COMPARING SOME CYTOLOGICAL AND MORPHOLOGICAL CHARACTERS OF DIPLOID AND AUTOTETRAPLOID PERENNIAL RYE *(Secale montanum Guss.)*

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Abstract

The objectives of the study were to obtain artificial tetraploids of perennial rye (*Secale montanum* Guss.) by using colchicine, and to compare tetraploid and diploid plants with respect to some cytological and morphological characteristics.

The seeds were germinated and 2500 seedlings with 2-3 mm root length were selected. A colchicine solution ($C_{22}H_{25}NO_6$) of 0.1% was applied for a period of 3 hours at 30^{o}C. they (200 control and 2500 treated seedlings) were planted to the growth flats. Control seedlings (untreated) were planted as a single row (50 seedlings) to 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 obtained at a rate of 5.97 %. Aneuploid, mixoploid and chimeric plants were also observed. In diploid plants, meiotic division was generally regular whereas in tetraploid plants, meiotic division was more irregular. In tetraploids of C_0 generation, the percentages of AI with regular segregation (14:14), irregular segregation (13/15; 12/16, etc.), lagging chromosomes, and a bridge were 59.87%, 11.18%, 19.74%, 9.21% respectively. The tetraploid plants grew more vigorously than diploids. Although demonstrating a lower number of tillers and seed set percentage, tetraploid plants showed higher leaf size, spike structures and stoma length, as compared to diploids. Induced tetraploid plants may be use as breeding material for the improvement of forage rye.

Key words: *Secale montanum Guss*, autotetraploid, meiosis, morphological characters

The wild perennial rye (*Secale montanum* Guss.) is believed to be the ancestor of the cultivated rye (*S. cereale* L.). Eastern Anatolian region of Turkey is one of the primary gene pool centers of *Secale montanum* Guss. (Hoffman W. *et al,* 1985). There are many different form of perennial rye in Turkey. Among these *Secale montanum* Guss *var. anatolicum* Boiss. and *Secale montanum* Guss *var.vavilovi* Grossh are important varieties (Kün E., 1988). These perennial species have large stature; high frost resistance strong tillering ability, slightly more prostrate growth habit and more tolerance to poor soils and drought, and their seeds are easily germinated (Evans G. M. *et al,* 1982; Reimann-Philipp R., 1986; Richard R., Wang C., 1987). Therefore, *Secale montanum* Guss has a low chromosome number (2n=14) and is cross-pollinated (Akgün I. *et al,* 1996). These characters are ideal for induced autoploidy breeding (Sağsöz S. *et al*, 2012).

After the reports on the efficacy of colchicine in the induction of chromosomal reduplication in plants, it has been used successfully in a number of plant species (Hague L. M., Jones R.N., 1987; Hassan L. *et al,* 1989; Sağsöz S. *et al,* 2002). The effects of doubling the chromosome number have been studied in many autotetraploid crops (Narasinga P.S.R.L., Pantulu J.V., 1982; Arundhati K. *et al,* 1983; Klinga K., 1986; Evans G.M., Rahman, M.M., 1990).

The objectives of the study were to obtain artificial tetraploids of perennial rye (*Secale montanum* Guss.) by using colchicine, and to compare tetraploid and diploid plants with respect to some cytological and morphological characteristics.

MATERIAL AND METHODS

The seeds of perennial rye *(Secale montanum* Guss*)* were collected from the plants naturally grown in Erzurum, Turkey. The seeds were germinated and 2500 seedlings with 2-3 mm root length were selected. A colchicine solution ($C_{22}H_{25}NO_6$) of 0.1% was applied for a period of 3 hours at 30 ^{o}C. After the seedlings were washed 6-7 times first with deionized water and then with tap water, they (200 control and 2500 treated

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seedlings) were planted to the growth flats. Control seedlings (untreated) were planted as a single row (50 seedlings) to each growth flat. After 2-2.5 months, surviving seedlings were transplanted individually to the pots and were grown under greenhouse conditions. Some cytological and morphological characteristics of the diploid and tetraploid plants were examined. Tetraploid plants were determined through microscopic evaluations.

Root-tip samples for mitotic chromosome counting were taken from treated seedlings. Five or six root-tips were selected from each plant. The root tips were pretreated in α-monobromnaphthalene saturated with water, for 3 hours at room temperature, fixed in Farmer's solution (3:1 ethyl alcohol : acetic acid), stained by the Feulgen method after hydrolyzing 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

In meiotic studies, spikes were fixed in Carnoy's solution. After 48 hours, the spikes were transferred to 70% alcohol solution and stored at 4-5 °C. Squash preparations of pollen mother cells (PMCs) were prepared using 2% aceto-carmine staining. The frequency of the various chromosome configurations in the cell at the first metaphase, the number of chromosomes in anaphase I plates and the number of micronuclei in tetrad cells were recorded in a number plants from each group. Chromosome associations and distribution were recorded in PMC at metaphase I and anaphase I from 15-30 cells per plant. Since the plants were maintained in the greenhouse, floret fertility was also determined in these plants at maturity.

Spike length, spikelet number, stoma length, tiller number, 1000-grain weight, the length, the width and the thickness of the leaves, and seed-set were determined in diploid and tetraploid plants.

The morphological characteristics of diploid and tetraploid groups were compared using t- test

RESULTS AND DISCUSSIONS

Chromosome Number after the Colchicine Treatment: 5.36% of colchicine treated seedlings survived, and they showed retarded growth, had thick coleoptiles, dark-colored, wide and short leaves among treated seedlings. Five showed full albino and 6 demonstated partial albinos characteristic. Most of the abnormal seedlings died within the first month. Mitotic chromosomes of seedlings were evaluated 35-40 days after planting to the pots. After the colchicine treatment, the rate of tetraploid plants was 5.97 %, and aneuploid and mixoploid plants with high levels of chimeric structures were also obtained (*table 1*). Moreover, it was observed that diploid perennial rye plants had 2n= 14 chromosomes and their tetraploids had 2n=28 chromosomes (*figure 1, 2*) Tissues from sectorial chimeras were vegetatively propagated and separated from diploid and poliploid clones differing in the number of chromosomes. Plants showing mixoploid characteristics were grouped based on their appearance and separated from diploids.

Table 1

Mitotic chromosome counting in surviving seedlings after colchicine treatment

Cytological characters	Percentage of seedling survival after treatment
Tetraploid	5.97
Diploid	83.58
Aneuploid	2.98
Mixoploid	2.24
Chimeric	7.46

Investigation of Meiosis: The stage of metaphase I could be examined from 361 polen mother cells of the control plants and 150 polen mother cells of tetraploid plants.

The stage of anaphase I (AI) was examined using 870 polen mother cells of control plants and 152 polen mother cell of tetraploid plants (*table 2*). In diploid plants, the percentages of AI showing regular segregation (7:7) and irregular segregation were 91.5 % and 0.8% respectively. In addition, 1.8% and 5.8 % of AI had lagging chromosomes and/or chromatids, and a bridge, respectively (*figure 3*)

In tetraploids of C_0 generation, the percentages of AI with regular segregation (14:14), irregular segregation (13/15; 12/16, etc.), lagging chromosomes, and a bridge were 59.87%, 11.18%, 19.74%, 9.21% respectively.

The tetrad stage was examined in diploid and tetraploid plants *(table 2*). The number of micronuclei per tetrad (M/Q) and percentages of tetrads without micronuclei were determined ((*figure 4*). The results are presented in Table 3. In control and tetraploid groups, M/Q was 0.038 and 0.298 respectively, while the percentage of tetrads without micronuclei were 96.18% and 70.16% respectively

Seed-Set: The seed set was estimated from the same plants used for analysis of meiosis. The seed-set in the tetraploids was significantly lower than that in diploids ($P<0.01$). In diploid and tetraploid plants, the percentages of seed-set were 42.38 % and 19.36 % respectively (*table 3)*.

Characteristics of Spikes: The data regarding spikes characteristics in the diploids and tetraploids were shown in *table 3*. Effect of ploidy

level on the spike length and number of spikelets per spike was statistically significant (P<0.01). The mean spike lengths in the diploid and tetraploid plants were 8.07 cm and 10.79 cm respectively; the numbers of spikelets in each spike were 26.08 and 33.81 respectively.

1000 Grain Weight: The size of seeds of tetraploid plants increased significantly as compared to diploid plants (P<0.01). While 1000 grain weight of tetraploid plants was 10.95 g, it was 6.39 g in the diploid plants (*table 3*).

Number of Tillers: The increase in ploidy level did not significantly reduce the numbers of tillers per plant. While it was 75.50 in diploids, the number of tillers per plant was 63.10 in tetraploids (*table 3*).

Leaf Characteristics: In perennial rye plants, the width and thickness of leaves increased significantly (P<0.01) depending upon ploidy level. The leaf lengths of diploids and tetraploids were 19.86 cm and 21.50 cm and did not differ significantly. While leaf width and thickness in diploids were 4.73 mm and 0.49 mm respectively, in tetraploids they were 5.87 mm and 0.70 mm respectively (*table 3*).

Stoma Lenght: Depending upon doubling the chromosomes in perennial rye, stoma length was significantly increased (P<0.01). The lengthes of the stomata in diploids and tetraploids were 19.82 and 22.65 µm respectively (*table 3*).

After the colchicin treatment to germinated seeds of perennial rye *(Secale montanum* Guss*),* tetraploid plants (2n=28) were obtained with a ratio of 5.97%, and aneuploid and mixoploid plants with chimeric structures were also observed. Additionally, it was also found that the ratio of unaffected plants were at a high level. The results showed that meiotic (anaphase I and tetrad) division was generally regular in diploid plants whereas in tetraploid plants, meiotic division was found to be mostly irregular.

Anaphase I data revealed a greater irregularity (irregular segregation, lagging chromosomes and/or chromatids and bridge) in the tetraploids as compared to the diploids. Tetraploids had a higher proportion of tetrads with micronuclei and a lower percentage of seed-set with respect to diploids. Higher micronuclei number per tetrad may result from higher lagging chromosomes and bridges at anaphase I. Sağsöz S. *et al,* (2002) reported a significant positive correlation between the frequency of anaphase I with lagging chromosomes and the frequency of the micronuclei in tetrad in the autotetraploid ryegrass populations. In addition, the bridges observed in our study could originate from paracentric inversions (Sybenga J., 1992). Evans G.M., Rahman M.M., (1990) reported that the segregation of the chromosomes in anaphase I has been regular in the pollen mother cells which do not contain trivalent and univalent in autotetraploid barley. Moreover, they observed that some quadrivalents may not show balanced segregation (2:2). The low fertility problem originates from cytological instability and physiological inharmony due to unbalanced gene combinations (Elgersma A., 1990). A number of previous studies demonstrated that the reason for low fertility in different species was univalents and trivalents (Narasinga P.S.R.L., Pantulu J.V., 1982; Evans G.M., Rahman M.M., 1990). The proposal that the meiotic irregularities are responsible for low fertility through the production of genetically imbalanced products is widely held and is based on the correlations between the pattern of chromosome pairing and disjunction at meiosis. It is known that after artificial tetraploids are obtained, seed-set will increase depending on the selection (Arundhati K. *et al,* 1983). On the other hand, genetic factors as well as environmental factors also influence seed-set (Elgersma; 1990)

Autotetraploid perennial rye plants grew more vigorously as compared to their diploids. In tetraploids the length, the width and the thickness of the leaves, the spikelet numbers of spikes and 1000-grain weight increased while the tiller number decreased, and the differences among these characters were highly significant. Rachis and the stoma length were higher in tetraploids than those in diploids. Our results are in agreement with the observations of (Phafler P.L. *et al*, 1984; Poehlman J.M., 1987).

CONCLUSIONS

The results of this investigation suggest that tetraploid plants often larger than diploid plants of the same species. Induced autoploidy may be one way of improving these perennial diploid species and the tetraploid plants may be use as breeding material for the improvement of forage rye for Turkey conditions.

Table 2

Anaphase I chromosome distribution, number of micronuclei per tetrad (M/Q) and percentages of tetrads without micronuclei in diploid and autotetraploid perennial rye (*Secale montanum* Guss)

Ploidy	N		Regular (7/7 or 14/14)	Irregular	Lagging	Bridge	Tetrad		
							N	M/Q	%
2n=14	870	No	796	7	16	51	996	0.038	96.18
		%	91.50	0.8	1.8	5.8			
2n=28	152	No	91	17	30	14	248	0.298	70.16
		%	59.87	11.18	19.74	9.21			

Table 3

The means and standard errors (SE) of morphological characters in the diploid and autotetraploid plants

Morphological characters	Ploidy	No. of sample	N	Mean ± SE	t-value
Seed –set	2n	50	10	42.38 ± 16	3.02**
	4n	50	10	19.36 ± 18	
Spike length (cm)	2n	50	10	8.07 ± 1.95	4.05 **
	4n	50	10	10.79 ± 0.85	
No.of spikelets per spike	2n	50	10	26.08 ± 5.13	3.39**
	4n	50	10	33.81 ± 5.03	
1000 grain weight	2n	400	4	6.39 ± 0.02	22.42**
	4n	400	4	10.95 ± 0.03	
No. of tillers	2n	10	10	75.50 ± 29.48	0.95ns
	4n	10	10	63.10 ± 28.47	
Leaf length (cm)	2n	150	10	19.86 ± 1.20	1.25ns
	4n	150	10	21.50 ± 0.53	
Leaf width (mm)	2n	150	10	4.73 ± 0.5	4.38**
	4n	150	10	5.87 ± 0.6	
Leaf thickness (mm)	2n	150	10	0.49 ± 0.13	4.23**
	4n	150	10	0.70 ± 0.08	
Stoma length (µm)	2n	500	10	19.82 ±1.11	2.88**
	4n	500	10	22.65 ± 3.13	

* Significant at 5 % level; ** Significant at 1 % level; ns nonsignificant

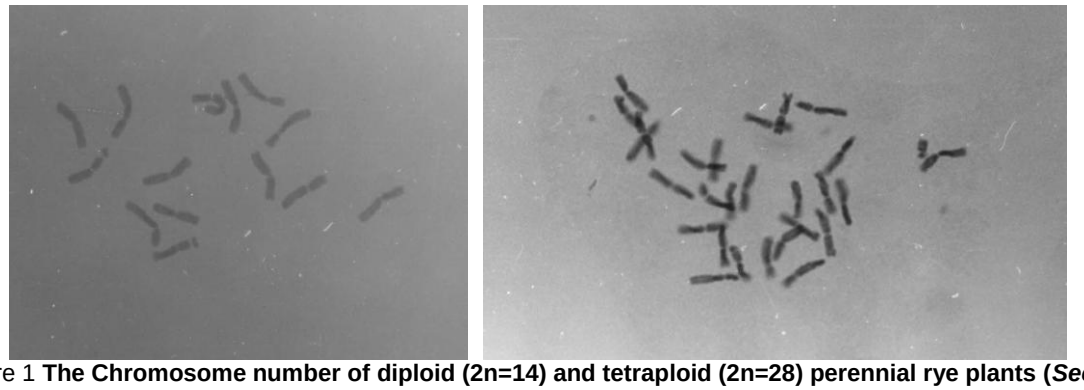

Figure 1 **The Chromosome number of diploid (2n=14) and tetraploid (2n=28) perennial rye plants (*Secale montanum* Guss.).**

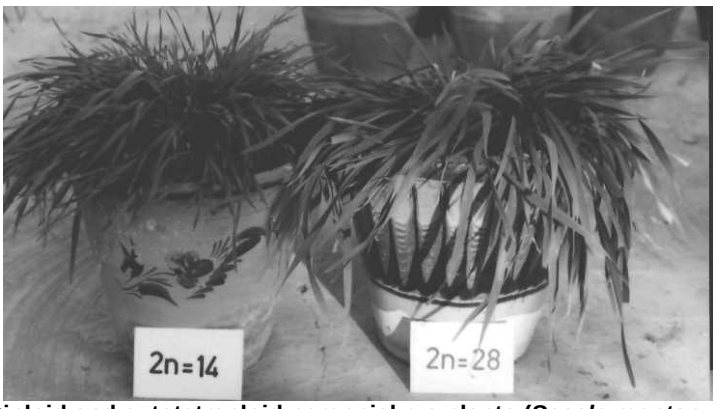

Figure 2 **Diploid and autotetraploid perennial rye plants (*Secale montanum* Guss.).**

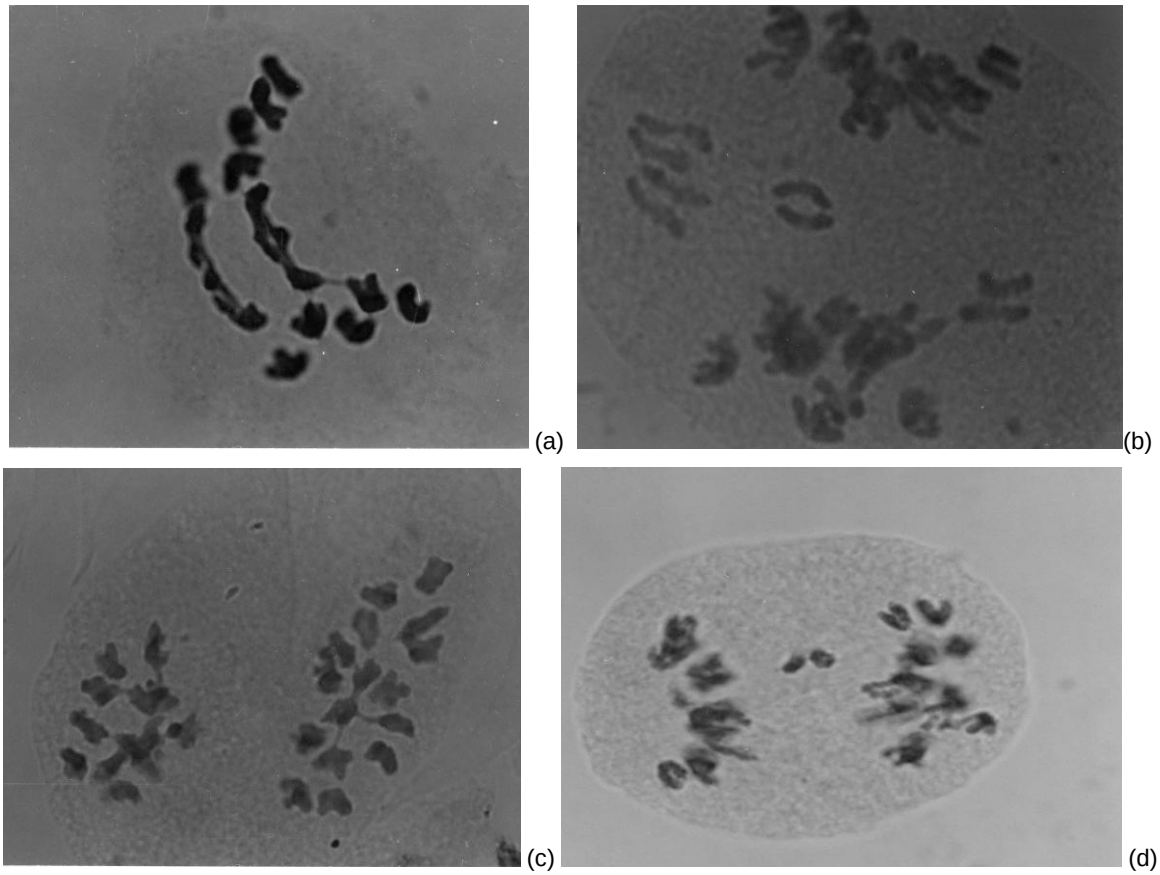

Figure 3 **Irregular chromosome segregations at AI in diploid (a) and autotetraploid (b,c,d) perennial rye plants**

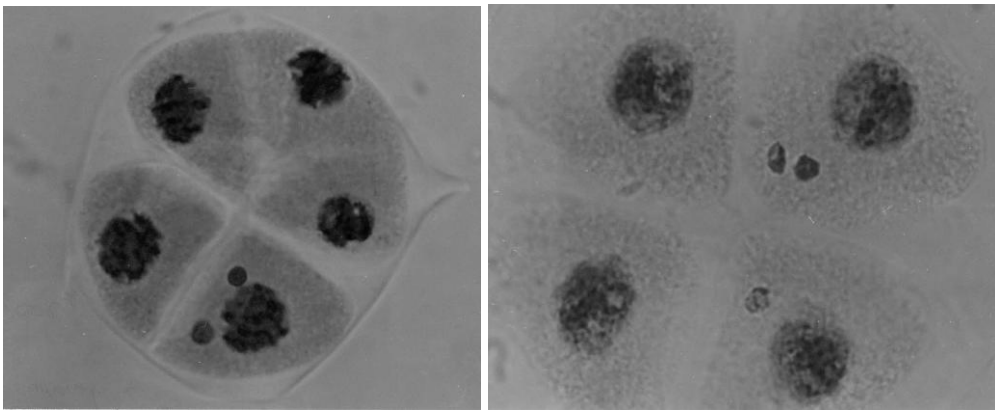

Figure 4 **Tetrad with micronuclei and abnormal tetrads in autotetraploid perennial rye plants**

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