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Agricultural and Cytological Characteristics of M_1 Perennial Rye (*Secale montanum* Guss.) as Effected by the Application of Different Doses of Gamma Rays

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Abstract: Perennial rye seeds were exposed to different doses of gamma rays (cobalt-60) (0, 2, 4, 6, 8, 10, 12, 14, 16, 20, 25 and 30 krad) to examine their effects on some agricultural and cytological characteristics in M_1 generation. The research was carried out under field and greenhouse conditions. To determine physiological injury and LD_{50}, seeds were sown according to completely randomized block design with three replications. Plants grown in field were used to identify morphological and cytological characteristics. Higher gamma ray doses decreased seed emerging rate, seedling height, spike length, spikelet number and seed set. The lethal dose (LD_{50}) was 18-20 krad and growth reduction dose (GR_{50}) was 12 krad. In M_1 generation, as doses of gamma rays increased, the frequency of metaphase I with univalent and the frequency of irregular anaphase I cells increased. In M_1 plants, anaphase I irregularities were especially seen as chromatin bridge. However, the frequency of anaphase I cells with laggards was higher than anaphase I cells with bridge at 20 and 25 krad doses of gamma rays.

Key words: *Secale montanum*, gamma radiation, morphology, meiosis

INTRODUCTION

Wild plant species are getting more important in the development of new cultivars. Several perennial wild genotypes contributed to the origination of cultivated rye (*Secale cereale* L.). Perennial species are of particularly important for grain and green matter yields[1].

Secale montanum Guss. is believed to be the ancestor of the cultivated rye. Eastern Anatolia region (Turkey) is one of the primary gene pool centers of *Secale montanum* Guss.[2] *S. montanum* is a wild plant in natural populations of Erzurum. Perennial rye is diploid with 2n = 14 chromosomes[3]. This perennial species has large stature, frost resistance, high tillering capacity, slightly more prostrate habit and easily germinating[4,5]. On the other hand, perennial rye is an important plant to prevent soil erosion in steep, arid and insufficient depth of soil area because of hairlike roots. However, wild perennial rye has poor agronomic value as a forage crop because of small and scant leaf and breaking peduncle these are problems for seed production[4,6].

To extent acreage of perennial rye, new cultivars with desired characteristics should be developed. Plant breeder must extent natural variation. At this point, mutation breeding is an effective method to create new variation. Number of mutant cultivars was 50 in 1964 and currently it has been reached to 1300 by large use of mutational methods in breeding programs. It was reported that three mutant rye cultivars had short-stemmed and, lodging and shatter resistant[7].

Induced mutations had been benefited by plant breeders to explore genetical variations resulted by the mutagens. Many report are available for the successful use of mutation breeding in the production of new cultivars in many crops[8,7,9]. For the induction of mutations in breeding programs, determining the most suitable doses of physical and chemical mutagens is important. High doses induce physiological injuries. Treatment doses range according to mutagens, plant species and materials. It is also reported that GR50 (Growth reduction 50) dose varied between 20 and 30 krad and benefit dose varied between 10 and 20 krad in mutation breeding of rye (*S. cereale*)[10].

In order to determine effects of physical and chemical mutagens, seedling height, number of viable plants, number of spike per plant, number of seed per spike and seed weight must be examined[6,11,12]. This information may be useful in selecting mutagens or doses/concentrations if there is any relationship existing between M_1 injury and the occurrence of beneficial macro- or micro-mutations.

The aims of this study were;

I to determine accurate gamma rays doses (LD_{50} and GR_{50}) for perennial rye,

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ii to investigate effects of doses of gamma rays on some agricultural and cytological characteristics of perennial rye and
iii to develop an ideal forage crop genotype of perennial rye.

MATERIALS AND METHODS

Seeds of diploid (2n=14) perennial rye (*Secale montanum* Guss.) collected from natural population were grown on the experimental Farm of the Faculty of Agriculture, University of Atatürk, Erzurum. The dry seeds (9% moisture) obtained from these plants were subjected to gamma rays emitted from cobalt-60 at The Center of Nuclear Research and Education in Turkey. Treatment doses were 0, 2, 4, 6, 8, 10, 12, 14, 16, 20, 25 and 30 krad. 1000 seeds were used for each application.

Greenhouse Experiment: To determine physiological injury and LD_{50}, seeds were sown into wooden box containing a mixture of soil: farm manure: sand (1:1:1) according to completely randomized block design with three replications. Fifty seeds were sown in each replication. The seedlings were counted on 15th day from sowing, thus percentage of seeds emerged was determined for lethal dose (LD_{50}; mutagen dose should be sufficient to kill about 50% of the seeds) and then seedling height was measured for physiological injury (GR50 dose).

Field Experiment: Seeds remaining after greenhouse experiment were sown in single rows at each block according to completely randomized block design with three replications at field for examining morphological and cytological characteristics. Blocks consisted 12 rows (control and 11 treated seed group) of 2 m length with 50 cm row spacing. Same number seed was sown for each different dose.

For each replication, a total of 60 spikes (20 spike from each row) were collected and number of spikelets, number of seeds per spike, spike length, seed set and some cytological characteristics (metaphase I, anaphase I and tetrad) were investigated[6,13,14].

Spike length: Spike length was found by measuring the length between the lowest node and the toppest node[6,14].

Spikelet number: Spikelet number was determined as average spikelet number per spike by counting all spikelets (total 60 spike) on the sample spike from each treatment. The top and bottom spikelets in all cases excluded[15,6].

Number of seed per spike: Number of seed per spike was determined by counting seeds from the spikelet samples that spikelet numbers were counted[6,15].

Seed set ratio: The studies seed set has been determined by counting the number of flowers and grains on the spikes[15]. Seed set was calculated as follow:

$$\%\text{Seed Set Per Spike} = \frac{\text{Number of grains}}{\text{Number of flowers}} \times 100$$

To determine meiotic characteristics, 15 spikes were collected from each replication and metaphase I univalents, regular and irregular (laggards and chromatin bridges) anaphase I segregations and number of micronuclei per quartet (M/Q) were examined. Sample preparation and staining were done as for Elçi[16] and Sagsöz *et al.*[17]. In meiotic studies, spikes were fixed in Carnoy's solution. After 24 h, the spikes were transferred to 70% alcohol and stored at 4 to 5°C. Aceto-carmin method was used for staining and spear preparations.

Spikes, which were little out or completely inside of the flag leaf, were selected. Pollen mother cells were investigated in anthers, which take place on the middle of the spike at the suitable phase. All data were subjected to analysis by using MSTATC statistical package program and significant differences were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Seedling Emergence Rate : The differences in emerging rate were significant ($P<0.01$) for treatment doses and when dose of gamma rays was increased, the percentage of emerging seedling was decreased. The highest percentage of seedling emergence was in control group (92%) and differences among control and 2, 4, 6, 8 doses were not significant, but differences between control and other doses were statistically significant. The percentage of emergence varied between 31.00 -92.00% according to doses and the lowest emergence rate was determined at 30 krad dose. In perennial rye, LD_{50} doses were calculated as 18 and 20 krad (Table 1).

The emergence was 3-4 days late in the seeds treated with rays, especially in 20 krad and higher doses. In higher doses (especially 30 krad), many seedlings died in following stages.

Previous researches investigating the effects of radiation applications on germination reported that germination rate was decreased by high radiation doses [11,18-20]. In addition, in high radiation doses, mitotic

Table 1: The percentage of seedling emergence and height in m_1 generation of perennial rye treated with different doses of gamma rays

Treatment Doses (Krad)	Sowing seed number	Seedling emergence (%)	Relative value	Seedling height (cm)	Relative value
Control	150	92.00a[1/]	100.00	14.33a[1/]	100.00
2	150	91.34a	99.28	14.43a	101.00
4	150	90.00a	97.83	12.58b	87.79
6	150	90.66a	87.67	10.83c	75.58
8	150	85.00ab	84.78	10.41c	72.64
10	150	78.66bc	85.50	7.98d	55.69
12	150	76.00c	82.61	7.23de	50.45
14	150	71.66c	77.89	6.17ef	43.06
16	150	73.00c	79.34	5.75f	40.13
20	150	44.00d	47.82	4.90fg	34.19
25	150	38.00de	41.30	4.30g	30.01
30	150	31.00e	39.70	2.99h	20.86

[1/] Means with the same letter(s) in a column are not significantly different from each other at P< (0.01).

Table 2: The spike length, spikelet number, seed number per spike and seed set rate in m_1 generation of perennial rye treated with different doses of gamma rays

Treatment doses (Krad)	Spike length (cm)	Spikelet number	Seed number	Seed set rate (%)
Control	13.94a[1/]	45.31a[1/]	74.58a[1/]	82.28a[1/]
2	13.88a	44.89a	69.43b	77.36a
4	13.61a	37.55bc	48.00bc	63.33b
6	14.11a	38.06bc	42.63b	56.13b
8	12.92ab	33.31cd	42.30b	63.50b
10	14.22a	40.67ab	32.17cd	39.57c
12	13.45a	32.00cd	18.77de	29.38cd
14	14.14a	32.33cd	19.42de	30.11cd
16	11.33bc	28.33d	16.44de	29.02cd
20	9.50c	19.00e	9.47de	24.99d
25	10.33c	18.05e	3.33e	9.39e
LSD	1.919	5.842	17.130	10.56

[1/]Means with the same letter(s) in a column are not significantly different from each other according to Duncan test at P< (0.01).

division capacity was retarded and seedlings died. In lentil, doses of different mutagens (gamma rays (4, 8, 12 krad), ethyl methane sulphonate, nitroso ethyl urea and sodium azide) were applied[12]. All mutagenic treatments caused considerable reduction in germination of the treated seeds. There was almost linear decrease in germination rate with increasing radiation doses or concentrations of the chemical mutagens.

Seedling height: Greenhouse grown seedling heights was measured and means values were calculated for each replication. Seedling height varied with doses of gamma rays and these variations were statistically significant. While seedling height was 14.33 cm in control group, it was 2.99 cm in 30 krad group.

The dose having 50% growth reduction (GR_{50}) rate was 12 krad (50.45%). On the other hand, in 2 krad dose treatment, seedling height increased according to control, but this increment was not statistically significant.

In mutation breeding, determination of limits of accurate mutagen dose is important. However mutagen dose is expected to create less physiological injury and more genetic variation. A quick and simple method to determine the effect of a mutagenic seed treatment is, in many plant species, the measurement of seedling height[6,12,21].

Other researchers reported that while radiation dose increased, seedling height decreased. Sarker and Sharma[12] reported in lentil that reduction in seedling height was positively correlated with dose/concentrations in all the mutagens. Among the mutagens, gamma rays caused maximum reduction in height. Similar observations were also reported by Gaul[11], Datta and Biswas[19], Amono[22] and Tutluer[6].

GR_{50} (Growth reduction 50) dose of gamma and neutron rays varied between 20 and 30 krad according to species, beneficial dose limits were between 20 and 30 krad for mutation breeding in rye[10]. In the present study, GR_{50} dose was calculated as 12 krad and this value showed that beneficial mutagen dose limit for perennial rye was similar to that of rye.

Field experiment: The seeds treated with 30 krad gamma rays had insufficient seedling emergence under (Table 2). Accordingly, this treatment was excluded from the experiment providing plant materials for generative and cytological examinations. Thus the effects of 11 treatments were examined in this stage of experiment.

Spike length: The effects of gamma rays on spike length were statistically significant and spike length varied between 9.50 and 14.22 cm depending upon the treatment

doses. The longest spike was found in 10 krad group, but the differences among this group and control, 2, 4, 6, 8, 12, 14, krad doses were not significant. The shortest spike was obtained from 20 krad dose, but differences among this dose and 16, 25 krad doses were not significant (Table 2).

Spikelet number per spike: The mean number of spikelets per spike varied depending upon the treatments and differences were statistically significant. The highest number of spikelets per spike was obtained in control group (45.31), 2 krad (44.89) and 10 krad (40.67) and these treatments statistically placed in the same group. The spikelet number varied between 18.05 and 45.31 and the lowest number of spikelets per spike was found in 25 krad dose (Table 2).

Seed number per spike: In control group and other treatments, seeds obtained from sample spikes were counted. As gamma ray dose increased, seed number per spike decreased significantly ($P<0.01$). The highest number of seeds was found in control group (74.58) and differences between this group and other treatments were significant. The seed number per spike varied between 3.33 and 69.43 according to doses of gamma rays. On the other hand, the lowest number of seeds per spike was obtained from 25 krad dose and spikes were mostly sterile in this group (Table 2).

Yıldırım *et al.*[23] reported that average spike length, spike number and grain number were higher than the control plants whereas plant height was shorter than the control in Kaya and Quantum barley mutant populations which were treated with 15 and 30 krad gamma rays. On the other hand, Yıldırım and Budak[24] noted that there were not any differences between Kaya mutant lines and control based on spike length and plant height.

Seed set rate: As doses of gamma rays increased, seed set rate decreased (Table 2). The percentage of seed set was highest in control group (82.28%) and was lowest in-group 25 krad dose (9.39%). The difference between control group and 2 krad dose was not significant, however differences between control group and other treatment doses were statistically significant.

Mutagens may create defects such as abnormal spike, inhibition of generative growth, unforming of reproductive organs in floret, undevelopment of embryo as if it is syngamy and development of abnormal seed[21,25,26]. In different plant species, mutagens increased sterility in spike and thus seed set decreased[6,12,27].

Table 3: Metaphase I and anaphase I stages in m_1 generation of perennial rye treated with different doses of gamma rays

	Metaphase I			Anaphase I				
Treatment doses (Krad)	Total cell numbers	Without univalent (%)	With univalent (%)	Total cell numbers	regular (%)	Irregular (%) lagging	Bridge	Total
Control	242	98.33	1.67	257	99.22	0.38	0.38	0.76
2	356	97.75	2.25	340	97.65	0.59	1.76	2.35
4	511	95.11	4.89	446	96.41	1.57	2.02	3.59
6	222	96.40	3.6	340	96.47	0.59	2.94	3.53
8	243	94.65	5.35	103	88.35	0.97	10.68	11.65
10	174	93.68	6.32	392	88.52	2.55	8.93	11.48
12	197	94.42	5.58	78	83.33	2.56	14.10	16.67
14	144	90.97	9.03	92	85.87	3.26	10.87	14.13
16	130	90.00	10.00	191	94.76	0.52	4.71	5.24
20	138	87.68	12.32	213	89.67	8.92	1.41	10.33
25	71	83.09	16.90	217	93.55	4.61	1.84	6.45

Table 4: The percentage of tetrads with and without micronuclei and number of micronuclei per tetrad (M/Q) in M_1 generation of perennial rye treated with different doses of gamma rays.

Treatment doses (Krad)	Total cell numbers	Without micronuclei (%)	With micronuclei (%)	M/Q
Control	1615	99.81	0.19	0.00
2	1547	98.45	1.62	0.02
4	1806	98.34	1.66	0.03
6	1898	99.05	0.95	0.01
8	671	98.51	1.42	0.02
10	1412	92.16	2.83	0.05
12	210	91.90	8.10	0.11
14	583	97.26	2.74	0.04
16	291	95.19	4.81	0.08
20	1113	90.39	9.61	0.23
25	454	91.85	8.15	0.14

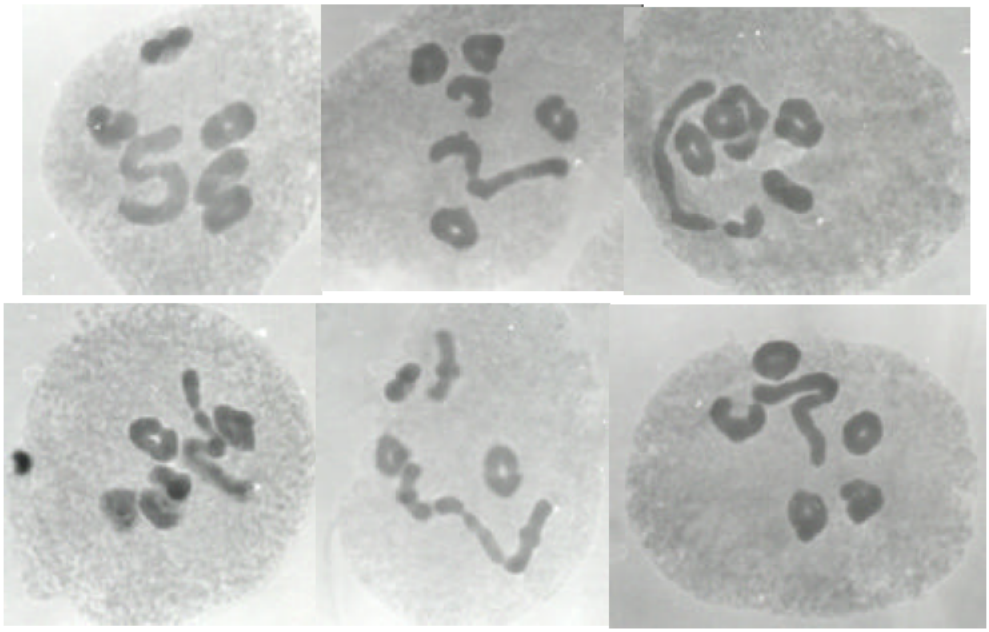

Fig. 1: Chromosome pairings at M I in M_1 generation of perennial rye

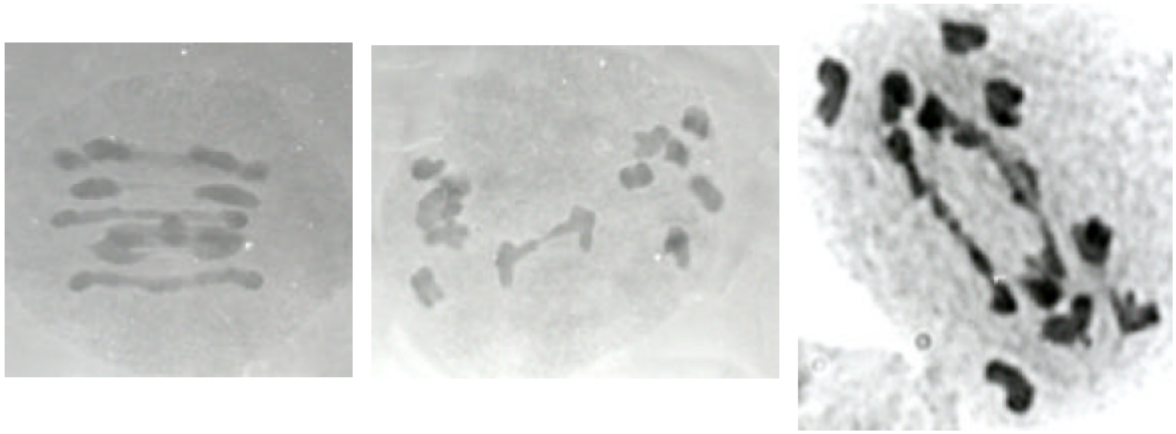

Fig. 2: Irregular chromosome segregations at AI in M_1 generation of perennial rye

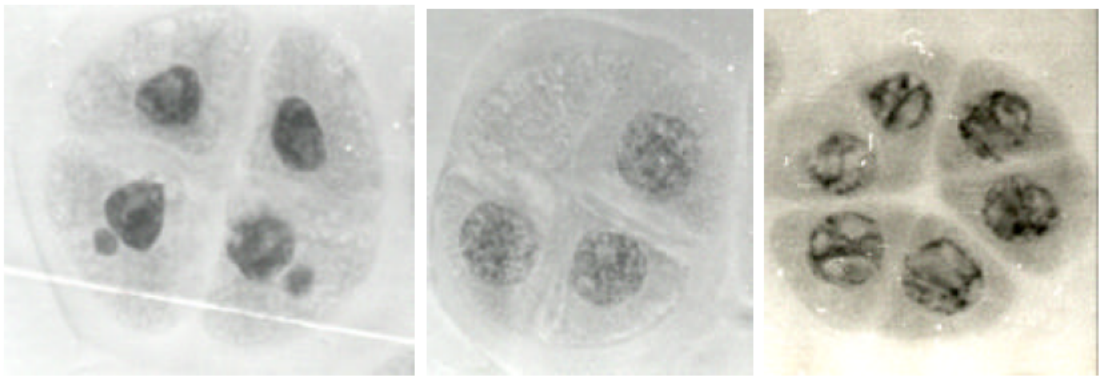

Fig. 3: Tetrad with micronuclei and abnormal tetrads in M_1 generation of perennial rye

Cytological effects

Metaphase I (MI): In control group, the frequency of MI with univalent was the lowest (1.67%). While doses of gamma rays increased, the frequency of MI with univalent increased and the highest frequency was found in 25 krad dose (16.90%). In sample spikes from control group, 98.33% of MI cells were of only bivalent pairings. On the other hand, when spikes from M_1 plants treated with different gamma rays (especially 6-12 krad doses) were examined, chromosomes paired both bivalents and quadrivalents (Table 3; Fig. 1).

Anaphase I (AI): In AI, regular and irregular chromosome segregations were examined. The highest frequency of regular AI (99.22%) was determined in control plants and this frequency according to gamma rays treatments decreased when the frequency of irregular AI cells increased. The highest frequency of irregular AI cells was observed in 12 krad treatment (16.67%) and followed by 14 (14.13%), 8 (11.65%), 10 (11.48%) and 20 (10.33%) krad doses, respectively. The percentage of irregular AI cells was lower in other treatments. AI irregularity was generally chromatin bridges (Fig. 2). Bridge formation was the most frequent in 12 krad gamma rays treatment and followed by 14, 8 and 10 krad doses, respectively. The AI cells with lagging chromosome/chromatid were observed in 20 and 25 krad doses treatments (Table 3).

Tetrad: As the criterion of meiotic stability, the percentage of tetrads without micronuclei was the highest in control group (99.81%) and radiation treatments reduced this percentage. The highest frequency of tetrads with micronuclei was determined in 20 krad dose (9,61%).

The number of micronuclei per tetrad (M/Q) changed between 0.001 and 0.235. The highest M/Q was observed in 20 (0.23), 25 (0.14) and 12 (0.11) krad doses. In tetrads with micronuclei, the percentages of tetrads with 1, 2 and 3 micronuclei were generally higher. The number of tetrads with 4 and over micronuclei was higher in 20 krad dose than other treatments. On the other hand, high doses of gamma rays were created abnormal tetrads (Table 4; Fig. 3).

As results, effect of low doses of gamma rays (2, 4 and 6 krad) on the cytological characteristics (MI pairing, AI segregations and micronuclei in tetrads) was found to be lower than effect on morphological characteristics. In high radiation doses with high physiological injury, laggards were high frequent and this result could be good indicator of chromosomal breakage. On the other hand, in most of spike samples, florets do not grow and normal structure of spike was not formed. In spike samples, seeds were not formed in most of normal florets. In *Vigna radiata*, 5-20 krad doses of radiation were applied and some cytological characteristics were investigated. While radiation dose increased, the number of multivalent pairing and univalent in MI, lagging chromosome/chromatid in AI and micronuclei per tetrad increased and fertility decreased. Furthermore, there was positive and significant correlation between chromosomal abnormalities and pollen sterility[28].

Sing and Khanna[29] investigated effects of gamma radiations in different plant species and determined that univalent frequency in MI, laggards and chromosome bridges in AI increased in plants treated with gamma rays. On the other hand, such abnormalities related with radiation doses and the frequencies of abnormalities were higher in M_1 generation than those of M_2 generation[30,31].

Datta and Biswas[19] applied different X-ray doses (4, 6, 8, 10, 20 and 30 krad) on seeds of *Nigella sativa*. They found that germination and seedling height decreased when dose were increased. These studies showed that LD_{50} dose was between 8-10 krad and determined that X-ray doses were increased on the other hand, chromosome cluster, frequency of MI with univalent, lagging chromosome and fragment increased.

In many studies, chromosome cluster, fragment, laggard, chromatin bridge and micronuclei were observed as the effects of physical and chemical mutagens[6, 30, 32, 33].

As a result of these studies, mutants for brittle spike as an important problem in domestication of perennial rye were not observed in M_1 generation. But, mutant plants having different morphological characteristics were determined. Because of the allogamous characteristics of perennial rye, genetical segregations should be carefully observed following generations. In addition to physiological injuring from gamma rays, many cytological irregularities formed. Genetic structure of our material was highly affected, favorable new genetically changes can be create in following generations.

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