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EFFECTS OF ETHYL METHANESULFONATE ON GROWTH AND YIELD PARAMETERS OF WHEAT AND TOLERANCE TO IMAZAMOX

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Abstract

In the present study objective bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) lines resistant to Imazamox herbicides and effective lethal ethyl methanesulfonate (EMS) doses for plants. During first year (2019/2020), 1000 seeds of bread wheat and durum wheat treated with 11 different EMS doses were sown under field and greenhouse conditions. During second year (2020/2021), M1 (first generation) cereal seeds were grown as M2 (second generation) in field experiments. Herbicide containing Imazamox at a rate of 125 ml per decare was applied to M2 generation plants when they reached approximately 15 cm in height. In bread and durum wheat lines, emergent plants were observed up to a dose of 60mM EMS. The effective EMS doses for the wheat species were determined as 80 mM for bread wheat and 50 mM for durum wheat.

Introduction

Cereals are cultivated in many parts of the world due to their high adaptability. Wheat is used in crop rotation systems and is a nutrition source for many countries. It provides half of the calories needed by a third of the world's population and almost half of the protein of the world's population (Abd-El-Haleem *et al.* 1998, Adams *et al.* 2002).

All plants are naturally tolerant to some herbicides. For this reason, researchers use this natural resistance to establish tolerance to cultivated plants (Gray *et al.* 2010). For this purpose, herbicide-tolerant plants are obtained using biotechnological genetic modification techniques (transgenic) and traditional production techniques (non-transgenic). Most plants have genes to make them tolerant to herbicides (Duke 2005). Some of the cultivars with wide genetic variation for herbicide tolerance are maize, wheat, rice, soybean, chickpea, and alfalfa (Prakash *et al.* 2020).

Plant breeders have used plant breeding methods (especially selection) for thousands of years. Mutation breeding is a method applied in many plant species, including field crops such as wheat, barley and rice, which gives rapid results in cultivar development. Mutation can be divided into natural and artificial mutations on which chemical and physical mutagens create artificial mutations. Variations in the desired direction can be created using physical mutagens such as x-ray, gamma, and neutron. Ethyl methanesulfonate (EMS) is widely used to create mutations (chemical mutagens). Ethyl methanesulfonate causes the alkalinization of guanine in nucleotides, leading to point mutations for each gene.

The objective of the present study were determine the mutant impacts of various EMS doses during the M1 generation seedling growth stage and adult response in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) lines, as well as to select the herbicide-resistant mutant line of the M2 generation.

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Materials and Methods

The experiments were carried out in the field, greenhouse, and laboratory conditions of the Faculty of Agriculture of Dicle University-Türkiye in the 2019/2020 and 2020/2021 growing seasons. Eleven different EMS doses were used in the study along with the Empire (bread wheat) and Burgos (durum wheat) as experimental materials. The experiments were designed in a randomized complete block with four replications.

The ethyl methanesulfonate (EMS) amounts calculated for the different EMS doses used in the research are shown in Table 1. For each dose, 1000 seeds of each variety were first kept in pure water for 6 hours, thus increasing the permeability of the seed coat. Afterwards, these seeds were thoroughly filtered and kept in 11 different EMS solutions (0, 10 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM) for 6 hours. The seeds were stirred every hour with a metal spoon for better mutagen penetration into the seeds. Then the seeds were rinsed with distilled water to remove EMS mutagens. The washed seeds were dried in a laboratory with air circulation and planted in the field and greenhouse conditions.

Table 1. EMS doses used in the present research work.

EMS doses	Amount of EMS used (ml)	Amount of pure water used (ml)
Control	0.00	500.00
10 mM	0.54	499.46
20 mM	1.08	498.92
30 mM	1.63	498.37
40 mM	2.17	497.43
50 mM	2.71	497.29
60 mM	3.25	496.75
70 mM	3.79	496.21
80 mM	4.34	495.66
90 mM	4.88	495.12
100 mM	5.42	494.58

The M1 (first generation) seeds were sown in the controlled greenhouse to examine the seedling parameters of wheat. Peat (2/3) and perlite (1/3) were used as the soil in the greenhouse, and thirty seeds for each EMS dose were sown on 24.12.2019 according to a randomized plot design with three replications. The temperature and humidity values during seedling growth are shown in Fig. 1. Plant emergence rate (%), first leaf length (cm), root length (cm), seedling height (cm), fresh seedling weight (g), and dry seedling weight (g) were measured on the 28th day of M1 plants.

In both years of the research in the field, the plot area was set as 4.80 m^2. Sowing was carried out in the first year on 23 November and the second year on 12 November, 500 seeds/m^2. At sowing fertilizers, 6 kg da-1 nitrogen (N) and 6 kg da-1 phosphorus (P2O5) were applied, with an additional 6 kg da-1 nitrogen (N) during the tillering stage. The experimental plots were harvested at full maturity using a small plot combine harvester machine on 30.06.2020 in the first year and by hand on 01.06.2021 in the second year.

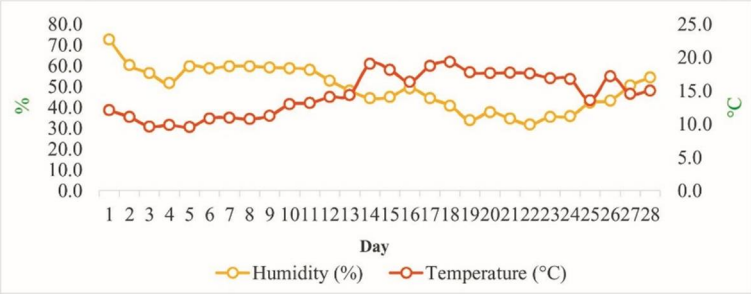

Fig. 1. Temperature and humidity data in the greenhouse.

In the first year of the study, the plants of the M1 generation were harvested for each species and dose. The yield (kg/ha) was determined by weighing the grains of the plants. 125 ml/da Imazamox (40 g/lt Imazamox with active ingredient, Intervix Pro (BASF)) was used to determine herbicide tolerance in cereals. Herbicide containing Imazamox at a rate of 125 ml per decare was applied to M2 generation plants when they reached approximately 15 cm in height. The atomizer spraying at a pressure of 0.45 mpa per second was used in the application of Imazamox. As a result of the application, the surviving plants were harvested separately. The spike number, the grain number, and the grain weight were determined for each dose in these plants. The soil analysis results of the experimental field and the precipitation and temperature data are shown in Table 2 and Fig. 2, respectively.

All parameters were analysed using JMP Pro 13 statistical package.

Table 2. Soil analysis results of the experiment field

Analysis Results			
Saturation (%)	:	63.00	Clay loam
Salinity (Saturation Sludge) (dS / m)	:	0.92	Without salt
% Salt (by calculation) TS 8334	:	0.04	Without salt
pH (Saturation Sludge)	:	8.11	Light Alkali
Lime (Calcimetric) (%)	:	11.24	Middle
Organic Matter (Walkley Black) (%)	:	0.71	Low
Nitrogen (%)	:	0.04	Low
Phosphorus (Olsen Spectrometer) (ppm)	:	4.00	Low
Potassium (A. Acetate-ICP) (ppm)	:	314.45	Very High
Calcium (A. Acetate-ICP) (ppm)	:	10717.89	Very High
Magnesium (A. Acetate-ICP) (ppm)	:	471.78	Middle
Sodium (A. Acetate-ICP) (ppm)	:	26.65	Low
Iron (DTPA-ICP) (ppm)	:	9.29	Very High
Copper (DTPA-ICP) (ppm)	:	1.61	Middle
Manganese (DTPA-ICP) (ppm)	:	16.50	Middle
Zinc (DTPA-ICP) (ppm)	:	0.08	Low

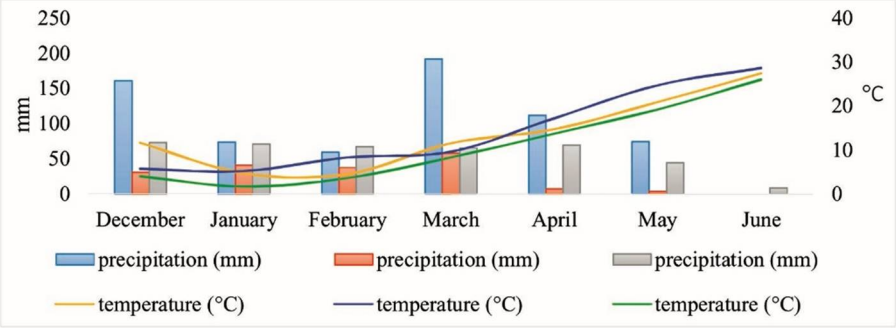

Fig. 2. Precipitation and temperature data for the research period.

Results and Discussion

Plant emergence rate, root length, seedling height, fresh seedling weight, dry seedling weight, and first leaf length were recorded in M1 stage bread and durum wheat lines generated with eleven different EMS doses under greenhouse conditions. Different EMS doses resulted in changes in all parameters assessed across the wheat species (Tables 3 and 4).

Table 3. Mean values of emergence rate, first leaf length and root length characteristics of wheat.

EMS doses	Emergence rate (%)		First leaf length (cm)		Root length (cm)	
	Bread wheat	Durum wheat	Bread wheat	Durum wheat	Bread wheat	Durum wheat
Control	97.78 a	85.56 a	10.10 a	8.30 a-c	28.53 a	36.85 a
10 mM	95.56 ab	75.56 a	9.30 ab	8.72 a	27.03 ab	38.29 a
20 mM	94.44 ab	74.44 a	7.53 cd	8.52 ab	20.00 cd	38.09 a
30 mM	86.67 bc	47.78 b	7.96 cd	7.21 b-d	24.17 a-c	22.59 b
40 mM	84.44 c	37.78 bc	8.36 bc	6.83 de	21.63 b-d	21.90 b
50 mM	82.22 cd	31.11 cd	6.50 ef	7.07 c-e	17.63 d	24.34 b
60 mM	73.33 de	21.11 d	6.23 e-g	5.85 e	18.93 cd	13.46 c
70 mM	64.44 e	0.00	7.03 de	0.00	19.20 cd	0.00
80 mM	53.33 f	0.00	5.36 g	0.00	18.27 cd	0.00
90 mM	43.33 g	0.00	5.83 fg	0.00	18.10 cd	0.00
100 mM	28.89 h	0.00	4.30 h	0.00	15.90 d	0.00
Mean	73.13	53.30	7.13	7.50	20.90	27.90
LSD	9.81**	11.63**	0.93**	1.30**	6.09**	5.92**

**Significant at 1% level.

Plant emergence up to 60 mM dose was observed with increased EMS doses in bread durum wheat lines. The emergence rate varied between 28.89% (100 mM EMS dose) and 97.78% (control dose) in bread wheat, while it varied between 21.11% (60 mM EMS dose) and 85.56% (control dose) in durum wheat.

Table 4. Mean values of seedling height, fresh and dry seedling weight characteristics of wheat grown in greenhouse conditions.

EMS	Seedling height (cm)		Fresh seedling weight (g)		Dry seedling weight (g)	
Doses	Empire	Burgos	Empire	Burgos	Empire	Burgos
Control	28.60 a	27.65 a	0.60 a	0.81 a	0.08 a	0.07 a
10 mM	28.13 a	27.56 a	0.49 b	0.80 a	0.06 ab	0.06 a
20 mM	23.53 b	27.33 a	0.41 cd	0.83 a	0.05 b-d	0.07 a
30 mM	22.43 bc	20.32 b	0.45 bc	0.52 b	0.05 bc	0.05 b
40 mM	22.47 bc	15.58 c	0.46 bc	0.44 bc	0.05 b-d	0.04 cd
50 mM	20.27 cd	19.18 bc	0.38 de	0.51 b	0.04 cd	0.05 bc
60 mM	16.97 ef	9.58 d	0.34 ef	0.32 c	0.04 cd	0.03 d
70 mM	20.50 cd	0.00	0.39 de	0.00	0.05 cd	0.00
80 mM	15.07 f	0.00	0.29 fg	0.00	0.03 d	0.00
90 mM	18.70 de	0.00	0.32 fg	0.00	0.04 cd	0.00
100 mM	10.13 g	0.00	0.26 g	0.00	0.03 cd	0.00
Mean	20.61	21.02	0.40	0.60	0.04	0.05
LSD	2.87**	3.84**	0.05**	0.13**	0.018**	0.008**

**Significant at 1% level.

Arisha *et al.* (2015) reported that EMS and other mutation sources used as mutagens have different effects on M1 plants. These effects vary according to the varieties, and the mutagen effect increases with the increase in dose.

The effectiveness of EMS doses varies depending on the genotype, and the application time and temperature of the dose are also the main factors for EMS (Olaolorun *et al.* 2019). Çetin (2021) determined that the germination rate in seeds decreased to approximately 30% with EMS applied at high concentrations. The plant emergence rate in mutagen applications in the M1 generation was determined by Olaolorun *et al.* (2019) 71.26-75.92% (EMS application-control) and Yorulmaz *et al.* (2021) as 0.00-93.33% (30 mM-control).

Regarding the cereal species' seedling parameters examined in the present study, the highest values were obtained from the control and 10 mM EMS doses. Values for these parameters decreased proportionally with increasing EMS doses. Seedling height was determined between 25.93-33.73 cm (Olaolorun *et al.* 2019) and 21.87-26.83 cm (Yorulmaz *et al.* 2021). The first leaf length in cereal species was determined between 7.80-8.39 cm (Yorulmaz *et al.* 2021).

In previous research, the values of root length varied between 12.08-16.15 cm (Olaolorun *et al.* 2019) and 27.38-32.69 cm (Yorulmaz *et al.* 2021). These researchers observed that the fresh and dry seedling weight of plants with mutations decreased. Yorulmaz *et al.* (2021) found that values of fresh seedling weight in M1 generation varied between 0.850-0.647 g, and dry seedling weight varied between 0.071-0.051 g.

The research determined the dose that caused a 50% decrease in plant emergence rate from 11 different EMS doses applied in cereal species as ED50. The ED50 dose was determined to be 90 mM for the bread wheat cultivar and 30 mM for durum wheat. The ED50 dose resulting in a 50% reduction in seed germination, is used to compare the mutagen effect in plants (Bharathi *et al.* 2013, Beyaz *et al.* 2016). ED50 dose may vary depending on the mutation application's plant type, genotype, mutagen source, and environmental conditions. (Aparna *et al.* 2013, Liamngee *et al.* 2017). The ED50 dose differed according to genotypes, and EMS doses of 0.4% and 0.7% were effective doses for wheat (Olaolorun *et al.* 2019).

The grain yield gradually decreased from 20.35 to 0 kg/ha with increasing EMS doses in bread wheat. The grain yield could be recorded in the durum wheat at only four doses (control, 10, 20, and 30 Mm), but it could not be recorded after 30 mM doses. It was determined that the grain yield in durum wheat varied between 2.11-24.85 kg/ha and obtained the highest grain yield from the 30 mM dose, while 30 mM in bread wheat (Table 5). According to the data obtained under field conditions, the lethal dose in bread wheat was found as 20 mM EMS and in durum wheat 30 mM EMS. Abaza *et al.* (2020) reported that the maximum increase in all parameters related to yield was achieved at the dose of 0.4% EMS in bread wheat.

Table 5. Mean grain yield (kg/ha-1) of wheat at M1 generation.

EMS doses	Empire (*Triticum aestivum* L.)	Burgos (*Triticum durum* Desf.)
Control	17.5	6.56
10 mM	14.35	9.70
20 mM	15.72	24.85
30 mM	20.35	2.11
40 mM	11.66	0.00
50 mM	9.09	0.00
60 mM	8.68	0.00
70 mM	16.24	0.00
80 mM	2.89	0.00
90 mM	2.99	0.00
100 mM	0.00	0.00

Table 6. The mean values of plant number, spike number, grain number and grain weight in herbicide-tolerant wheat.

EMS Doses	Plant number (piece)	Spike number (piece)	Grain number (piece)	Grain weight (g)
Bread wheat-20 mM	2	2	44.00	1.25
Bread wheat -30 mM	4	19	28.11	1.18
Bread wheat -60 mM	4	16	30.63	0.98
Durum wheat-30 mM	5	11	26.73	0.87
Mean	3.75	12	32.37	1.07
Total	15	48	129.47	4.30

Herbicide spraying in the field was done in the second year of the study to make imazamox tolerant plant selection among mutant M2 plants. Fifteen herbicide-tolerant plants were obtained in the field research (Table 6).

Resistant plants were obtained in bread wheat at doses of 20, 30, and 60 mM EMS and in durum wheat at a dose of 30 mM EMS. The number and weight of grains per spike are optimal in imazamox-tolerant plants (Table 6).

Plant growth was negatively affected by increasing EMS dosages in wheat species, with the harmful effect increasing linearly as the dose was raised. On the other hand, the doses that cause a decrease of more than 50% (ED50) in the plant emergence rate, 90 mM for bread wheat and 30 mM for durum wheat. According to these results, an effective variation can be created by applying

the optimum dose at the ED50 level. The ability to obtain plants tolerant to imazamox herbicide in field conditions in the M2 generation demonstrates that both acceptable mutant variety is created after EMS, and selection may be made based on desired agronomic parameters following the M2 generation.

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