Journal of Applied Biological Sciences 3 (1): 59-64, 2009
ISSN: 1307-1130, www.nobel.gen.tr

# Effects of EMS Treatment on the Seed Germination in Wheat

Bilge BAHAR* Mahinur S. AKKAYA

Middle East Technical University, Department of Chemistry, Biochemistry and Biotechnology Research Programs, Inonu Blvd., Ankara, TR-06531, TURKEY

* Corresponding Author
e-mail: bilgebahar@gmail.com

Received: October 10, 2008
Accepted: December 11, 2008

## Abstract

The aim of this study was to identify the effects of different percentages of Ethyl-methane Sulphonate (EMS) mutagen on the seed germination, root and shoot growth so that the most effective EMS dose can be applied for successful mutagenesis research studies like TILLING. For this purpose, the seeds of "Gerek-79" bread wheat cultivar were treated with 0.1, 0.2, and 0.3% EMS doses. Seed germination, coleoptyl existing, first leaf existing, embryonic root length, coleoptyl length, seedling length, seedling growth rate, first leaf length, fresh and dry weights of root and shoot, and root to shoot ratio were evaluated in comparison to the untreated materials. Mostly, there were statistically significant differences among EMS treatments for all the evaluated characters. The levels of differences were more pronounced with the increased doses of EMS. Thus, the selected characters evaluated in this study are relevant for the assessment of effective and optimum EMS treatments. In this way, M1 plants can be selected at early stages of growth based on the indicator characteristics of the EMS mutagen treatments to elicit the best response prior to the large scale mutant production for later experiments.

**Key words:** Wheat, EMS, germination, root, and shoot growth.

## INTRODUCTION

By means of inducing genetic variations, mutations have been used successfully in several crops for breeding of agronomically important traits,. Conventional mutation techniques have mostly been used to improve yield, quality, and disease and pest resistance in crops. Many mutant varieties involving of more than 100 plant species have been officially released. Especially, some economically important crop (barley, wheat, and cotton) mutant varieties occupy the majority of cultivated lands [1]. The accurate selection techniques determines the success of induced variation and obtaining desired characteristics in breeding [2,3]. One way to induce mutation is through the use of chemical mutagens. EMS is a common, powerful, and one of the most effective chemical mutagen, especially recommended using when mutation is introduced to the seed materials, since the application and the monitoring of the outcome of mutations are relatively easy. In plants, EMS usually causes point mutations, on the other hand, loss of a chromosome segment or deletion can also occur in lesser extent [4]. Therefore, EMS has the potential of altering loci or a candidate gene of specific interest without inducing large deletions. This creates an advantage for the plant breeders to obtain useful alleles, over using exotic or wild germplasms in which the group of linked deadly alleles can be present. The most important parameters for inducing mutation with EMS are concentration, duration of treatment, and solution temperature [5]. Thus, prior to the large scale generation of variants initial studies on induced mutations are usually conducted for finding optimum combination of these parameters together with the optimum dose to elicit the best response. Both physical and chemical mutagens were tested in various crop species such as wheat, barley, rice, tobacco, corn, Brassica, fruit crops and vegetables [6]. Obviously any mutagenesis as gamma ray or EMS treatment makes plants vulnerable to negative effects on characters of M1 and the following generations, such germination, production of vital seeds, root and seedling lengths etc [7]. Thus, it is important to optimize the best possible condition for generating large number of mutants having good seed germination for segregation without detrimental genetic damages. Besides the wide use of mutagenesis to obtain genetic variation for plant breeding purposes, the generation of induced mutations is also applied with intentions for altering gene function with changed phenotype in basic research. One such purpose is to confirm a function of a candidate gene, especially for the elucidation of biochemical, developmental, and disease resistance mechanisms [8]. Since mutations induced with EMS are mostly point mutations resulting in a broad range of alleles in a relatively small population [9], such genetic changes at the nucleotide level of candidate genes with loss of functions can be generated without loosing the gene itself. Thus, with applications of accurate selection methods well characterized phenotyping and TILLING experiments, the candidate genes can be easily confirmed in they are the genes involved in the disease resistance for example, or any other particular trait [10]. In some studies [11], it is shown that about 50% of the mutations are mismatched mutations, only 4.5 % is nonsense, of which 98% are GC/AT transitions due to the fact that EMS alkylation of G residues at the O6 position, thereby causing mis-pairing with T [12].

The phenotypic changes indicate that most mutations fall into more than a single category (pleitropic), with some organs such as leaves more prone to alterations than others. However, each identified mutant can be classified into phenotypic categories according to predetermined criteria in a research. For example mutations cause embryo lethality, slow germination, extremely small plant size, aborted growth, rough or smooth, narrow or wider, different colored (yellow, purple, white, yellow-green, gray, and dark green) leaves, late or early flowering, and partial or full sterility [13]. Flippetti et al. [14] showed that the plants of the M1 and M2 generations have reduced emergence, survival, and fertility in Vicia faba L., and EMS was more effective than gamma rays. In another study in Vicia faba L., all the germination, and seedling parameters were affected adversely due to mutagenic treatment. Severe reduction in germination, frequency of normal seedlings, reduction in plumule to radicle length and physiological injuries of radicles indicated effective mutagenesis [15]. In addition, Khan et al. [16] stressed that all the mutagenic treatments brought reduction in seed germination, pollen fertility, and survival at maturity, such reduction with an exception of survival, were found to be dose dependent.

The objective of this study is to determine the effects of EMS doses on the germination of wheat seeds and the growth of seedling and roots, so that the most effective of conditions can be determined in these preliminary experiments and later can be applied for successful mutagenesis in basic research studies such as knock-out of genes for confirming particular functions.

## MATERIAL AND METHOD

### Plant material

"Gerek-79", bread wheat cultivar, has been used as the plant material in this study. It is registered by Anatolian Agricultural Research Institute in 1979. This cultivar is susceptible to yellow rust disease at seedling stage and it is soft white grained with brown spikes and awn. Gerek-79 is has been recommended for Middle Anatolia and passage regions, since it is earlier.

### EMS treatment

In our study, the experimental procedure for induced EMS mutagenesis of Williams et al. [17] was followed and triple technical repeats of three different percentages of EMS (Ethyl-methane Sulphonate) doses (0.10%, 0.20%, 0.30%) and a control dose (0% EMS) were applied. First of all, 25 seeds for each technical replicates were presoaked in 15 mL (0.6 mL/seed) for 8 h in 0.05 M phosphate buffer, pH 8.0 for 16 h, at 20 oC by 100 rpm constant shaking. Treated seeds were rinsed under running tap water for 1 min to remove excess EMS solution from seed surfaces and transferred onto the Petri dishes containing water soaked filter paper and let grown in growth chamber at 20 oC as triplicates of 25 seeds of each dose treatment. Following the next day of the treatments, the seeds were continuously assessed for the germination and developmental stages daily.

### Assessment of developmental stages

ZGS, Growth Scale of Zadoks et al. [18] was used in the assessment of developmental stages. Some seedling characters such as embryonic root length (ZGS 0.5, radicle emerged from caryopsis), coleoptyl length (ZGS 0.9, first leaf just at coleoptyl tip), seedling growth rate (ZGS 1.1, first leaf unfolded on main shoot, second appears; ZGS 1.2, second leaf unfolded on main shoot, third appears), first leaf length (ZGS 1.1), fresh and dry weights of root and shoot (ZGS 1.2) were evaluated according to Growth Scale of Zadoks scale of assessments.

### Experimental design and statistical analysis

Experiments were conducted onto three replicated randomized block design. Analysis of variance was based on the procedure of Statistical Analysis System [19] for each character. Least significance difference (LSD) at a 0.05 probability level was used to detect the differences between treatment means.

### Results and Discussion

Seed germination values (%) showed significant difference at 0.001 probability level for only one day after EMS treatment (except for the lowest dose treatment) (Table 1, Figure 1A). On the other hand, the seed germination values did not differ significantly for the 2nd, 3rd and 4th days. The highest % germination was observed on the control samples as expected when compared to the all the assessed days of treatment with increasing EMS amounts. The range of percent germination was between 1.3 (0.30% EMS dose) and 97.3 (control samples of the first day) (Table 1). In the following days, germination values were very close to each other with high percentage values. Rupinder and Kole [15] stated that the severe reduction in germination is an indication of effective mutagenesis. Also, Khan et al. [16] pointed out mutagenic treatments brought reduction in seed germination. In our assays, such reduction is present only at the first day with the highest dose of EMS, thus it maybe concluded that there is only a single day delay in germination. Observed slight slow germination with the highest dose EMS treatment is indeed considered less than expected [13].

Coleoptyl existence (CE) values showed significant differences at 0.001 probability level on the 2nd day after EMS treatment, but not on the 4th day (Table 1, Figure 1B). These values on the 2nd day were the highest for control, 0.10% and 0.20% EMS doses (97.3, 93.3, and 93.3%, respectively). The lowest value was observed in 0.30% EMS dose (52.0%). These values were close to each other on the 4th day of EMS treatment, with an observed trend of decreased percentages with increased doses. However, these decreases were not statistically significant on the 4th day.

The percentages of the first leaf existence (FLE) values (Table 1, Figure 1C) were statistically significant for the 3rd and the 4th day of the EMS treatment but not for the 5th day. Values have emphasized the delay of the immerging first leaf formation. In addition, the highest first leaf existence values were found in control and 0.1% EMS doses on the 3rd and the 4th day of EMS treatment (Table 1, Figure 1C ). On the 3rd day of EMS treatment, no FLE was observed in 0.20 and 0.30% EMS doses. Similarly in 0.30% EMS dose samples on the 4th day of EMS treatment, no first leaf existence was observed. Nonetheless, all doses showed first leaf existing on

the 5th day, but these values were not statistically significant. It was expectedly obvious that the increased doses of treatment delayed the first leaf existence.

days, SL decreased by increasing of EMS doses. Seedling growth rate (SGR) decreased in parallel with the SL values (Table 2). In addition, Rupinder and Kole [15] showed that

**Table 1.** . Values of SG (Seed Germination), CE (Coleoptyl Existence), and FLE (First Leaf Existence) at different days after EMS treatment.

| EMS Doses | Days after EMS treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 2 | 4 | 3 | 4 | 5 |
| | SG (%) | | | | CE (%) | | FLE (%) | | |
| **Control** | 97.3 a† | 98.6 | 98.6 | 98.6 | 97.3 a | 98.6 | 81.3 | 97.3 a | 98.6 |
| **0.1 %** | 94.7 a | 97.3 | 98.6 | 98.6 | 93.3 a | 97.3 | 61.3 | 94.7 a | 96.0 |
| **0.2 %** | 61.3 b | 96.0 | 97.3 | 97.3 | 93.3 a | 97.3 | 0.0 | 73.3 b | 94.6 |
| **0.3 %** | 1.3 c | 96.0 | 96.0 | 96.0 | 52.0 b | 94.7 | 0.0 | 0.0 c | 89.3 |
| **Mean** | 63.7 | 97.0 | 97.6 | 97.6 | 84.0 | 97.0 | 35.7 | 66.3 | 94.6 |
| **CV (%)** | 8.49 | 2.62 | 2.84 | 2.84 | 4.72 | 3.10 | 31.99 | 16.8 | 0.59 |
| **LSD $_{0.05}$** | 10.19*** | NS | NS | NS | 7.48*** | NS | 21.48*** | 5.67*** | NS |

*** P<0.001, ** P<0.01, * P<0.05.

† Means within a column followed by the same letters were not significantly different based on a LSD test at P = 0.05.

Embryonic root length (ERL) values showed significant differences at 0.001 probability level on the 3rd day after

seedling parameters were affected adversely due to mutagenic treatment.

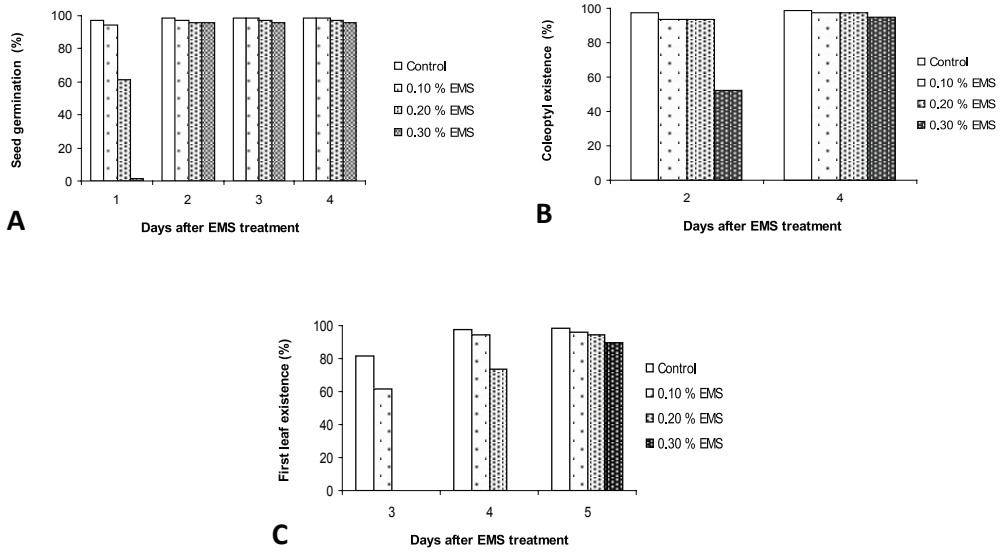


**Figure 1.** Observed traits of seed germination (**A**) coleoptyl existence (**B**), first leaf existence (**C**) at different time points of treatment and EMS doses (**H**).

EMS treatment (Table 2, Figure 2A). ERL values decreased by increasing of EMS dose; changed between 11.5 mm (0.3% EMS) and 54.0 mm (control). Coleoptyl length (CL) on the third day after EMS treatment was similar to ERL (Figure 2A). While the highest CL was found in control dose with 24.7 mm, the lowest CL was observed in 0.3% EMS dose with 6.9 mm (Table 2). Rupinder and Kole [15] stated that radicle length was reduced by effective mutagenesis.

Seedling Length (SL) showed statistically significant differences at 0.001 probability level on the 7th and 14th day after EMS treatment (Table 2). On the both measurement

First leaf length (FLL) was measured on the 14th of EMS treatments, and found that significant differences among EMS doses at 0.001 probability level (Table 2, Figure 2C). Also, the highest FLL value was observed in the control samples (8.25 cm). On the other hand, the lowest values were observed in the 0.2% (5.63cm) and 0.3% (5.22cm) EMS doses.

Values of fresh and dry weight of roots and shoots, and root to shoot ratio showed statistically significant differences, except shoot dry weight (Table 3, Figure 3A, 3B). The highest root and shoot fresh weights were observed at control (respectively, 357.3 and 594.3 mg); however, the lowest root and shoot fresh

*** P<0.001, ** P<0.01, * P<0.

**Table 2.** Values of ERL (Embryonic Root Length), CL (Coleoptyl Length), SL (Seedling Length), SGR (Seedling Growth Rate) and FLL (First Leaf Length) at different days after EMS treatment.

| EMS Doses | Days after EMS treatment | | | | | |
|---|---|---|---|---|---|---|
| | 3 days | | 7 days | 14 days | | |
| | ERL | CL | SL | SL | SGR | FLL |
| | (mm) | (mm) | (cm) | (cm) | (mm day$^{-1}$) | (cm) |
| **Control** | 54.0 a† | 24.7 a | 9.86 a | 17.44 a | 10.83 a | 8.25 a |
| **0.1 %** | 36.0 b | 21.5 b | 9.33 a | 15.66 a | 9.05 ab | 7.51 b |
| **0.2 %** | 23.4 c | 14.0 c | 6.65 b | 12.73 b | 8.67 ab | 5.63 c |
| **0.3 %** | 11.5 d | 6.9 d | 5.40 c | 9.56 c | 5.94 b | 5.22 c |
| **Mean** | 31.2 | 16.8 | 7.81 | 13.85 | 8.62 | 6.65 |
| **CV (%)** | 9.22 | 8.53 | 6.60 | 10.33 | 19.89 | 5.30 |
| **LSD $_{0.05}$** | 5.4*** | 2.7*** | 0.97*** | 2.7*** | 3.23* | 0.67*** |

*** P<0.001, ** P<0.01, * P<0.05. † Means within a column followed by the same letters were not significantly different based on a LSD test at P = 0.05.

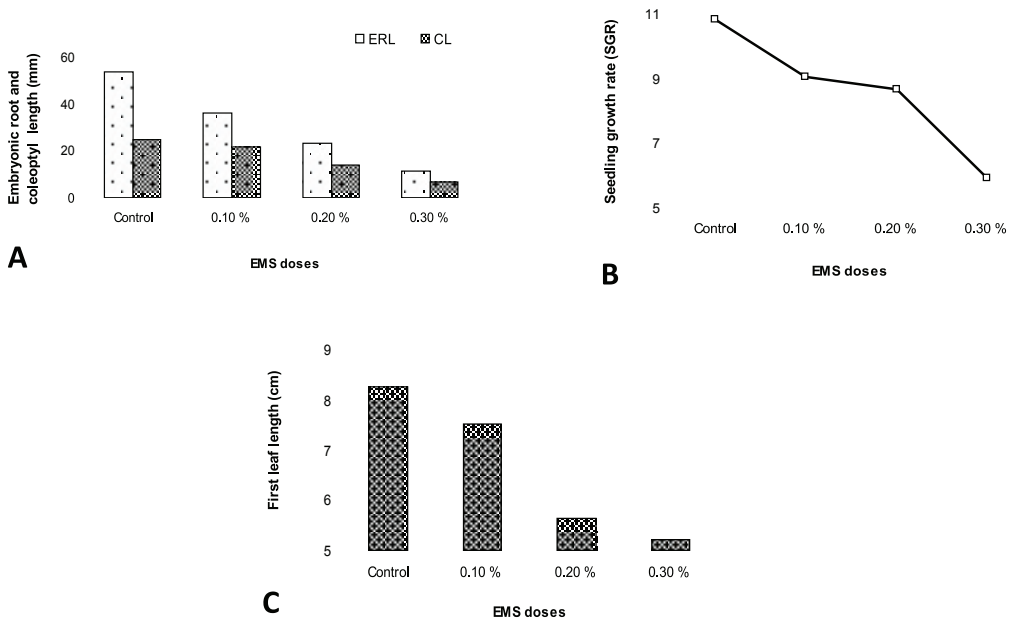


**Figure 2.** Observed traits of embryonic root length and coleoptyl length (A), seedling growth rate (B), fresh and dry weights of shoots and roots, first leaf length (C) at different time points of treatment and EMS doses.

**Table 3.** Values of RFW (Root Fresh Weight), SFW (Shoot Fresh Weight), RDW (Root Dry Weight), SDW (Shoot Dry Weight) and R:S (Root to Shoot) Ratio on the 23rd day after EMS treatment.

| EMS Doses | RFW | SFW | RDW | SDW | R:S Ratio |
|---|---|---|---|---|---|
| | (mg) | | | | |
| **Control** | 357.3 a† | 594.3 a | 73.5 a | 106.6 | 0.69 a |
| **0.1 %** | 235.5 b | 529.3 ab | 57.1 b | 100.1 | 0.57 b |
| **0.2 %** | 199.7 b | 445.2 bc | 45.4 c | 91.7 | 0.52 b |
| **0.3 %** | 102.2 c | 426.9 c | 36.3 c | 87.6 | 0.40 c |
| **Mean** | 223.6 | 498.9 | 53.1 | 96.5 | 0.55 |
| **CV (%)** | 16.65 | 9.67 | 9.27 | 8.61 | 5.32 |
| **LSD $_{0.05}$** | 70.1*** | 90.8** | 9.3*** | NS | 0.07*** |

*** P<0.001, ** P<0.01, * P<0.05. † Means within a column followed by the same letters were not significantly different based on a LSD test at P = 0.05.

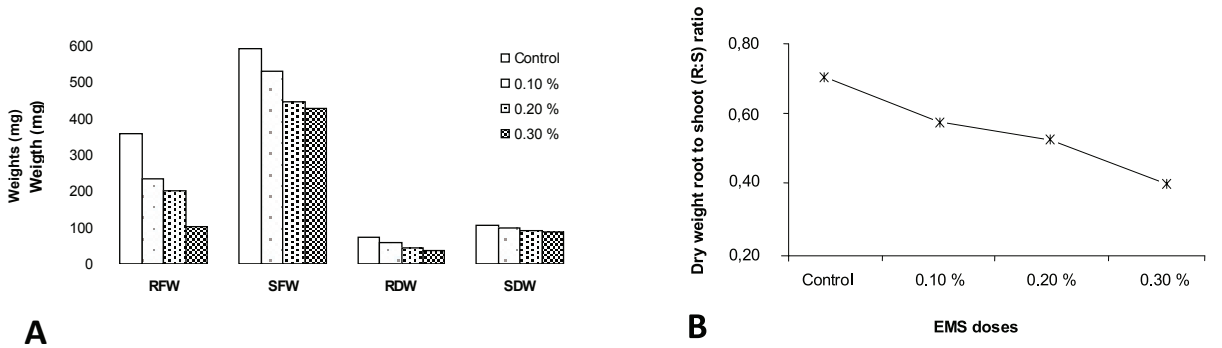


**Figure 3.** Observed traits of weights **(A)**, root to shoot ratio at different time points of treatment and EMS doses **(B)** on the 23rd days of treatment and EMS doses.

weights were indicated at 0.3% EMS dose (respectively, 102.2 and 426.9 mg). While the highest root dry weight was at control (73.5 mg), the lowest root dry weight values were observed at 0.2% (45.4 mg) and 0.3% (36.3 mg) EMS doses. Nevertheless, there weren't statistical significant differences among EMS doses; shoot dry weights ranged between 87.6 mg (0.3% EMS) and 106.6 mg (control). Also, root to shoot ratio (R:S) showed differences significantly changing between 0.40 (0.3% EMS) and 0.69 (control) (Table 3, Figure 3B).

## CONCLUSIONS

In all observed traits (coleoptyl existence, embryonic root length and coleoptyl length, first leaf existence, first leaf length, seedling growth rate, fresh and dry weights of shoots and roots, root to shoot ratio) related to shoots and roots were decreased by increased EMS doses. On the other hand, such reduction for seed germination was only at the first day with the highest dose of EMS, in the following days the mutants recovered germination even at the highest EMS treatment dose (0.3%). As a result, we are considering that 0.3 % EMS dose treatment is high enough to produce variability as followed by the other trait assessments (such as Figures 2A, 2B, 3A and 3B), but low low enough to obtain level of germination similar to control untreated samples at the later days. Thus, we believe that our statically determined observations on some traits affected by EMS mutation will be useful when large number of mutants is required for TILLING experiments to identify and confirm function of the candidate genes. For which purpose, the highest dose of EMS treatment (0.3%) is considered to be the best dose, since it not only produce highest variability but also not detrimental for the survival of the plants.

**Acknowledgement**

The authors gratefully acknowledge the support from State Planning Organization (DPT2004K120750) and TUBITAK (The Scientific & Technological Research Council of Turkey) postdoctoral study support to BB (PhD).

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