivated rye (*S. cereale* L.) (Stutz 1972), and the Eastern Anatolian region of Turkey is one of the primary gene pool centres of *Secale montanum* (Hoffman et al. 1985). There are many different forms of perennial rye in Turkey. Among the species, *Secale montanum* Guss var. *anatolicum* Boiss and *Secale montanum* Guss var. *vavilovi* Grossh are important (Kün 1988). These perennial species have large stature, high frost resistance, strong tillering ability, slightly prostrate growth habit and tolerance to poor soils and drought, and their seeds are easily germinated (Reimann-Philipp 1986; Wang 1987; Evans et al. 1982). However, wild perennial rye has poor agronomic value as a forage crop because of its small and sparse leaves and its breaking peduncles that are a problem for seed production. If these faults could be overcome, wild perennial rye could be particularly important for forage production.

Induced autoploidy may be one way of improving these perennial diploid species. *Secale montanum* has a low chromosome number (2n=2x=14) and is cross-pollinated (Akgün et al. 1996). These characters are ideal for autoploidy. Generally, autotetraploid plants are more vigorous than their diploid parents; their stems are thicker and stouter, leaves are thicker, larger, and deeper green, and roots, flowers and seeds are larger. On the other hand, autopolyploid plants in general have a reduced number of seeds per plant (Poehlman 1987). This reduction comes partly from reduced numbers of flowers per plant, and partly from a reduction in the percentage of fertile flowers (Aasveit 1968). Meiotic irregularities are probably responsible for low fertility through the production of genetically imbalanced products since there is a correlation between the pattern of chromosome

pairing and disjunction at meiosis. A number of previous studies have determined that fertility in different species is reduced by the presence of univalents and trivalents (Hossain & Moore 1975; Narasinga & Pantulu 1982; Evans & Rahman 1990). Moreover, increasing numbers of univalents in pollen mother cells decrease pollen viability (Roseweir & Rees 1962), and chromosomes at anaphase I should segregate evenly to the poles for high fertility in induced autotetraploids (Arundhati et al. 1983; Sağsöz et al. 2002). However, many physiological characteristics, such as plant vigour, in addition to cytological factors affect seed set and fertility in autotetraploid populations (Hossain & Moore 1975).

The objectives of the study were to obtain artificial tetraploids of perennial rye (*Secale montanum*) by using colchicine, and to determine some cytological characteristics and fertility in advanced generations of autotetraploid perennial rye.

MATERIAL AND METHODS

Seeds of *Secale montanum* were collected from plants growing naturally in Erzurum, Turkey. The plants were selected based on their morphological characteristics such as vigour and high tillering capacity. Seeds of selected plants were grown on the experimental farm of the Faculty of Agriculture, Atatürk University.

The seeds were germinated, and 2500 seedlings with roots 2–3 mm long were selected. A 0.1% colchicine solution was applied for 3 h at 30°C. After the seedlings were washed six to seven times, first with deionised water and then with tap water, 200 control and 2500 treated seedlings were planted into growth flats. Control seedlings were planted as a single row (50 seedlings) in each growth flat. After 2–2.5 months, surviving seedlings were transplanted individually to the pots and were grown under greenhouse conditions. Tetraploid plants were determined by microscopic evaluations and cytological and morphological characteristics of the diploid and tetraploid plants were examined. Chromosome numbers were determined by a modification of conventional root tip squash methods as follows. Five or six root tips were collected from each plant. The root tips were pretreated in α-monobromnaphthalene saturated with water for 3 h at room temperature, fixed in Farmer's solution (3:1 ethyl alcohol:acetic acid), stained by the Feulgen method after hydrolysing for 20 min in 1 *N* HCL at 60°C, and squashed in 45% acetic acid. To determine the chromosome number, at least five good metaphase plates from each plant were examined.

C_1 plants produced from C_0 plants were grown in 10 cm diameter plastic pots containing a mixture of soil:farm manure:sand (1:1:1) under greenhouse conditions. Vigorous and high tillering capacity plants were selected from the C_1 population, and the plants were transplanted in the field into the plots at a spacing of 15 × 30 cm within and between rows, respectively. To obtain the future generations (C_2 and C_3), vigorous and large-spiked plants were selected and used for seed production. After one generation of multiplication the populations were grown as spaced plants. Spike samples from 25 plants of each generation (C_1, C_2 and C_3) were taken (total 75 spikes) and meiotic characters were investigated in these spikes. In addition, seed setting of the same plants was recorded on two spikes for each generation from each plant (2 × 25 = 50 spikes from each generation) and seed set rate was calculated for each plant. The plant samples used to determine both the meiotic stage and seed setting were cross-pollinated progenies of the plants of the same population. Within the population, different generations were separated by approximately 1000 m to prevent cross-pollination. Thus, cross-pollination occurred only among plants in the same generation.

The spike samples used in meiotic studies were fixed with modified Carnoy's solution (6:3:1; ethanol: glacial acetic acid:chloroform) and were stored in 75% alcohol in a refrigerator. Squash preparations from two or three anthers were stained with 2% aceto-orcein. To determine meiotic irregularities in each generation, a minimum of 100 metaphase I (MI), 50 anaphase I (AI) and anaphase II (AII), and 150 tetrads in each plant's pollen mother cells (PMC) were examined. In AI and AII phase, irregular segregations (lagging chromosomes or chromatids and bridges) were examined whereas in MI and tetrads, univalents and number of micronuclei were examined, respectively. Relationships between chromosomal behaviour in different stages of meiosis and seed set were investigated. Data obtained from three populations were compared by using the *t*-test for significance.

RESULTS

Cytological investigations

After colchicine treatment, 134 seedlings, corresponding to 5.36%, survived. They showed retarded

growth, had thick coleoptiles and dark-coloured, wide and short leaves. Mitotic chromosomes of seedlings were evaluated 35–40 days after transplanting to pots. The frequency of tetraploid plants was 5.97% (eight plants), aneuploid 2.98% and mixoploid plants 2.24%, with high levels of chimeric structures also obtained. Tetraploid plants had 2n=28 chromosomes. Shoots from sectorial chimeras were vegetatively propagated and separated from diploid and polyploid clones differing in the number of chromosomes.

Metaphase I

The frequencies of cells with univalents at metaphase I among the generations were significantly different ($P < 0.01$). The frequency of cells with univalents at metaphase I was 18.51% in C_1, 14.55% in C_2 and 9.38% in C_3 generations (Table 1). The number of univalents per cell was highest in the C_1 generation (0.24), whereas the C_3 generation had the least (0.12). Generally, PMCs had one univalent, and the percentage of PMCs with one univalent in C_1, C_2 and C_3 populations was 13.44, 12.16 and 6.89%, respectively. Pollen mother cells with four univalents were rarely observed in any population.

Anaphase I and II

Regular and irregular segregation of lagging chromosomes or chromatids and cells with bridges were determined at anaphase I and II (Fig. 1, 2). Generally, lagging chromatids or fragments were observed in the cells containing bridges and these cells were scored together with cells with bridges. The frequency of irregular segregation at anaphase I and II was significantly different between generations ($P < 0.01$). The rates of irregular segregating cells at anaphase I and II were 23.43 and 20.92%, in C_1, 15.93 and 16.47% in C_2 and 7.86 and 7.16%, in C_3 populations, respectively. In C_1, C_2 and C_3 populations, rates of cells with lagging chromosome at anaphase I were 17.07, 9.90 and 5.52%, at anaphase II were 18.51, 13.87, and 6.54% respectively (Table 2).

Tetrads

The ratios of tetrads with micronuclei were 18.17% in C_1, 14.06% in C_2 and 6.17% in C_3 (Table 3). The numbers of micronuclei per tetrad (M/Q) were 0.30 in C_1, 0.24 in C_2 and 0.10 in C_3. The percentage of tetrads without micronuclei increased in advanced generations, i.e., the number of micronuclei per tetrad decreased. In all populations, the percentage of tetrads with one micronucleus was higher than those with two, three and four micronuclei. In C_1, C_2 and C_3 populations, the ratio of tetrads with one micronucleus was 8.64, 6.78 and 3.26%, respectively. Tetrads with four or more micronuclei were very rare (Table 3).

Seed set

The percentage seed set of autotetraploid perennial rye is given in Table 3. The highest seed set was obtained in C_3 (59.59%), while the lowest seed set was obtained from C_1 (27.72%). The differences among generations were statistically significant ($P < 0.01$).

The interrelationships between a number of characters were studied (Table 4). Seed set was negatively correlated with the four meiotic characteristics (percentage of metaphase I cells with univalents, percentage of irregular anaphase I and II and percentage of quartets with micronuclei), the coefficients all being highly significant (–0.841**, –0.792**, –0.733**, –0.744**, respectively). On the other hand, the relationships among meiotic characteristics were positive and significant.

Table 1 The numbers and percentages of metaphase I cells with univalents in different generations of autotetraploid perennial rye (%). C_1/C_2 $t = 2.926$**; C_1/C_3 $t = 7.426$**; C_2/C_3 $t = 3.549$**. **, $P < 0.01$.

Generations	*N*	No. of cells examined		Univalents in metaphase I					Mean univalent I/cell
				1	2	3	4	Total	
C_1	25	3571	(no.)	480	157	23	1	661	0.24
			(%)	13.44	4.4	0.64	0.3	18.51	
C_2	25	4391	(no.)	534	91	12	2	639	0.17
			(%)	12.16	2.07	0.27	0.05	14.55	
C_3	25	2611	(no.)	180	56	8	1	245	0.12
			(%)	6.89	2.14	0.31	0.04	9.38	

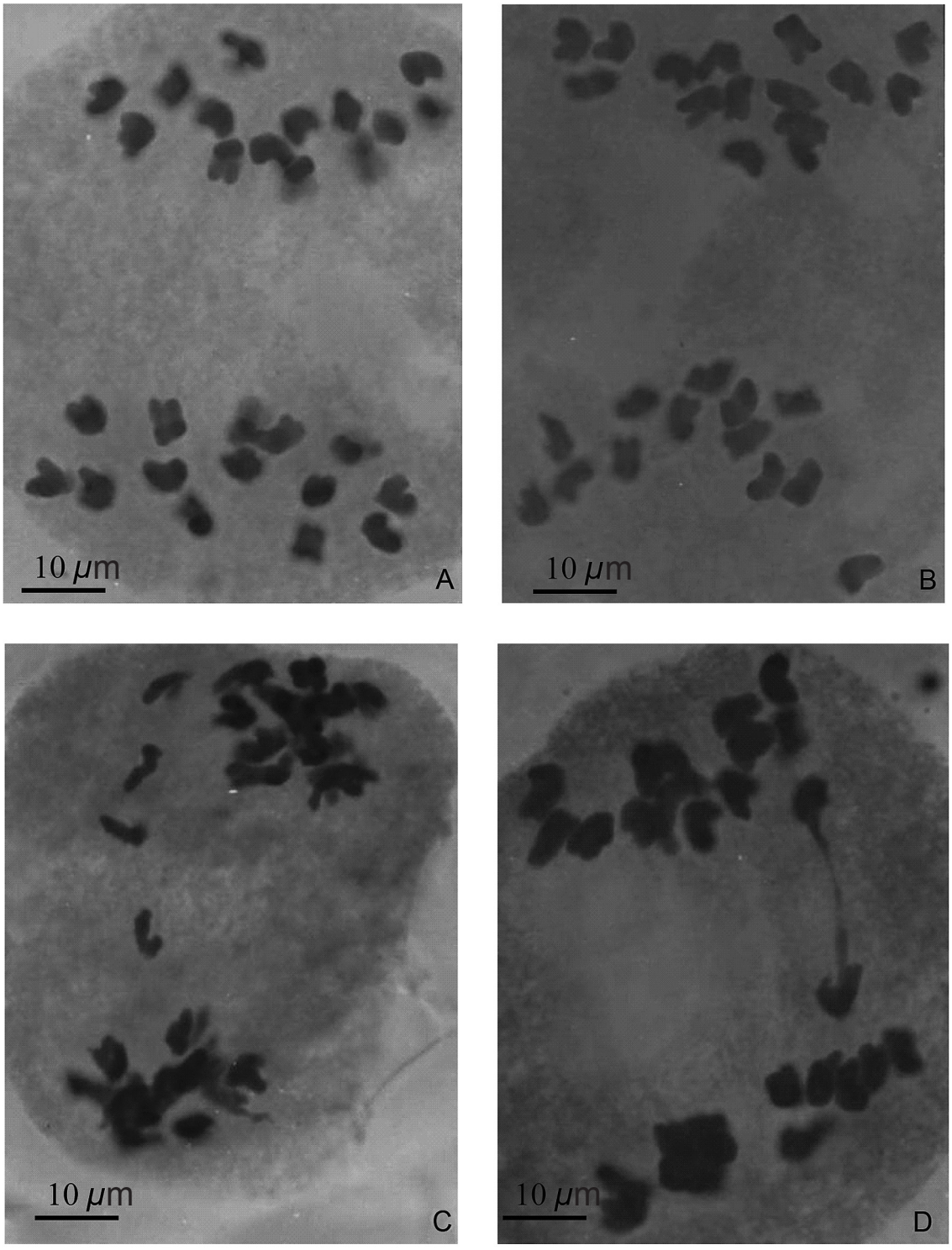

Fig. 1 The anaphase I cells of autotetraploid perennial rye: **A**, balanced segregation (14:14); **B**, unbalanced (13:15); **C**, lagging chromatids; **D**, bridge.

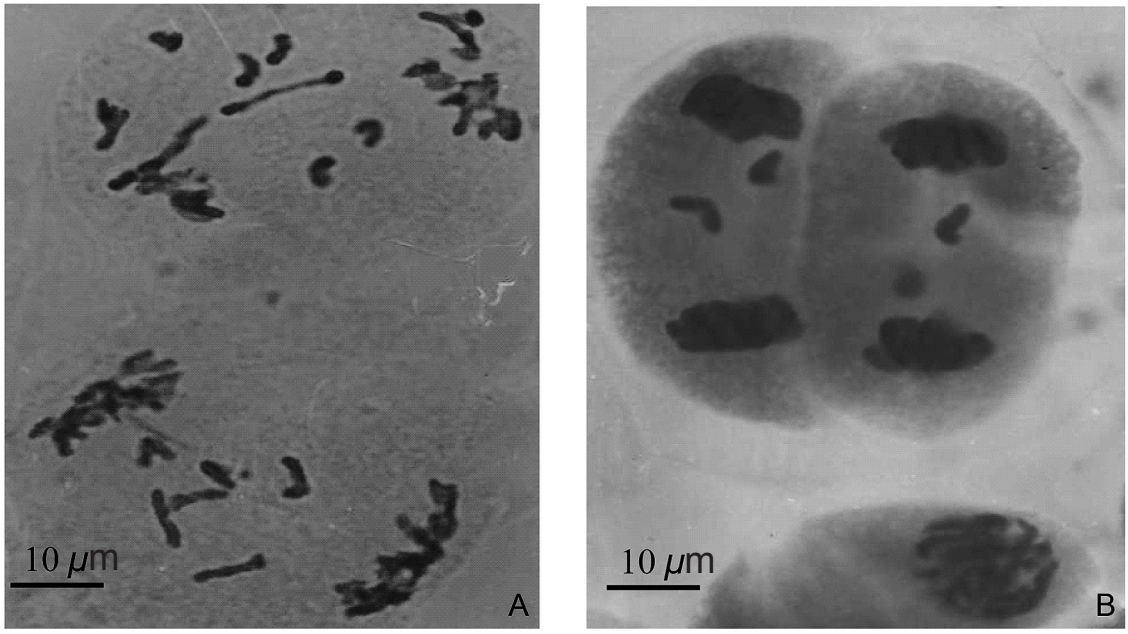

Fig. 2 **A**, The anaphase II cell with lagging chromosomes and bridges; **B**, the anaphase II cell with lagging chromosomes.

Table 2 The numbers and percentages of irregular anaphase I and II in different generations of autotetraploid perennial rye (%). AI, anaphase I C_1/C_2 $t = 4.806$**; C_1/C_3 $t = 10.360$**; C_2/C_3 $t = -5.064$**. AII, anaphase II C_1/C_2 $t = 2.463$*; C_1/C_3 $t = 11.099$**; C_2/C_3 $t = 5.782$**. *, $P < 0.05$; **, $P < 0.01$.

		No. of cells examined			Irregular anaphase segregations					
					Lagging chromosome		Bridge		Total	
Generations	*N*	AI	AII		AI	AII	AI	AII	AI	AII
C_1	25	2390	1869	(no.)	408	346	152	45	560	391
				(%)	17.07	18.51	6.36	2.41	23.43	20.92
C_2	25	3132	2113	(no.)	310	293	189	55	499	348
				(%)	9.9	13.87	6.03	2.6	15.93	16.47
C_3	25	2191	2081	(no.)	121	136	51	13	172	149
				(%)	5.52	6.54	2.33	0.62	7.85	7.16

This shows that abnormal chromosomal behaviour formed in the first stage of meiosis continued in further stages of meiosis.

DISCUSSION

After colchicine treatment of germinated seeds of perennial rye (*Secale montanum*), tetraploid plants (2n=4x=28) were obtained, and aneuploid and mixoploid plants with chimeric structures were also observed. Additionally, the ratio of unaffected plants was at a high level. Induced autopolyploids have many superior characteristics; however, they have important problems such as low fertility. This problem originates from cytological instability and physiological disharmony because of unbalanced gene combinations (Elgersma 1993). In an autotetraploid, four homologous chromosomes can be paired as 1 quadrivalent, 1 trivalent + 1 univalent, 2

Table 3 The percentages of tetrads with micronuclei and seed set in different generations of autotetraploid perennial rye. M/Q, the number of micronuclei per tetrad; CV, coefficient of variation. Micronuclei in tetrads: C_1/C_2 $t = 2.6767$*; C_1/C_3 $t = 14.3423$**; C_2/C_3 $t = 6.3447$**. Seed set: C_1/C_2 $t = –6.0928$**; C_1/C_3 $t = –15.2506$**; C_2/C_3 $t = –5.7942$**. *, $P < 0.05$; **, $P < 0.01$.

Generations	*N*	No. of cells examined		No. of micronuclei in tetrads					M/Q	Seed set (%)	
				1	2	3	4	Total		Mean	CV
C_1	25	4435	(no.)	383	330	60	33	806	0.31		
			(%)	8.64	7.44	1.35	0.74	18.17		27.72	26.91
C_2	25	5014	(no.)	340	267	58	40	705	0.24		
			(%)	6.78	5.33	1.16	0.8	14.07		43.51	24.56
C_3	25	5222	(no.)	170	112	32	8	322	0.10		
			(%)	3.26	2.14	0.61	0.15	6.17		59.59	12.21

Table 4 The correlation coefficients among examining characters in different generations of autotetraploid perennial rye ($n = 75$). **, $P < 0.01$.

Characters	1	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**

bivalents or 1 bivalent + 2 univalents in metaphase I or they can all remain as univalents. According to this, cells having one univalent would also have one trivalent. The association of one trivalent and one univalent will cause irregular segregation of chromosomes in the sister cells, and laggards at anaphase I may be lost in the second division. Previous studies have shown that the cause of low fertility in different species was the formation of univalents and trivalents (Hossain & Moore 1975; Narasinga & Pantulu 1982).

Evans & Rahman (1990) have reported that the segregation of chromosomes in anaphase I is regular in PMCs that do not contain trivalents and univalents in autotetraploid barley. They also found that some quadrivalents may not segregate equally (2:2). Unbalanced segregation of quadrivalents has also been observed by researchers studying different species (Simonsen 1975; Arundhati et al. 1983). In the C_3 generation, the average proportion of cells with univalents was higher than the proportion of cells showing irregular segregation in the anaphase I. It has been thought that this may be the result of balanced segregation of some univalents to the poles, or of the segregation of bivalents in the early phase (desynapsis), especially in the cells containing more than one univalent, resulting in balanced segregation of chromosomes (O'Mara 1943).

Anaphase I and II data revealed a greater irregularity in the C_1 than in the other generations. The first generation had a higher proportion of tetrads with micronuclei and a lower percentage seed set than the C_2 and C_3 generations. Higher micronuclei number per tetrad may result from higher numbers of lagging chromosomes and bridges at anaphase I and II. Sağsöz et al. (2002) reported a significant positive correlation between the frequency of anaphase I with lagging chromosomes and the frequency of the micronuclei in tetrads in autotetraploid ryegrass populations.

In this study, there has been important progress in seed setting and improvements of meiotic stability of autotetraploid perennial rye by selecting the plants with well developed large ears. The mean seed set in the C_3 generation was greater than that of both the C_1 and C_2. This is correlated with a lower frequency of meiotic irregularities. Positive and important correlations were found among cytological

characters in meiotic divisions. This shows that any abnormalities starting in the early stage of meiosis (especially during chiasmata formation) were carried into the following stages resulting in aneuploids or non-functional microspores. These aneuploid gametes will hinder successful fertilisation, but selecting individual plants with high seed set will help to eliminate aneuploids.

In all groups, frequent meiotic irregularities were observed. However, in the C_3 generation, the average proportion of cells with univalents and the proportion of cells showing irregular segregation in anaphase I and II were lower than that of both the C_1 and C_2. This confirms the results of Hossain & Moore (1975) that selection directed towards the plants that grow well is just as important as cytological factors related to increasing the fertility in autotetraploid rye. Furthermore, they suggested that the significant positive correlation between seed set and spike characters in the high seed setting population may indicate that spike length and spike number represent physiological vigour during initiation of the reproductive phase, and may be exploited in practical plant breeding. Similar investigations in other crops commonly show that after artificial tetraploids are obtained seed set can be increased by selection (Bremer & Bremer-Reinders 1954; Vitvitskii 1982; Arundhati et al. 1983). Similarly in our study, average seed set increased from 27.72 to 59.59%. Consequently, seed set in these populations would be highly influenced by plant vigour; plants with the best phenotype could be genetically superior and have regular meiotic segregation. Usually, euploids are superior to aneuploids, and the effects are stronger on the generative characters. In addition, it is known that environmental factors influence seed set (Hebblethwaite & McLaren 1979; Elgersma 1990).

Thus, our results show that seed set can be affected by chromosome pairing behaviour at meiosis, and both the establishment of cytological balance and the vigour of plants have effects on seed set. In addition, positive and significant correlations were found among cytological characters at meiosis. This shows that any abnormality starting at the early stage of meiosis (especially during chiasmata formation), was maintained into the following stages.

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