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Full Length Research Paper

Response of selected wheat genotypes to ethylmethanesulphonate concentration, treatment temperature and duration

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Ethylmethanesulphonate (EMS) is the most useful chemical mutagen to induce genetic variation in plant breeding programs. Key factors in inducing mutations with EMS include dose, genotype, temperature and duration of treatment. This study was conducted to determine the optimum EMS concentration, treatment temperature and duration for effective mutagenesis in selected wheat varieties. Seeds of four varieties (B936, B966, SST387 and SST875) were treated with four EMS concentrations (0, 0.3, 0.5 and 0.7%), three temperature regimes (30, 32.5 and 35°C) at four treatment durations (0.5, 1, 1.5 and 2 h) with two replicates. Percentage seedling emergence, germination and seedling height were recorded for the treatment combinations. The most effective treatment in variety B936 was 0.7% EMS at 30°C for 1.5 h exposure. B966 responded best at 0.5% EMS at 35°C for 1.5 h; SST387 at 0.5% EMS, 32.5°C and 2 h and SST875 at 0.5% EMS, 32.5°C and 1 h. Increased EMS dose, temperature and exposure time were detrimental to seeds of the respective varieties. The study established varietal specific EMS dose and treatment conditions to be used in inducing large-scale mutagenesis in wheat.

Key words: Chemical mutagenesis, ethylmethanesulphonate (EMS), *Triticum aestivum.*

INTRODUCTION

Induced genetic variations have been used successfully in several crops to extract mutants with suitable agronomic traits such as herbicide resistance, early maturity and improved nutrition (van Harten, 1998; Singh and Kole, 2005). Artificial mutations in plants can be induced using chemical alkylating (ethylmethanesulphonate and ethidium bromide) or physical (ionic radiation, X-rays, UV light, gamma rays and neutrons) mutagenic agents (Predieri, 2001; Mba et al., 2010).

Chemical mutagenesis using Ethylmethanesulphonate ($CH_3SO_2OC_2H_5$) is particularly efficient when used on self-fertilized crops such as wheat (*Triticum aestivum* L.). Induced mutations using ethylmethanesulphonate (EMS) have been applied in cultivar development of small grain cereals for traits such as stem rust resistance, early maturity and modified kernel colour in wheat (Chopra, 2005), starch mutants in oats (Maluszynski et al., 1995; Verhoeven et al., 2004), ALS herbicide resistance in wheat (Pozniak and Hucl, 2004), and imidazolinones herbicide resistance in wheat (Newhouse et al., 1991; Pozniak and Hucl, 2004). In Sweden, mutation induction in a barley cultivar, Bonus, created a short straw mutant, Pallas, and a very early cultivar, Mari. These two cultivars were used in crosses with other cultivars and produced the cultivars Hellas, Kristina, Mona, Eva and Salvo (van Harten, 1998).

EMS is helpful in pre-breeding or genetic enhancement aimed to develop suitable germplasm (Minocha and Arnason, 1962; van Harten, 1998). It is known to induce a broad spectrum of mutations in plants (van Harten, 1998; Henikoff et al., 2004; Chen et al., 2012). Recently the technique is being applied to generate mutants with altered agronomic traits for genetic studies and to predict

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the gene function through identification of an allelic series by Targeting Induced Local Lesions IN Genomes (TILLING) (Till et al., 2003; Xin et al., 2008). EMS usually renders point mutations (Okagaki et al., 1991) which is recommended for use on seed materials, since its application and monitoring of mutational events are relatively easy (Weil and Monde, 2007; Mba et al., 2010).

Mutation experiments require careful selection of a mutagen and an appropriate treatment regime with characteristics suited to the tissue source and the objective of the mutagenesis (Ahloowalia et al., 2001). The effectiveness of mutagenic treatments and amount of induced genetic variation in crops is dependent on the variety, the mutagen dose, temperature and the time of exposure to the mutagen (Maluszynski and Khan, 2002; Rupinder and Kole, 2005; Mba et al., 2007, 2010; Xin et al., 2008). Temperature influences the rate of hydrolysis of the mutagenic solution; at low temperatures the hydrolysis rate is decreased, implying that the mutagen remains stable for a longer time period (Mba et al., 2007).

Previous studies (Till et al., 2003; Xin et al., 2008; Mba et al., 2010) reported the usefulness of pre-testing a range of EMS doses on target crop genotypes before large-scale mutagenesis undertaken. However, there is limited information that compared the combined treatment effect of EMS doses, genotypes, treatment temperature and durations. Furthermore, there are varied protocols and recommendations in the literature on EMS doses *per se* for mutagenesis studies. For seed propagated crops such as barley (*Hordeum vulgare* L.), Mba et al. (2007, 2010) suggested a preliminary ranges of EMS (0.05 to 0.2 M), two to three treatment temperatures varying from 30 to 35°C and two treatment conditions of 2 to 6 h duration that should be tested to determine the optimal treatment condition. In Bulgaria, mutant wheat cultivars were reportedly developed using EMS doses varying from 0.1 to 0.3% (Tomlekova, 2010).

Systematic determination of the optimum treatment combination is crucial to determine the ideal treatment condition on the target plant materials for successful mutation induction (Mba et al., 2010). The number of days for germination, germination percentage and seedling height could serve as a criterion in determining the biological effects of a mutagenic treatment in plants (Singh and Kole, 2005; Mba et al., 2010). The lethal dose causing 50% reduction in seed germination (LD_{50}) is an important parameter for comparing the effectiveness of different mutagenic treatments in mutation experiments (Hohmann et al., 2005; Mba et al., 2010).

Common wheat (*T. aestivum* L.; 2n=6x=42, AABBDD) is the second most important crop after maize in South Africa. However, wheat production is constrained by several challenges such as competition by weeds, heat stress, recurrent drought, diseases, poor soil fertility all resulting in lower than expected yields. Developing wheat germplasm with increased grain yield potential, superior end use quality, tolerance to biotic and abiotic stresses remains important to boost productivity (Pozniak and Hucl, 2004).

The objective of this study was to determine the optimum EMS concentration, treatment temperature and duration that would provide desired emergence, germination percentage and vigorous seedlings in four selected wheat genotypes. Selected treatment combinations could be used for large-scale mutagenesis to isolate mutants for herbicide resistance and other agronomic traits.

MATERIALS AND METHODS

Study site and plant material

Studies were conducted at the Controlled Environmental Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. Seeds of four wheat varieties (SST387, SST875, B936 and B966) used in the study were supplied by Sensako, wheat breeding company, South Africa. The varieties were selected based on suitable agronomic traits, market potential and adaptability to production in the dry semi-arid zones (SST387, B936 and B966) and irrigation regions (SST875) of South Africa.

Seed mutagenesis, planting, experimental design and data collected

Mutagenesis was performed according to the procedure described by Mba et al. (2007, 2010) as suggested and validated for barley. Seeds of the four wheat varieties were subjected to four EMS concentrations (0, 0.3, 0.5 and 0.7% v/v), at four exposure times (0.5, 1, 1.5 and 2 h) and three temperature regimes (30, 32.5 and 35°C). This resulted in a total of 192 treatment combinations. For mutagenesis, seeds of each variety were placed into specially designed mesh bags (nylon mesh, 7 cm width and 11 cm length) with 40 seeds allocated per treatment in two replicates. Seeds were surface sterilized soaking in 70% ethanol for 1 min and rinsing 3 times and soaking again in 30% sodium hypochlorite bleach solution (2% NaOCl) for 5 min and rinsing 3 times under cold tap water. The seeds were then soaked in distilled water for about 16 to 20 h before EMS treatment. Seeds in mesh bags prepared for different treatment combinations were immersed in their respective EMS dose trays at a specific temperature and for a specific period of time in a water bath. Treated seeds were then washed under running tap water for about 2 to 3 h to remove the excess EMS and to eliminate the mutagen for safe handling and dried on a paper towel. EMS residue was collected in a specially designed container and disposed by EnviroServ Waste Management (Pty Ltd./South Africa), the company that removes toxic waste.

Seeds were planted at a depth of 1 cm in seedling trays containing a pine bark seedling mix growth media (National Plant Foods/South Africa) which was pasteurized in a steam chamber for 4 h. The trays were watered immediately after sowing and transferred to the CEF.

The experiment was laid out as a completely randomized design with two replications. For each replicate 40 seeds per treatment were planted. Data collected included number of days to 50% emergence, germination percentage and seedling height in millimeters. Days up to 50% emergence was recorded when 50% of seedlings emerged for each treatment. Germination percentage was recorded by counting the number of seeds that germinated and emerged for each treatment. Fourteen days after planting, seedling height of 10 randomly selected plants from each treatment was measured as the length from the base of the plant to the tip leaf.

Table 1. Analysis of variance on number of days to 50% emergence, germination percentage and seedling height among four wheat varieties when tested using four EMS doses at three temperature regime and four exposure time.

Source of variation	d.f.	Days to 50% emergence		Germination (%)		Seedling height	
		m.s.	F prob.	m.s.	F prob.	m.s.	F prob.
EMS	3	321.84	<0.001	1108.87	<0.001	10632.07	<0.001
Temperature (Temp)	2	168.23	<0.001	2425.01	<0.001	1252.34	<0.001
Time	3	473.60	<0.001	3204.59	<0.001	1587.56	<0.001
Variety	3	365.52	<0.001	1282.53	<0.001	533.42	<0.001
EMS x Temp	6	54.23	<0.001	634.12	<0.001	213.17	<0.001
EMS x Time	9	94.74	<0.001	903.89	<0.001	1232.12	<0.001
Temp x Time	6	23.32	<0.001	268.51	<0.001	573.10	<0.001
EMS x Variety	9	111.23	<0.001	695.88	<0.001	1475.23	<0.001
Temp x Variety	6	72.68	<0.001	252.50	<0.001	873.39	<0.001
Time x Variety	9	108.94	<0.001	403.12	<0.001	523.29	<0.001
EMS x Temp x Time	18	36.72	<0.001	274.42	<0.001	170.33	<0.001
EMS x Temp x Variety	18	146.78	<0.001	443.40	<0.001	286.33	<0.001
EMS x Time x Variety	27	70.05	<0.001	263.76	<0.001	654.38	<0.001
Temp x Time x Variety	18	38.02	<0.001	422.83	<0.001	244.63	<0.001
EMS x Temp x Time x Variety	54	36.32	<0.001	129.93	<0.001	245.68	<0.001
Residual	192	2.84		18.12		27.23	

d.f. = degrees of freedom; m.s. = mean square; F.prob. = Probability values.

Data analysis

Data on number of days to 50% emergence, germination percentages and seedling heights were subjected to analysis of variance (ANOVA) using Genstat® Release 11th edition (Payne et al., 2008). Mean comparisons were conducted using the Fisher's Least Significant Difference Procedure when significant interactions were detected in the ANOVA. The associations between emergence, germination percentages and seedling heights were determined using the Pearson correlation coefficients.

RESULTS

Highly significant interactions ($P < 0.001$) were observed between EMS, temperature, time and variety on days to 50% emergence, germination percentage and seedling height (Table 1). This suggests that the application of EMS to induce artificial mutations in wheat is significantly influenced by the EMS concentration, varietal differences, length of exposure time and temperature.

Table 2 summarizes the average number of days for 50% seedling emergence. Seeds treated with the highest EMS concentration, temperature and longest duration of exposure that is, 0.7% EMS, 35°C, and 2 h, respectively, showed delayed emergence. Varieties B936, B966 and SST875 subjected to 0.3% EMS concentration and a temperature of 30°C for 0.5 h emerged earlier (50%) compared to a higher EMS concentration (0.7%) that drastically reduced emergence (Table 2). Early seedling emergence was recorded after seven days when EMS concentration was 0.5% at a temperature of 35°C and exposure time of 1 h for all the varieties (Table 2).

The average seed germination of the varieties is presented in Table 3. Germination varied from 27 (0.70% EMS, 35°C, 2 h treatments) to 95% (0.30% EMS, 32.5°C, 0.5 h). Test varieties showed low seed germination at high EMS concentrations, temperature and treatment duration (0.7% EMS, 35°C, and 2 h) ranging from 27 to 62% (Table 3) which was associated with delayed emergence varying from 12 to 30 days (Table 2). Aiming the LD_{50}, varieties B936 and SST387 showed the optimal germination at 50% while varieties B966 and SST875 recorded higher germinations at 53% and 57%, respectively. These were noted at treatment conditions of EMS at 0.7%, 30°C and 1.5 h exposure time in B936; EMS at 0.5%, 35°C and 1.5 h (B966); EMS at 0.5%, 32.5°C and 2 h (SST387). SST875 required an EMS level at 0.5%, 32.5°C and 1 h treatment.

Seedling height significantly varied among genotypes, EMS dose and treatment conditions (Table 4). Seedling height decreased with increased EMS dose, high treatment temperatures and exposure duration. In the test varieties the seedling height varied from 14 mm (0.7% EMS, 35°C, 2 h) to 112 mm (0.3% EMS, 30°C, 1.5 h) (Table 4). On both measurements, seedling heights were affected adversely due to the mutagenic treatment compared controls subjected to varied temperatures and duration only (Table 4). Although all doses of EMS mutagen elicited a reducing effect on seedling height, some of the seedlings at 2 h treatment, 0.7% EMS concentration and at 35°C displayed an increase in height, especially SST387.

In this study there were significant negative associations between days to 50% emergence with germination percentage and seedling height (Table 5).

Table 2. Effect of four doses of EMS (0, 0.3, 0.5 and 0.7%), three treatment temperatures (30, 32.5 and 35ºC), four exposure times (0.5, 1, 1.5 and 2 h) and variety on mean days to 50% emergence.

Treatment conditions			Varieties			
EMS (v/v)	**Temperature (°C)**	**Time (h)**	**B936**	**B966**	**SST387**	**SST875**
0.3	30	0.5	7	7	8	7
		1	6	8	6	6
		1.5	7	7	6	6
		2	9	8	6	7
	32.5	0.5	8	7	7	6
		1	6	8	9	7
		1.5	7	9	10	7
		2	11	10	9	8
	35	0.5	9	10	6	7
		1	6	7	10	6
		1.5	7	9	9	7
		2	6	9	8	6
0.5	30	0.5	6	7	9	6
		1	7	8	6	10
		1.5	6	10	8	10
		2	7	30	6	8
	32.5	0.5	9	8	8	7
		1	7	7	7	7
		1.5	8	30	9	7
		2	8	30	11	9
	35	0.5	6	8	7	7
		1	7	7	8	7
		1.5	8	11	7	21
		2	8	30	8	30
0.7	30	0.5	7	6	9	6
		1	8	7	6	6
		1.5	10	7	7	7
		2	8	8	30	9
	32.5	0.5	8	9	7	30
		1	7	7	7	8
		1.5	9	10	8	30
		2	11	30	9	30
	35	0.5	7	6	5	6
		1	6	30	7	6
		1.5	11	9	7	7
		2	12	30	13	20
Control (0)	30	0.5	5	6	5	5
		1	5	6	6	6
		1.5	6	8	8	7
		2	8	7	7	7
	32.5	0.5	5	6	6	5
		1	7	7	7	6
		1.5	8	7	6	7
		2	8	8	6	7
	35	0.5	6	7	6	7
		1	7	7	7	8
		1.5	9	8	6	8
		2	8	9	8	9

Least Significant Difference (p=0.05) = 2.42; Degrees of Freedom = 192; Coefficient of variation % = 11.3.

Table 3. Effect of four doses of EMS (0, 0.3, 0.5 and 0.7%), three treatment temperatures (30, 32.5 and 35°C), four exposure times (0.5, 1, 1.5 and 2 h) and variety on mean germination (%).

Treatment conditions			Varieties			
EMS (v/v)	**Temperature (ºC)**	**Time (h)**	**B936**	**B966**	**SST387**	**SST875**
0.3	30	0.5	71.7	68.0	55.6	72.4
		1	83.4	64.5	78.5	76.8
		1.5	78.4	71.2	88.4	82.7
		2	58.3	64.3	78.4	81.4
	32.5	0.5	68.3	68.7	43.4	95.4
		1	88.4	76.4	56.7	82.7
		1.5	80.0	60.0	51.6	81.7
		2	46.7	61.7	55.0	83.5
	35	0.5	60.0	58.3	90.0	85.2
		1	80.0	76.7	55.2	82.7
		1.5	73.4	61.7	53.3	76.4
		2	86.7	63.4	70.2	92.5
0.5	30	0.5	85.0	61.7	66.7	88.4
		1	83.2	63.4	73.4	72.3
		1.5	85.0	61.6	63.2	70
		2	81.7	40.0	85.6	70
	32.5	0.5	65.0	66.7	63.4	73.35
		1	76.7	71.7	58.0	56.6
		1.5	83.3	42.7	47.4	70
		2	60.0	43.3	50.3	42.6
	35	0.5	85.0	60.0	45.0	71.6
		1	78.4	63.4	61.6	70.0
		1.5	73.3	53.3	70.0	48.4
		2	68.4	35.0	56.7	46.7
0.7	30	0.5	83.3	81.7	86.7	86.7
		1	71.7	78.4	78.4	95.0
		1.5	50.0	73.3	71.7	81.7
		2	70.0	40.0	43.4	68.4
	32.5	0.5	65.0	65.0	60.0	40.0
		1	81.7	66.7	73.2	76.7
		1.5	68.4	56.7	61.8	43.3
		2	26.7	40.0	56.6	21.7
	35	0.5	80.0	72.4	95.0	81.7
		1	86.7	31.7	61.5	93.3
		1.5	73.4	66.6	65.0	75.0
		2	41.7	26.7	61.7	60.0
Control (0)	30	0.5	86.9	63.6	70.1	93.4
		1	85.0	68.8	74.0	92.5
		1.5	82.0	71.4	75.0	94.3
		2	75.1	72.6	74.4	93.4
	32.5	0.5	82.0	69.8	73.0	91.5
		1	80.2	70.1	72.3	84.5
		1.5	80.3	71.1	73.3	80.2
		2	74.3	74.5	65.5	80.4
	35	0.5	76.4	65.5	71.3	82.5
		1	73.2	62.3	74.5	83.2
		1.5	74.4	59.4	73.4	84.4
		2	70.5	66.6	72.7	75.6

Least Significant Difference (p=0.05) = 7.34; Degrees of Freedom = 192; Coefficient of variation % = 8.2

Table 4. Effect of four doses of EMS (0, 0.3, 0.5 and 0.7%), three treatment temperatures (30, 32.5 and 35°C), four exposure times (0.5, 1, 1.5 and 2 h) and variety on mean seedling height (mm).

Treatment conditions			Varieties			
EMS (v/v)	**Temperature (ºC)**	**Time (h)**	**B936**	**B966**	**SST387**	**SST875**
0.3	30	0.5	97.2	101.1	50.3	85.0
		1	90.2	100.0	86.3	79.3
		1.5	98.9	111.7	101.1	87.0
		2	73.2	105.0	80.2	88.1
	32.5	0.5	97.6	89.4	84.1	91.4
		1	98.1	106.1	62.6	73.7
		1.5	92.7	90.6	85.0	85.3
		2	37.5	102.5	64.56	61.7
	35	0.5	89.7	54.6	46.6	72.0
		1	86.0	81.3	40.9	101.9
		1.5	109.0	95.9	95.1	67.4
		2	84.2	89.0	41.8	79.1
0.5	30	0.5	65.6	82.4	73.5	76.7
		1	100.4	86.1	82.3	78.7
		1.5	89.2	84.8	80.1	78.4
		2	60.0	96.8	76.1	78.9
	32.5	0.5	89.4	107.9	100.3	81.3
		1	86.0	88.3	75.9	79.4
		1.5	69.6	68.7	55.8	64.7
		2	59.3	46.4	105.2	73.3
	35	0.5	87.4	93.4	107.2	72.7
		1	88.0	80.7	78.3	78.3
		1.5	80.5	73.1	84.8	60.7
		2	66.4	53.9	76.0	62.3
0.7	30	0.5	57.7	112.9	108.9	82.1
		1	73.6	63.5	83.4	95.2
		1.5	47.8	103.2	70.1	56.9
		2	66.9	31.1	42	55.3
	32.5	0.5	52.8	100.6	55	45.8
		1	83.7	70.4	69.8	70.6
		1.5	55.5	55.8	59.0	42.1
		2	33.4	13	66.4	30.6
	35	0.5	95.8	86.8	98.5	92.5
		1	82.9	30.25	60.9	75.5
		1.5	40.6	37.5	60.2	56.8
		2	37.4	14.0	75.9	32.5
Control (0)	30	0.5	87.4	89.6	74.5	79.6
		1	112.2	88.0	84.3	84.5
		1.5	105.2	87.4	52.5	69.7
		2	96.9	88.2	54.5	55.6
	32.5	0.5	78.5	78.9	72.6	82.2
		1	98.8	76.5	85.3	75.2
		1.5	106.2	78.8	64.5	75.0
		2	95.6	70.0	56.8	68.9
	35	0.5	87.6	86.6	78.2	68.2
		1	84.6	85.5	76.3	72.2
		1.5	88.5	80.6	88.9	75.5
		2	95.7	78.8	105.2	74.0

Least Significant Difference (p=0.05) = 9.73; Degrees of Freedom = 192; Coefficient of variation % = 6.24.

Table 5. Correlation coefficients of the pair-wise associations between days to 50% emergence, germination (%) and seedling height in wheat.

Character	DTE	GPT	SH
DTE	1	-0.96**	-0.82**
GPT		1	0.94**
SH			1

**=correlation is significant at P=0.01. DTE=Days to 50% emergence; GPT=Germination percentage; SH=Seedling height.

Thus, early days to emergence could not provide high germination percentage and seedling height in the tested varieties. There was a significant positive correlation between germination percentage and seedling height (r=0.94, P<0.01) (Table 5).

DISCUSSION

The present study found significant interactions among varieties, EMS doses, and temperature regime and exposure time on seed germination, seedling emergence and seedling height in wheat (Table 1). This enabled to identify suitable treatment combinations before large scale mutagenesis can be implemented in the selected wheat genotypes. The ideal treatment in variety B936 was 0.7% EMS, 30°C and 1.5 h. While B966 required 0.5% EMS, 35°C and 1.5 h; 0.5% EMS, 32.5°C and 2 h (SST387) and 0.5% EMS, 32.5°C and 1 h (SST875) treatments. These treatment combinations rendered 50 to 60% seedling emergence and germination percentage associated with relatively healthy and tall growing seedlings in the test varieties (Tables, 2, 3 and 4). A similar EMS concentration (0.5%) should be used for the three varieties (B966, SST387 and SST875) with varied treatment temperatures and durations.

High doses of EMS mutagenesis resulted in drastically reduced seed germination, delayed seedling emergence and reduced seedling growth consistently in the varieties (Tables 2, 3, and 4). This is not suitable for large scale mutagenesis since few plants would survive and sampled for selection which in turn limits the success of artificial selection in the subsequent mutation generations to identify useful mutants. Chen et al. (2012) applied EMS at 0.8% for 18 h using the Chinese common wheat cultivar 'Jinmai 47' and found a germination rate of 40%. The authors found significantly low germination at 25 and 8% when using EMS at 1 and 1.2%, respectively. This suggested the necessity to apply lower EMS concentrations to obtain healthy and fertile mutants that will be grown to maturity. Jayakumar and Selvaraj (2003) suggested that high EMS dose may destruct growth promoters, increase growth inhibitors and metabolic status of the seed and induce various chromosomal aberrations. As expected wheat seeds treated at low EMS dose and reduced temperatures and exposure time showed increased percentage emergence and germination with healthy and vigorous seedlings. However, this would provide low mutation frequency associated with reduced selection response in identifying target and desired mutants. Van Harten (1998) indicated that normally genetic mutations occur spontaneously at low frequencies (10^{-5} to 10^{-8} per locus). Greene et al. (2003) and Weil and Monde (2007) reported that lowering the treatment dosage will decrease the overall mutation rate. Therefore, the key for determining the optimal mutagen dosage is to maximize mutational density while minimizing lethality and aneuploidy.

Reduced seed germination and delayed seedling emergence have been reported with increased mutagenic concentrations (Leonard, 1967; Maluszynski et al., 1995; Adamu et al., 2002; Khan et al., 2004; Rupinder and Kole, 2005; Chen et al., 2012). Other studies (Kleinhofs et al., 1978; Mba et al., 2007, 2010) indicated that increased EMS concentration could be accompanied by a corresponding greater amount of injury to seedlings with subsequent lethality. Singh and Kole (2005) suggested that the effect of EMS in reducing germination could probably be attributed due to water potential difference, in which the higher EMS concentration may have lowered the water potential outside the seed and therefore the seeds could not imbibe enough water for proper germination. In other cereals such as sorghum Xin et al. (2008) used one genotype and varied EMS concentrations (0.1 to 0.6%) without describing the treatment temperature and durations. The authors recommended EMS concentration of 0.25% as the highest dosage at which treated seeds developed into healthy M_1 plants that produced viable M_2 seeds.

In the present study, seedling height decreased in all varieties with increased EMS concentrations (Table 4). The shortest seedling height (14 mm) was noted at the 14^{th} day after sowing at 0.7% EMS, 35°C and 2 h treatment. Conversely, tall seedling height (111 mm) was measured when seeds were exposed at low EMS concentration (0.3%), reduced temperature (30°C) and 1.5 h treatment duration. Other studies indicated that time and rate of seedling emergence and growth are influenced by the genetic constitution of the variety, seed dormancy, seed vigor, depth of planting, soil aeration, temperature and water supply (Forcella et al., 2000; Samarah and Al-Kofahi, 2008).

The study concluded the requirement of variety specific EMS concentration, treatment temperature and duration to induce large scale mutations and select targeted mutant individuals in wheat. Increased EMS dose, temperature and exposure time were detrimental to all the traits considered in the study.

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