African Journal of Biotechnology Vol. 10(59), pp. 12602-12613, 3 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.329
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Seed viability, germination and seedling growth of canola (*Brassica napus* L.) as influenced by chemical mutagens

S. N. Emrani*, A. Arzani and G. Saeidi

Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan-84156 83111, Iran.

Accepted 7 July, 2011

Mutation induction is considered as an effective way to enrich plant genetic variation, particularly for traits with a very low level of genetic variation. The objectives of this study were to evaluate the effect of different dosages of chemical mutagens on seed germination, seed viability and seedling growth characteristics and to identify optimum treatment conditions for chemical mutagens based on the LD_{50} criterion in canola (*Brassica napus* L.). Two pretreatment conditions of soaking in distilled water and non-soaking, different concentrations of chemical mutagens, and four treatment periods were investigated. The effect of mutagen dosage on seed viability was also assessed using the tetrazolium staining test. Results revealed the significant effects of mutagen dosages and treatment periods on seed viability and seed germination as well as on seedling characteristics for all the mutagens tested. Additionally, it was found that increased dosage and period in each treatment led to significant reductions in seed viability for the tested mutagens. Pretreatment did not significantly influence most of the studied characteristics. The 0.8% ethyl methanesulfonate (EMS) for 6 h, 12 mM N-nitroso-N-ethylurea (ENU) and 6 mM sodium azide for 8 h and 9 mM N-nitroso-N-methylurea (NMU) for 4 h were considered as optimum treatment conditions.

Key words: *Brassica napus*, canola, chemical mutagen, germination, seed viability, seedling growth.

INTRODUCTION

Canola (*Brassica napus* L.) is one of the most important sources of vegetable oils and protein-rich meals worldwide. Canola ranks third in global production of oilseed crops and fifth among economically important crops following wheat, rice, maize, and cotton (FAOSTAT, 2011). With 7% saturated fats, canola oil contains the least amount of saturated fats among the common edible oils. The polyunsaturated fats in canola oil include the essential fatty acid α-linolenic acid (omega-3) and linoleic acid (omega-6) which help reduce cholesterol in the blood stream. Canola oil is also a good source of vitamins E and K and plant sterols which may keep the heart healthy (McDonald, 2011). Therefore, canola oil is promoted as one of the healthiest vegetable oils for human consumption.

Availability of genetic diversity and genetic variation is the heart of any breeding program which plays a critical role in developing well-adapted and improved varieties. Mutation induction is an effective tool to enhance the genetic variation available to plant breeders, particularly for traits with a very low level of genetic variation (Szarejko and Forster, 2007). The high frequency with which certain radiations and chemicals can cause genes to mutate made it feasible to perform genetic studies that were not possible when only spontaneous mutations were available. Consequently, much of our knowledge of genetics of higher organisms is based upon works utilizing induced mutations for analyzing gene function

*Corresponding author. E-mail: nazgol_6532@yahoo.com Tel: +98 311 391 3453. Fax: +98 311 391 2254.

Abbreviations: EMS; Ethyl methanesulfonate, **ENU;** N-nitroso-N-ethylurea and **NMU;** N-nitroso-N-methylurea.

(McCallum et al., 2000). To date, several well-documented examples of successful applications of mutation breeding to oilseed crops have been reported in the literature (Ahmad et al., 1991; Bacelis, 2001; Bhatia et al., 1999; Ferrie et al., 2008; Fowler and Stefansson, 1972; Kott et al., 1996; MacDonald et al., 1991; Newsholme et al., 1989; Osorio et al., 1995; Parry et al., 2009; Rowland, 1991; Sala et al., 2008; Schnurbush et al., 2000; Spasibionek, 2006; Swanson et al., 1989; Velasco et al., 2008). Induced mutations have been used mainly to generate variation that could rarely be found in germplasm collections. Mutation techniques have been applied to improve such traits as earliness, semi dwarfness, lodging resistance, disease resistance, yield and quality (Bhatia et al., 1999; Newsholme et al., 1989; Osorio et al., 1995; Parry et al., 2009; Rowland, 1991; Schnurbush et al., 2000).

About 3088 mutant varieties have been developed according to FAO/IAEA mutant varieties database (FAO/IAEA, 2011). To date, 198 mutant cultivars of annual oilseed crops including soybean, sesame, canola, sunflower and linseed have been released (FAO/IAEA, 2011). Soybean with 155 mutant cultivars possesses the highest number of mutant cultivars, followed by sesame with 24 and canola with 15 cultivars. In canola, oil modification has been achieved by using seed and microspore mutagenesis (Ferrie et al., 2008; MacDonald et al., 1991; Velasco et al., 2008). In spring canola, radiation treatment has been applied to the seeds of "Regent" cultivar and M_5 lines selected with increased oleic acid contents varying from 63 to 79%. In winter canola, chemical mutagenesis was used to isolate two canola mutants of the cultivar "Winfield" with high oleic acid content (Wong and Swanson, 1991). Mutation breeding in canola has been also used to improve herbicide resistance (Ahmad et al., 1991; Sala et al., 2008; Swanson et al., 1988, 1989), disease resistance (Ahmad et al., 1991; MacDonald et al., 1991; MacDonald and Ingram, 1986; Newsholme et al., 1989), and lower glucosinolate content (Barro et al., 2002; Kott, 1998; Kott et al., 1996). Chemical and physical mutagens are available for mutagenic treatment of crop plants. Nevertheless, several chemical mutagens have been applied of which ethyl methane sulfonate (EMS), N-nitroso-N-methylurea (NMU), N-Nitroso, N-Ethylurea (ENU) and sodium azide are the preferred agents in plant mutation induction (Medrano et al., 1986; Szarejko and Forster, 2007). Alkylating agents are the most important chemical mutagens used in mutation breeding. They add ethyl or methyl groups to bases in the nucleotide structure, which leads to activating a silent gene, silencing an active gene, or altering a particular gene action (Snustad and Simmons, 2006). Chemical mutagens have not only been used for forward genetic screens but also used for reverse genetic screens. To date, databases of many gene sequences of model plant species are available, and the prediction of gene function on the basis of comparisons among genomes is feasible. It is still necessary to validate those predictions, and the 'reverse genetics' that is based on the mutagenesis of the target gene can been employed. Chemical mutagenesis has a number of inherent attractions such as the ability to use different mutagens, change mutagen doses and to easily scale the size of the mutagenesis procedure.

Optimization of the mutation induction conditions in each plant species plays a critical role in the successful employment of the mutagenic events (Padma and Reddy, 1977). Breeders must be aware of the genetic structure and responses of plant genotype to a mutagen because frequency and type of induced mutation depends on plant genotypic background, mutagen concentration and pre and post-treatment conditions. Mutagen dosage, temperature, pH, pre-treatment and post treatment influence mutagen action, production of M_1 plants, and M_1 viability. These factors vary from plant to plant and from mutagen to mutagen (Fowler and Stefansson, 1972; Kharkwal, 1998). Mutagen dosage is the most important factor that affects mutation frequency. Hence, defining the optimal dose of a chemical mutagen is one of the most critical steps that have often been complicated by limited knowledge of the effects of environmental conditions and environment by mutagen interaction on both mutagenic and toxic impacts on plant tissues.

Optimal dose can be defined as the dosage leading to adequate genetic variation accompanied by the lowest plant lethality (Snustad and Simmons, 2006). Mutagen dose, treatment period and their interaction can be considered as the main factors also influenced by pretreatment, temperature, pH, and post-treatment (Hu and Rutger, 1992). Lethal dose 50 (LD_{50}) is generally used as a criterion to define the optimum mutagenic dose. Bacelis (2001) investigated the effects of different concentrations of EMS, ENU and NMU on variability of two flax varieties and reported 0.025% ENU, 0.012% NMU and 0.3% EMS as their optimal doses. Patil et al. (2011) also introduced 0.1 to 0.2% EMS concentrations as optimum dosages to induce maximum variations in soybean populations. Fowler and Stefansson (1972) evaluated EMS for mutagenesis in rapeseed (*B. napus* L.) and observed that increasing EMS concentration from 0 to 1.0% adversely affected germination percentage, plant vigor and seed yield. Germination test is an indication of the potential of a seed lot to emerge under field conditions. On the other hand, tetrazolium test is a timely and accurate test for determining seed viability (AOSA, 2000; Karrfalt, 2011). Landho and Jorgensen (1997) used the tetrazolium test for evaluating *Brassica* wild species and hybrids and found stained seeds which did not germinate after 2 to 3 days due to dormancy. Therefore, application of both germination and tetrazolium tests, rather than by either one alone, provides complementary evidence of seed viability (Elias et al., 2006).

When developing mutagenized populations for breeding

purposes, forward or reverse genetic analyses, ascertaining the optimum mutation frequency and thus appropriate size of a desirable mutagenized population is crucial. Mutagen treatment is usually applied in such a manner that it produces sufficient lethality while allowing sufficient fertility, so that a high frequency of induced mutations may be recovered in mutagenized population. The objective of this study was to determine the optimal doses and treatment conditions for four chemical mutagens (EMS, NMU, ENU and sodium azide) in canola using seed germination and tetrazolium test.

MATERIALS AND METHODS

Seeds of spring canola cultivar "RGS003" were exposed to four chemical mutagens obtained from Sigma-Aldrich (St. Louis, Missouri, USA) which comprised of ethyl methane sulfonate (EMS, Sigma M0880), N-nitroso-N-methylurea (NMU, Sigma, N4766), N-nitroso-N-ethylurea (ENU, Sigma N8509), and sodium azide (NaN_3, Sigma S2002). A 4 × 2 × 4 × 4 factorial design with a completely randomized design having five replications was used. Each replication consisted of a 120 × 20 mm Petri-dish with 100 seeds. Four mutagens, two levels of pre-treatment period including soaking in distilled water for 3 h and non-soaking, four dosages of each mutagen along with control and four treatment periods comprised the experimental factors. Seeds were treated with EMS concentrations of 0 (control), 0.4, 0.8, 1.2 and 1.6% (v/v) for 3, 6, 9 and 12 h periods. For NMU and ENU treatments, the treatments included solutions of 0 (control), 3, 6, 9 and 12 mM for 2, 4, 6 and 8 h. And for sodium azide treatments, seeds were treated with 0 (control), 2, 4, 6 and 8 mM solutions for 2, 4, 6 and 8 h. After mutagen treatments, seeds were rinsed for 30 min with running tap water to completely remove mutagens.

One hundred seeds per treatment were placed on a filter paper in sterilized Petri dishes containing 15 ml distilled water. The Petri dishes were placed in an incubator with 12 h of darkness at the constant temperature of 25±1°C. Germination counts were made after 2, 4, 6 and 8 days of incubation. Seeds were considered germinated when the radicle was at least 3 mm long. For germination percentage, the number of seeds germinated on day 7 was considered. The germination rate index was determined by $\sum(Ni/Di)$ as described by Carlton et al. (1968), where Ni is the number of seeds germinated between two counting's and Di represents the day of counting. Seedling height and radicle length were determined in centimetres as the mean of 10 seven day-old seedlings per treatment.

Seed viability was tested using a standard tetrazolium test (AOSA, 2000). To evaluate the effects of different chemical mutagen dosages on seed viability, an experiment was conducted using a factorial experiment (4×4×4) with a completely randomized design replicated three times. Four mutagens, four dosages of each mutagen and four treatment periods were the factors of the experiment. For each treatment, 100 seeds were placed between moist paper towels for 8 h. They were then incubated in 1% (w/v) solution of 2,3,5-triphenol tetrazolium chloride for 24 h at 25 ±1°C. Seeds with stained embryos were scored as viable.

Statistical analysis

The germination percentage data was transformed using arcsin√x (Steel and Torrie, 1980) and then subjected to analysis of variance (ANOVA). Data from seed germination test was analyzed as a 4 × 2 × 4 × 4 factorial experiment with a completely randomized design (CRD), replicated five times. Data from the viability test were analyzed as a 4 × 4 × 4 factorial experiment with a CRD replicated three times. ANOVA was carried out using PROC GLM of SAS (SAS Institute Inc, 2008). Mean comparisons were conducted using the Fisher's (protected) least significant difference (LSD) test. Linear correlation coefficients (r) were also calculated between pairs of traits.

RESULTS

The results of analyses of variance indicated that mutagen, dosage and treatment period significantly influenced canola-seed germination percentage, germination rate index, radicle length and seedling height (Table 1). Pre-treatment significantly affected only germination rate and radicle. Among the first-order interactions, mutagen × treatment period and dosage × treatment period were significant for all the traits. For seedling height, second and third-order interactions were significant.

All the main effects (mutagen, dosage, treatment period) along with first and second-order interactions were highly significant for seed viability (Table 2).

Ethyl methane sulfonate

Average germination percentage reduced with increasing mutagen concentration and treatment period where germination percentage was reduced from 92.7% in the control to 7.9% in the treatment with 1.6% EMS (Table 3). This trait was also reduced by increasing treatment period from 3 to 12 h where the germination percentage changed from 65.1% in non-presoaked seeds treated for 3 h to 9.25% in presoaked seeds treated for 12 h with EMS. The treatment with 1.6% EMS acting similar to those of 12 h treatment with different concentrations of this mutagen almost blocked seed germination. The highest germination rate index (37.9) belonged to the presoaked control treatment and the least amount was related to the 9 h treatment with 1.2% of EMS in of non-presoaked seeds (Table 4).

Seedling height and radicle length also decreased with increasing EMS concentration and treatment period (Tables 5 and 6). Pre-soaking did not significantly alter seedling height and radicle length traits. In both pre-treatment conditions, treatment periods higher than 6 h affected neither the germination rate nor the seedling height of EMS-treated seeds. Non-presoaked seeds performed superior than presoaked ones in most of the treatments. Mean comparisons of seed viability for the EMS treatment are presented in Table 7. Seed viability varied between 0 for the treatment with 1.6% EMS for 12 h to 89.7% for the control. Means of germination percentage just like seed viability grouped canola genotypes into 9 different classes. The twelve hour treatment with 1.6% EMS induced the least amount of both germination percentage and seed viability.

Table 1. Analyses of variances for germination percentage, germination rate, radicle length and seedling height in canola mutants.

Source of variation	df	Mean square			
		Germination percentage	Germination rate index	Radicle length	Seedling height
Mutagen (M)	3	2.83**	2104.39**	716.42**	299.91**
Pre-treatment (P)	1	0.004	388.83**	2.81	0.01
Dosage (D)	4	1.79**	680.38**	54.82**	11.34**
Treatment period (T)	3	2.46**	2662.98**	120.69**	17.78**
M×P	3	0.01	160.81**	4.06*	0.91**
M×D	12	0.53**	439.44**	19.86**	1.72**
M×T	9	0.49**	248.99**	28.66**	2.72**
P×D	4	0.02	55.87*	1.30	0.79**
P×T	3	0.03	77.96**	9.90**	4.10**
D×T	9	0.04*	17.18	1.86	2.08**
M×P×D	12	0.03	40.32*	1.89	0.38*
M×P×T	9	0.02	16.30	5.23**	1.11**
M×D×T	27	0.05**	50.25**	4.01**	0.64**
P×D×T	9	0.04*	37.70	1.45	0.44*
M×P×D×T	27	0.02	17.31	1.53	0.79**
Residual	408	0.02	23.19	1.47	0.22
C.V.		17.87	30.41	23.76	16.67

* and ** significant at 0.05 and 0.01 of probability levels, respectively.

Table 2. Analysis of variance for seed viability in canola mutants.

Source of variation	df	Mean square
Mutagen (M)	3	0.59**
Dosage (D)	4	0.60**
Treatment period (T)	3	0.73**
M× D	12	0.08**
M×T	9	0.11**
D× T	9	0.05**
M× D×T	27	0.06**
Residual	136	0.01
C.V.		8.87

** Significant at P≤0.01.

N-Nitroso, N-ethyleurea

Increasing mutagen dosages decreased germination percentage in a way that the presoaked control and the 8 h non-presoaked treatment with 12 mM ENU led to the highest and the lowest amounts of germination percentages, respectively (Table 3). Treatment of soaked seeds with 6 mM ENU for 6 h yielded the lowest germination rate among the treatments. On the other hand, the 2 h treatment of non-presoaked seeds with 12 mM of this mutagen produced the highest germination rate which was even greater than that of the control (Table 4). Application of this treatment to non-presoaked seeds also induced the lowest amount of radicle length and seedling height.

The six hour presoaked seed treatment with 12 mM ENU had the highest amount of radicle length with no significant difference from the control treatment (Table 5). Increasing ENU dosage reduced seedling height. Presoaked seeds treated with 3 mM ENU for 8 h produced the highest seedling height, which was even higher than that of the control treatment (Table 6). Pre-treatment significantly affected germination rate and seedling height of ENU-treated canola seeds. Germination rate was reduced by soaking but pre-soaked seeds had a higher seedling height except for the 2 h treatment with this mutagen (Table 4). The highest seed viability belonged to the control treatment, while the seeds treated with 9 mM ENU for 8 h led to the highest reduction in this trait (Table 7). This trait divided mutant seeds to 13 different groups. Germination percentage also showed high genetic variation and grouped genotypes into 11 different classes.

N-Nitroso, N-methylurea

As expected, the increase of NMU concentration and treatment period reduced germination percentage, germination rate, radicle length and seedling height, but the changes in germination rate were irregular for different NMU concentrations. Control presoaked treatment had the highest germination percentage and

Table 3. Mean comparisons of germination percentage for dosages, pre-treatment and treatment period and their interactions in EMS, ENU, NMU and sodium azide treated canola seeds.

EMS concentration (%)	Soaking				Non-soaking				Total mean
	3 h	6 h	9 h	12 h	3 h	6 h	9 h	12 h	
Control			94[a]		91.5[ab]				92.75 [a]
0.4	84[a-c]	81[a-c]	56.25[c-f]	37[e-g]	91[ab]	73a-d	55.25[c-f]	38.5[e-g]	64.5[b]
0.8	83.75[a-c]	44.75[d-f]	0.75[h]	0[h]	63.25[b-e]	34.75[fg]	18.5[gh]	0.75[h]	30.81[c]
1.2	63[b-e]	15.5[gh]	0.5[h]	0[h]	60[c-e]	34fg	0.75[h]	0[h]	21.71[c]
1.6	12.5[gh]	0[h]	0[h]	0[h]	46.25[d-f]	4.25[h]	0h	0[h]	7.87[d]
Total mean	60.81[a]	35.31[b]	14.37[cd]	9.25[d]	65.12[a]	36.5[bc]	18.62[b-d]	9.81[d]	
ENU concentration (mM)	**Soaking**				**Non-soaking**				**Total mean**
	2 h	**4 h**	**6 h**	**8 h**	**2 h**	**4 h**	**6 h**	**8 h**	
Control	85.25[a 1]				75.50[b]				80.37 [a]
3	63.56[cd]	60.03[c-f]	57.81[c-h]	50.17[g-n]	57.38[c-i]	61.62[c-e]	56.21[c-j]	47.11[k-n]	56.73[b]
6	65.43[c]	56.37[c-j]	49.90[h-n]	49.40[h-n]	65.05[c]	48.05[i-n]	54.25[d-l]	53.26[c-l]	55.21[b]
9	56.87[c-i]	50.24[h-n]	47.49[j-n]	45.47[k-n]	54.51[d-k]	52.50[f-m]	43.75[mn]	45.25[k-n]	49.51[c]
12	64.25[c]	58.25[c-h]	51.25[f-m]	45.50[k-n]	64.75[c]	59.25[c-g]	52.50[f-m]	41.75[n]	54.68[b]
Total mean	62.52[a]	56.22[bc]	51.61[cd]	47.63[de]	60.42[ab]	55.35[c]	51.67[cd]	46.84[e]	
NMU concentration (mM)	**Soaking**				**Non-soaking**				**Total mean**
	2 h	**4 h**	**6 h**	**8 h**	**2 h**	**4 h**	**6 h**	**8 h**	
Control	88.5[a]				85.59[b]				87.04 [a]
3	67.70[d-g]	57.15[h-k]	56.16[i-l]	59.47[g-i]	73.46[c-e]	70.48[c-e]	68.25d-g	49.01[l-n]	62.71[b]
6	73.65[c-e]	65.72[e-h]	67.19[d-g]	46.25[mn]	75.78[cd]	73.09[c-e]	61.36f-i	49.25[k-n]	64.03[b]
9	66.14[e-h]	44.22[no]	52.63[i-m]	43.87[no]	78.30[bc]	73.09[c-e]	51.26j-n	38.30[o]	55.97[c]
12	67.50[d-g]	69.17[d-f]	54.58[i-m]	52.09[j-n]	68.56[d-f]	65.98[e-h]	44.14[no]	15.54[p]	54.69[c]
Total mean	68.74[b]	59.06[c]	57.64[c]	50.42[d]	74.02[a]	70.66[b]	56.25[c]	38.02[e]	
Sodium azide concentration (mM)	**Soaking**				**Non-soaking**				**Total mean**
	2 h	**4 h**	**6 h**	**8 h**	**2 h**	**4 h**	**6 h**	**8 h**	
Control	85.75[a]				87[a]				86.37 [a]
2	71.75[bc]	60.75[e-i]	62.5[c-g]	55.75[g-l]	67.5[b-e]	62[d-h]	60.75[e-i]	52.5[h-m]	61.68[b]
4	71[b-d]	66[b-f]	59.25[e-j]	51.25[i-n]	71.75[b]	70.75[b-d]	62.5[c-g]	51.25[i-n]	62.96[b]
6	57.75[f-k]	54.5[g-l]	48[l-o]	44.75[m-p]	55.75[g-l]	55[g-l]	48.25[k-o]	43[n-q]	50.87[c]
8	48.75[k-o]	43.25[n-q]	40.25[o-r]	34.75[qr]	50.5[j-n]	43.75[m-q]	37.25[p-r]	32[r]	41.31[d]
Total mean	62.31[a]	56.12[bc]	52.5[c]	46.62[d]	61.37[a]	57.87[ab]	52.18[c]	44.68[d]	

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

Table 4. Mean comparison of germination rate index for dosages, pre-treatment and treatment period and their interactions in EMS, ENU, NMU and sodium azide treated canola seeds.

EMS concentration (%)	Soaking				Non-soaking				Total mean
	3 h	6 h	9 h	12 h	3 h	6 h	9 h	12 h	
Control	37.90$^{a\ 1}$				35.43^{ab}				36.66^{a}
0.4	25.77^{bc}	21.16^{cd}	11.48^{d-g}	7.24^{f-h}	32.48^{ab}	21.57^{cd}	13.87^{d-f}	8.57^{e-h}	17.77^{b}
0.8	25.39^{bc}	7.34^{f-h}	0.37^{h}	0^{h}	18.13^{c-e}	6.75^{f-h}	3.39^{gh}	0.13^{h}	7.69^{c}
1.2	20.92^{cd}	3.29^{gh}	0.18^{h}	0^{h}	18.48^{c-e}	7.29^{f-h}	0.11^{h}	0^{h}	6.28^{c}
1.6	2.92^{gh}	0^{h}	0^{h}	0^{h}	14.89^{d-f}	0.80^{h}	0^{h}	0^{h}	2.32^{d}
Total mean	18.75^{a}	7.94^{b}	3.01^{b}	1.81^{b}	20.99^{a}	9.10^{b}	4.34^{b}	2.17^{b}	

ENU concentration (mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	26.12^{b}				18.39^{cd}				22.25^{a}
3	16.66^{c-g}	16.47^{c-g}	14.52^{f-k}	14.02^{f-k}	12.21^{i-k}	19.42^{c}	14.57^{e-k}	11.92^{jk}	14.97^{b}
6	16.46^{c-g}	15.33^{d-i}	11.59^{k}	14.96^{e-j}	18.60^{cd}	13.70^{f-k}	15.77^{d-h}	16.05^{d-h}	15.31^{b}
9	14.58^{e-k}	13.54^{g-k}	14.21^{f-k}	13.74^{f-k}	16.50^{c-g}	23.23^{b}	16.84^{c-f}	16^{d-h}	16.08^{b}
12	24.39^{b}	17.85^{c-e}	16.63^{c-g}	12.89^{h-k}	31.36^{a}	25.59^{b}	24.46^{b}	19.61^{c}	21.60^{a}
Total mean	18.02^{b}	15.79^{c}	14.23^{d}	13.90^{d}	19.66^{a}	20.48^{a}	17.91^{b}	15.89^{c}	

NMU concentration (mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	29^{a}				28.89^{a}				28.94^{a}
3	19.21^{c-f}	13.78^{k-m}	13.01^{m-o}	14.56^{j-m}	18.56^{d-g}	17.83^{f-i}	14.78^{j-m}	10.07^{p-r}	15.22^{c}
6	22.74^{b}	18.14^{e-h}	15.72^{i-l}	9.31^{q-s}	20.27^{c-e}	21.13^{bc}	13.55^{l-o}	9.57^{q-s}	16.30^{b}
9	14.53^{j-m}	10.20^{p-r}	11.54^{o-q}	7.51st	20.75^{b-d}	14.94^{j-m}	8.64^{rs}	5.67^{t}	11.72^{e}
12	19.43^{c-f}	16.80^{g-j}	12.18^{n-p}	9.50^{q-s}	22.67^{b}	16^{h-k}	9.17^{rs}	2.26^{u}	13.50^{d}
Total mean	18.97^{b}	14.73^{d}	13.11^{e}	10.22^{g}	20.56^{a}	17.47^{c}	11.53^{f}	6.89^{h}	

Sodium azide concentration (mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	26.77^{a-f}				30.31^{a}				28.54^{a}
2	25.68^{a-h}	18.44^{h-k}	17.67^{h-k}	14.55^{k}	26.51^{a-g}	28.54^{a-c}	20.51^{d-k}	14.51^{k}	20.80^{b}
4	21.07^{b-k}	19.25^{f-k}	15.41^{jk}	13.80^{k}	28.90^{ab}	25.55^{a-h}	24.30^{a-i}	18.12^{h-k}	20.80^{b}
6	20.41^{e-k}	15.64^{jk}	14.55^{k}	15.05^{jk}	28.48^{a-d}	24.11^{a-i}	21.51^{b-k}	17.71^{h-k}	19.68^{b}
8	20.51^{d-k}	18.73^{g-k}	15.60^{jk}	16.40^{i-k}	27.85^{a-e}	22.79^{a-j}	20.75^{c-k}	17.16^{i-k}	19.97^{b}
Total mean	21.91^{ab}	18.01^{bc}	15.80^{bc}	14.95^{c}	27.93^{a}	25.24^{a}	21.76^{ab}	16.87^{bc}	

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

Table 5. Mean comparison of radicle length for dosages, pre-treatment and treatment period and their interactions in EMS, ENU, NMU and sodium azide treated canola seeds.

EMS concentration (%)	Soaking				Non-soaking				Total mean
	3	6 h	9 h	12 h	3 h	6 h	9 h	12 h	
Control	$4.82^{a\ 1}$				4.25^{ab}				4.53^{a}
0.4	5.22^{a}	4.15^{ab}	1.70^{e-h}	1.47^{e-h}	4.65^{a}	3.85^{a-d}	1.95^{d-g}	1.27^{e-h}	3.03^{b}
0.8	3.87^{a-c}	1.91^{e-g}	0.08^{gh}	0^{h}	3.95^{a-c}	2.50^{b-e}	1.60^{e-h}	0.45^{f-h}	1.79^{c}
1.2	5.15^{a}	1.05^{e-h}	0.05^{gh}	0^{h}	4.42^{a}	2.07^{c-f}	0.45^{f-h}	0^{h}	1.65^{c}
1.6	1.72^{e-h}	0^{h}	0^{h}	0^{h}	3.90^{a-c}	0.45^{f-h}	0^{h}	0^{h}	0.75^{d}
Total mean	3.99^{a}	1.77^{b}	0.45^{c}	0.36^{c}	4.23^{a}	2.21^{b}	1^{bc}	0.43^{c}	

ENU concentration(mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	8.73^{a-c}				9.53^{ab}				9.13^{a}
3	6.96^{c-j}	7.08^{c-h}	8.33^{a-g}	7.47^{c-h}	8.24^{a-g}	6.94^{c-j}	6.30^{g-j}	7.77^{b-h}	7.39^{cd}
6	8.65^{a-c}	7.66^{b-h}	6.59^{d-j}	6.56^{e-j}	6.98^{c-i}	7.45^{c-h}	4.95^{j}	6.08^{h-j}	6.86^{d}
9	5.04^{ij}	6.89^{c-j}	9.68^{ab}	8.28^{a-g}	7.71^{b-h}	7.89^{a-h}	7.47^{c-h}	8.61^{a-d}	7.70^{bc}
12	8.36^{a-f}	8.84^{a-c}	9.91^{a}	7.18^{c-h}	8.08^{a-h}	8.47^{a-e}	6.43^{f-j}	7.48^{c-h}	8.09^{b}
Total mean	7.25^{ab}	7.61^{ab}	8.62^{a}	7.37^{ab}	7.75^{ab}	7.68^{ab}	6.28^{b}	7.48^{ab}	

NMU concentration(mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	9.08^{a}				9.59^{a}				9.33^{a}
3	7.64^{b}	5.42^{d-f}	6.39^{cd}	6.96^{bc}	9.68^{a}	7.92^{b}	6.27^{cd}	5^{e-h}	6.91^{b}
6	6.15^{cd}	4.39^{gh}	4.06^{hi}	2.71^{jk}	7.83^{b}	6.23^{cd}	4.58^{f-h}	2.35^{kl}	4.79^{c}
9	5.70^{de}	3.40^{ij}	1.69^{l-n}	1.40^{l-n}	7.10^{bc}	5.09^{e-g}	1.87^{k-m}	1.35^{mn}	3.45^{d}
12	5.17^{e-g}	4.21^{g-i}	2.28^{k-m}	1.42^{l-n}	6.97^{bc}	4.34^{g-i}	2.02^{k-m}	0.78^{n}	3.40^{d}
Total mean	6.16^{b}	4.35^{c}	3.60^{d}	3.12^{e}	7.89^{a}	5.89^{b}	3.68^{d}	2.37^{f}	

Sodium azide concentration (mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	5.16^{d-g}				6.23^{a-f}				5.69^{bc}
2	6.70^{a-d}	6.65^{a-d}	6.35^{a-f}	6.97^{a-c}	6.33^{a-f}	7.22^{ab}	7.80^{a}	6.01^{b-f}	6.75^{a}
4	6.65^{a-d}	6.92^{a-c}	6.53^{a-e}	5.60^{b-g}	6.72^{a-d}	5.79^{b-g}	4.81^{e-g}	6.14^{a-f}	6.14^{b}
6	5.20^{d-g}	6.08^{a-f}	4.21^{g}	5.45^{c-g}	6.49^{a-f}	6.28^{a-f}	4.86^{e-g}	5.56^{b-g}	5.52^{c}
8	5.67^{b-g}	5.14^{d-g}	5.77^{b-g}	4.81^{e-g}	6.52^{a-f}	5.97^{b-f}	5.15^{d-g}	5.26^{c-g}	5.53^{c}
Total mean	6.05^{a}	6.19^{a}	5.71^{a}	5.70^{a}	6.51^{a}	6.31^{a}	5.65^{a}	5.74^{a}	

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

rate (Tables 3 and 4). Two hour treatment of non-presoaked seeds with 3 mM NMU led to the highest radical length and seedling height (Tables 5 and 6). Non-presoaked seeds treated with 12 mM NMU for eight hours induced the lowest values for all the traits of germination percentage, germination rate index, radicle length, and seedling height. Means of seed viability for NMU treated seeds varied between 91% for control to 36% for the one with 12 mM NMU for 8 h (Table 7). These two treatment conditions caused the extreme amounts of germination percentage, too.

Sodium azide

Control and 8 mM canola non-presoaked seeds treated with NaN_3 resulted in the highest and lowest mean values of germination percentage, respectively (Table 3). Increased mutagen significant for the 4 h treatment duration. The least germination rate belonged to the presoaked significant for the 4 h treatment duration. The least germination rate belonged to the presoaked treatment with 4 mM sodium azide for 8 h (Table 4). In contrast, non-pre-soaked non-treated seeds (control)

Table 6. Mean comparison of seedling height for dosages, pre-treatment and treatment period and their interactions in EMS, ENU, NMU and sodium azide treated canola seeds.

EMS concentration (%)	Soaking				Non-soaking				Total mean
	3 h	6 h	9 h	12h	3 h	6 h	9 h	12 h	
Control	2.05$^{a\ 1}$				1.58^{a-d}				1.81^{a}
0.4	1.62^{a-c}	1.57^{a-e}	0.92^{c-h}	0.77^{d-i}	1.75^{ab}	1.52^{a-f}	0.97^{b-h}	0.80^{d-i}	1.24^{b}
0.8	1.37^{a-f}	0.96^{b-h}	0.08^{i}	0^{i}	1.47^{a-f}	1.07^{b-g}	0.75^{e-i}	0.80^{d-i}	0.81^{c}
1.2	2.07^{a}	0.47^{g-i}	0.05^{i}	0^{i}	2.10^{a}	1.10^{b-g}	0.20^{hi}	0^{i}	0.75^{c}
1.6	0.75^{e-i}	0^{i}	0^{i}	0^{i}	1.75^{ab}	0.22^{hi}	0^{i}	0^{i}	0.34^{d}
Total mean	1.45^{ab}	0.75^{cd}	0.26^{d}	0.19^{d}	1.76^{a}	0.97^{bc}	0.48^{cd}	0.4^{cd}	

ENU concentration(mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	3.31^{l-n}				3.60^{i-n}				3.45^{d}
3	4.07^{f-j}	4.73^{c-e}	3.93^{g-m}	5.86^{a}	4.56^{c-g}	4.96^{b-d}	4.12^{e-i}	4.37^{d-h}	4.57^{a}
6	4.17^{e-i}	4.67^{c-f}	5.58^{ab}	4.04^{f-k}	5.43^{ab}	3.69^{i-n}	3.15^{n}	3.77^{h-n}	4.31^{b}
9	3.88^{h-m}	4.75^{c-e}	3.45^{j-n}	3.58^{i-n}	5.17^{bc}	3.43^{k-n}	3.29^{mn}	3.46^{j-n}	3.87^{c}
12	3.34^{l-n}	3.67^{i-n}	3.94^{g-l}	3.33^{l-n}	3.67^{i-n}	3.75^{h-n}	3.63^{i-n}	3.40^{l-n}	3.59^{d}
Total mean	3.86^{c-e}	4.45^{ab}	4.22^{bc}	4.20^{bc}	4.70^{a}	3.95^{cd}	3.54^{e}	3.75^{de}	

NMU concentration(mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8	
Control	3.93^{b-d}				3.84^{b-f}				3.88^{a}
3	3.12^{j-l}	3.88^{b-e}	3.40^{f-j}	3.42^{e-j}	4.25^{ab}	3.42^{e-j}	3.75^{c-g}	3.61^{d-i}	3.61^{b}
6	3.35^{g-j}	2.84^{k-m}	2.46^{m-o}	2.85^{k-m}	4.19^{a-c}	3.44^{e-j}	3^{j-l}	1.99^{pq}	3.01^{c}
9	3.26^{h-k}	3.44^{e-j}	2.45^{m-p}	1.49^{rs}	3.71^{d-h}	3.19^{i-k}	2.07^{o-q}	1.31^{s}	2.61^{d}
12	2.67^{l-n}	2.28^{n-p}	2.37^{n-p}	1.81^{qr}	4.44^{a}	2.46^{m-o}	2.01^{o-q}	0.74^{t}	2.35^{e}
Total mean	3.1^{b}	3.11^{b}	2.67^{c}	2.39^{d}	4.14^{a}	3.12^{b}	2.70^{c}	1.91^{e}	

Sodium azide concentration(mM)	Soaking				Non-soaking				Total mean
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	
Control	3.34^{b-e}				3.58^{a-e}				3.46^{a}
2	3.56^{a-e}	3.74^{a-c}	3.18^{b-e}	3.41^{b-e}	3.09^{de}	3.41^{b-e}	3.42^{b-e}	3.57^{a-e}	3.42^{a}
4	3.25^{b-e}	3.80^{ab}	3.62^{a-e}	3.05^{de}	3.64^{a-e}	3.40^{b-e}	3.41^{b-e}	3.45^{a-e}	3.45^{a}
6	3.26^{b-e}	3.40^{b-e}	3.32^{b-e}	3.03^{e}	3.54^{a-e}	3.57^{a-e}	3.06^{de}	3.22^{b-e}	3.30^{a}
8	3.37^{b-e}	3.16^{b-e}	3.19^{b-e}	3.16^{b-e}	4.10^{a}	3.70^{a-d}	3.14^{c-e}	3.31^{b-e}	3.39^{a}
Total mean	3.36^{ab}	3.52^{a}	3.32^{ab}	3.16^{b}	3.59^{a}	3.52^{a}	3.25^{ab}	3.38^{ab}	

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

exhibited the highest mean value of germination percentage (Table 3). Changes in sodium azide concentration also affected radicle length of mutant seedlings. Non-presoaked seeds treated with 2 mM sodium azide for 6 h produced seedlings with longer radicles than any other treatment. On the other hand, the treatment with 6 mM sodium azide for 6 h induced the least radical length in pre-soaked seedlings (Table 5). The highest seedling height belonging to non-presoaked seeds treated with 8 mM sodium azide for 2 h did not vary significantly from the same value recorded for the control treatment under similar non-soaking conditions. The lowest amount of seedling height belonged to 8 h treatment with 6 mM sodium azide in presoaking conditions (Table 6). The highest seed viability belonged to control among the studied treatments of NaN_3 (Table 7). Means of seed viability varied between 51% (8 mM/8 h) and 86.7% (control). Treatment with 8 mM sodium azide for eight hours induced the lowest germination percentage, too. Seed viability means grouped genotypes into 9 different

Table 7. Mean comparison of seed viability for dosages and treatment period and their interactions in EMS, ENU, NMU and sodium azide treated canola seeds.

EMS concentration (%)	Treatment duration				Total mean
	3 h	6 h	9 h	12 h	
Control			89.7[a 1]		
0.4	85.7[a]	75.3[b]	58.3[c]	42.3[d]	65.4[a]
0.8	78[b]	60[c]	41[d]	28[ef]	51.75[a]
1.2	44.7[d]	36.7[de]	23[f]	5[g]	27.35[b]
1.6	38.9[d]	25.3[f]	7[g]	0[h]	17.8[c]
Total mean	61.82[a]	49.32[ab]	32.32[b]	18.82[c]	43.51

ENU concentration (mM)	Treatment duration				Total mean
	2 h	4 h	6 h	8 h	
Control			89.3[a]		
3	87[a]	74.3[c]	55.7[fg]	55.7[fg]	68.17[a]
6	80.3[b]	55.7[fg]	47[ij]	48.3[h-j]	57.82[b]
9	66.7[d]	54.7[f-h]	54[f-h]	44.7[j]	55.02[b]
12	63[de]	57.3[ef]	53.3[f-i]	49.7[g-j]	55.82[b]
Total mean	74.25[a]	60.5[b]	52.5[c]	49.6[c]	60.98

NMU concentration (mM)	Treatment duration				Total mean
	2 h	4 h	6 h	8 h	
Control			91 [a]		
3	80[b]	81.7 [b]	70[d]	54.7[f]	71.6[a]
6	74.7[c]	68.7[d]	57[ef]	45.3[hi]	61.42[b]
9	59.3[e]	49.3[gh]	43.3[i]	50[g]	50.47[c]
12	58.3[ef]	48[gh]	46.3[g-i]	36[j]	47.15[d]
Total mean	68.07[a]	61.92[b]	54.15[c]	46.5[d]	59.63

Sodium azide concentration (mM)	Treatment duration				Total mean
	2 h	4 h	6 h	8 h	
Control			86.7 [a]		
2	82.3[ab]	84.7[a]	77.7[bc]	79[bc]	80.92[a]
4	71.3[d-f]	74.7[c-e]	68.3[f]	71.7[d-f]	71.5[b]
6	77[cd]	55.3[g]	53.7[g]	53[g]	59.75[c]
8	70.3[ef]	66.7[f]	57.3[g]	51[g]	61.32[c]
Total mean	75.22[a]	70.35[b]	64.25[c]	63.67[d]	69.45

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

groups, but the variation between genotypes was higher for germination percentage which divided genotypes into 11 different groups.

Correlation coefficients

The results of correlation analysis indicated the highly significant positive relationships between germination percentage, on one side, and germination rate, radicle length, seedling height and seed viability, on the other, in EMS and NMU treated canola seeds (Tables 8). For ENU treatment, significant and positive correlations were observed between germination percentage and germination rate (r=0.56*) and between germination percentage and seed viability (r=0.83**). For sodium azide-treated seeds, no significant relationship was observed between germination percentage and seedling height (Table 8). A positive and significant relationship was observed between radicle length and seedling height for all the treatments with the exception of ENU treated seeds where this relationship was negatively significant (Table 8). Correlation coefficients between seed viability and other traits were positive for most of the treatments

Table 8. Correlation coefficients between variables measured on EMS, ENU, NMU and sodium azide treated canola seeds.

EMS	GP	GR	RL	SH	SV
Germination percentage (GP)	1	0.97[1]	0.94	0.92	0.93
Germination rate index (GR)		1	0.93	0.89	0.89
Radicle length (RL)			1	0.98	0.86
Seedling height (SH)				1	0.85
Seed viability (SV)					1
ENU	GP	GR	RL	SH	SV
Germination percentage (GP)	1	0.56	0.31	0.06	0.83
Germination rate index (GR)		1	0.51	-0.43	0.27
Radicle length (RL)			1	-0.50	0.26
Seedling height (SH)				1	0.27
Seed viability (SV)					1
NMU	GP	GR	RL	SH	SV
Germination percentage (GP)	1	0.96	0.88	0.83	0.80
Germination rate index (GR)		1	0.87	0.78	0.81
Radicle length (RL)			1	0.92	0.90
Seedling height (SH)				1	0.79
Seed viability (SV)					1
Sodium azide	GP	GR	RL	SH	SV
Germination percentage (GP)	1	0.73	0.53	0.43	0.76
Germination rate index (GR)		1	0.31	0.50	0.59
Radicle length (RL)			1	0.50	0.68
Seedling height (SH)				1	0.52
Seed viability (SV)					1

but seed viability was not correlated with germination rate index, radicle length and seedling height under ENU treatment conditions.

DISCUSSION

Flowering plants are particularly well adapted to random mutagenesis because large, saturated mutant populations can be generated through chemical mutagenesis. Such populations can then be screened for the particular phenotypes using 'reverse screened' tools, which are conducted based on gene sequence for mutations in the target gene (Stephenson et al., 2010). It is important, therefore, to determine the level of mutagen treatment necessary to achieve the utmost mutation load in an important oilseed crop species such as canola. The interdependence of treatment variables that influence the degree of M_1 seed lethality induced by a mutagen is clearly illustrated by the interactions between mutagen concentration, treatment period and pretreatment observed in this study. When one considers that these are only a few of the treatment variables that could have been investigated, it becomes even more apparent that the reaction of mutagen with the cellular constituents is complex, underscoring the necessity for close control of experimental conditions to ensure repeatable treatment effects (Fowler and Stefansson, 1972).

In this study, inverse relations were found between mutagen concentration and both rate and percentage of M_1 seed germination in canola. These results are in agreement with the findings of previous research with other plants (Afsar et al., 1980; Fowler and Stefansson, 1972; Padavai and Dhanavel, 2004; Singh and Kole, 2005). In the case of EMS, treatments with 1.2% for 12 h and 1.6% for 9 and 12 h brought complete lethality in both pretreatment conditions (Table 3). Fowler and Stefansson (1972) reported that increasing of EMS concentration from 0 to 1% adversely affected germination percentage. The interaction between dosage and duration of treatment for germination percentage was significant. This result shows the importance of duration of mutagen treatment in finding an optimal mutagenic dose. Pretreatment had no significant effects on traits in most of the treatments in this study. Soaking increases mutagen penetration into seeds and leads to higher metabolic activities, but there would be no need for presoaking if the duration of treatment with mutagen is long enough (Fowler and Stefansson, 1972).

In general, EMS treated seeds produced the lowest

values for all traits (Tables 3, 4, 5 and 6). From a germination percentage aspect, mutagens ranked in the following descending order: NMU>sodium azide> ENU>EMS. Therefore, EMS had the highest lethality dose in this experiment so that most seeds treated with 1.6% EMS or treated for 12 h did not even germinate. Hence, to obtain the highest variability and number of suitable mutants, it is inevitable to use lower dosages of this mutagen over shorter treatment periods. In flax, Bacelis (2001) studied the efficiency of chemical mutagens and found ENU as the most efficient mutagen followed by NMU and EMS. Although, a positive correlation is evident to exist between seedling failures and mutation frequency, this relationship is not linear (Afsar et al., 1980; Fowler and Stefansson, 1972). This is because at higher concentrations of the mutagen, some mutants were eliminated from the population in the first generation, or they became sterile if they did survive. This is due to mutagenic effects on plant genes and/or chromosomal aberrations. The extent of reduction in growth is related to the mechanism of action for a given mutagen. Mutagens may inhibit an energy supply system resulting in the inhibition of mitosis which can be associated with seedling growth depression. Seed's physiological conditions during treatment greatly influence the magnitude of growth depression (Afsar et al., 1980). Thus, breeders are interested in finding a mutagenic dose that induces adequate mutagenic outcome but which results in low sterility and lethality. Efficiency of the LD_{50} criterion has been validated by almost all researchers (Das and Haque, 1997; Gustafson, 1989; Hu and Rutger, 1992; Snustad and Simmons, 2006). According to this criterion, treatment with 0.8% EMS solution for 6 h has led to 50% lethality compared to that of control (Table 3). Nevertheless, this mutagenic treatment may be proposed as the appropriate treatment conditions when one considers overall genomic aberrations caused by a higher mutagenic dose. Jabeen and Mirza (2004) subjected *Capsicum annum* seeds to different treatment levels of EMS (0.01, 0.1 and 0.5%) and two durations of exposure (3 and 6 h) and suggested that using 0.5% EMS for 3 h could induce appropriate morphological mutations. Das and Haque (1997) also studied the responses of sesame seeds to gamma rays and EMS in M_1 generation. In their study, the optimum dosages for mutation induction were 0.7 to 0.9% EMS as determined by the LD_{50} criterion. The optimum dosage of EMS for rice was 8 h treatment with 1% EMS according to Padma and Reddy (1977). Compared to the control, treatment with the 12 mM ENU solution for eight hours and non-soaking pre-treatment induced 50% reduction in germination percentage in canola seeds (Table 3) and this treatment would, hence, be an optimal dose of ENU in mutagenic studies.

In the case of NMU, treatments of seeds with the 9 mM solution for 8 h could be proposed for enhancing the mutagen efficiency. This finding also confirms the earlier results of Ramulu (1972) with sorghum who observed that lower dosages of NMU are more efficient than higher concentrations. Mean comparisons of the effect of sodium azide treatment revealed that 8 h non-soaking seed treatment with 6 mM solution of this mutagen induced 50% reduction in germination percentage compared to that of the control treatment (Table 3). Treatment with the 8 mM sodium azide solution for 4 h was also suitable according to the LD_{50} criterion as the two treatments did not significantly differ. The choice of either of these two treatment conditions depends upon experimental conditions and supplements along with breeder's expert opinion. On one hand, application of lower mutagen concentrations is safer because it causes less sterility and abnormalities. From a breeding point of view, however, application of higher mutagen concentrations results in the higher frequency of induced mutations. Hence the first treatment would be a suitable sodium azide treatment condition in this study.

A positive relationship was observed between seed viability and other traits which were highly significant in most treatments (Table 8). The strong significant and positive correlation between germination percentage and seed viability revealed that the standard germination test could unbiasedly predict seed viability in canola. In the case of ENU treatment, there was a negative correlation between seedling height and radicle length (r=-0.50*). This inverse relationship may be due to the imbalanced allocation of seed storage to the development of radicle and seedling.

Conclusion

The significant effects of mutagen dosages and treatment periods on seed viability and seed germination as well as on seedling characteristics for the tested mutagens were observed. The 0.8% ethyl methanesulfonate (EMS) for 6 h, 12 mM ENU and 6 mM sodium azide for 8 h and 9 mM NMU for 4 h were considered as optimum treatment conditions. This study was one step toward exploring the most desirable treatment conditions for enhancing mutation efficiency in the canola breeding programs as well as genetic studies. Further research is required to determine the effects of other variables such as genotype, temperature, pH, and post-treatment on mutagen action and M_1 plant survival and reproduction.

ACKNOWLEDGEMENTS

This work was partially funded by Center of Excellence for Oilseed Crops at Isfahan University of Technology, Isfahan, Iran.

REFERENCES

Afsar AM, Konzak CF, Rutger JN, Nilan RA (1980). Mutagenic effects of sodium azide in rice. Crop Sci. 20: 663-668.

Ahmad I, Day JP, MacDonald MV, Ingram DS (1991). Haploid culture

and UV mutagenesis in rapid-cycling *Brassica napus* for the generation of resistance to chlorsulfuron and *Alternaria brassicicola*. Ann. Bot. 67: 521-525.
AOSA (2000). Tetrazolium testing handbook. Contrib. 29. Handbook on seed testing. Lincoln, NE: AOSA. p. 302.
Bacelis K (2001). Experimental mutagenesis in fiber flax breeding. Biologia, 1: 40-43.
Barro F, Fernandez-Escobar J, De La Vega M, Martin A (2002). Modification of glucosinolate and eruic acid contents in doubled haploid lines of *Brassica carinata* by UV treatment of isolated microspores. Euphytica, 129: 1-6.
Bhatia CR, Nichterlin K, Maluszynski M (1999). Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. Mut. Breed. Rev., 11: 1-36.
Carlton AE, Cooper CS, Wiesner LE (1968). Effect of seed pod and temperature on speed of germination and seedling elongation of sainfoin (*Onobrychis viciaefolia* Scop). Agron. J. 60: 81-84.
Das ML, Haque MM (1997). Response of sesame seeds to gamma rays and EMS in M_1 generation. Bangladesh J. Bot. 26: 75-78.
Elias S, Garay A, Schweitzer L, Hanning S (2006). Seed quality testing of native species. Native Plant, J. 7: 15-19.
FAO, FAOSTAT (2011). Available at: http://faostat.fao.org/site/339/default.aspx/. (Accessed 15 January, 2011).
FAO/IAEA Mutant Varieties Database. (2011). Available at: http://mvgs.iaea.org/Search.aspx/.
Ferrie AMR, Taylor DC, MacKenzie SL, Rakow G, Raney JP, Keller WA (2008). Microspore mutagenesis of *Brassica* species for fatty acid modifications: a preliminary evaluation. Plant Breed., 127: 501-506.
Fowler DB, Stefansson BR (1972). Effects of the mutagenic agent EMS on the M1 generation of rape *(Brassica napus*). Can. J. Plant Sci. 52: 53-62.
Gustafson A (1989). Mutation and gene recombination as spelling tools in plant breeding. In: Olsson G (ed). Res. Result Plant Breed., pp. 34-53.
Hu J, Rutger JN (1992). Pollen characteristics and genetics of induced and spontaneous genetic male sterile mutants in rice. Plant Breed., 109: 97-107.
Jabeen N, Mirza B (2004). Ethyl methane sulfonate induces morphological mutations in *Capsicum annuum*. Int. J. Agric. Biol. 6: 340-345.
Karrfalt RP (2011). Seed Testing. Available online at: http://www.nsl.fs.fed.us/wpsm/chapter5.pdf /.
Kharkwal MC (1998). Induced mutation in chickpea *(Cicer arietinum* L.). I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian J. Genet. 58: 159-167.
Kott L (1998). Application of doubled haploid technology in breeding of oilseed *Brassica napus*. Agric. Biotech. News Infor. 10: 69-74.
Kott L, Wong R, Swanson E, Chen J (1996). Mutation and selection for improved oil and meal quality in *Brassica napus* utilizing microspores culture. *In* Jain SM, Sopory SK, Veilleux RE (eds). *In vitro* haploid production in higher plants. Springer, Berlin Heidelberg New York, 2: 151-167.
Landho L, Jorgensen RB (1997). Seed germination in weedy *Brassica campestris* and its hybrids with *B. napus*: Implications for risk assessment of transgenic oilseed rape. Euphytica, 97: 209-216.
MacDonald MV, Ahmad I, Menten JOM, Ingram DS (1991). Haploid culture and in vitro mutagenesis (UV light, X-rays, and gamma rays) of rapid cycling *Brassica napus* for improved resistance to disease. *In* Plant mutation breeding for crop improvement, IAEA, Vienna, 2: 129-138.
MacDonald MV, Ingram DS (1986). Toward the selection *in vitro* for resistance to *Alternaria brassicicola* (Schw)Welts., in *Brassica napus* ssp. *oleifera* (Metzg.) Sinsk., winter oilseed rape. New Phytol. 104: 621-629.
McCallum CM, Comai L, Greene EA, Henikoff S (2000). Targeting Induced Local Lesions IN Genomes (TILLING) for Plant Functional Genomics. Plant Physiol. 123: 439-442.
McDonald BE (2011). Canola Oil: Nutritional Properties. Canola Council Publications. Available online at: http://www.canolacouncil.org/health_nutritional.aspx/ (Accessed 5 January 2011).
Medrano H, Millo E, Guerri J (1986). Ethyl-methane-sulphonate effects on anther culture of *Nicotiana tabacum*. Euphytica, 35: 161-168.
Newsholme DM, MacDonald MV, Ingram DS (1989). Studies of selection in vitro for novel resistance to phytotoxic products of *Leptospheria maculans* (Desm.) Ces.& De Not in secondary embryogenic lines of *Brassica napus* ssp. *oleifera* (Metzg.) Sinsk., winter oilseed rape. New Phytol. 113: 117-126.
Osorio J, Fernandez-Martinez J, Mancha M, Garces R (1995). Mutant sunflowers with high concentration of saturated fatty acid in the oil. Crop Sci. 37: 739-742.
Padavai P, Dhanavel D (2004). Effects of EMS, DES and colchicine treatment in soybean. Crop Res. 28: 118-120.
Padma A, Reddy GM (1977). Genetic behavior of five induced dwarf mutants in an Indica rice cultivar. Crop Sci. 17: 860-863.
Parry MA, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, Labhilili M, Phillips AL (2009). Mutation discovery for crop improvement. J. Exp. Bot. 60: 2817-2825.
Patil A, Taware SP, Raut VM (2011). Induced variation in quantitative traits due to physical (γ rays), chemical (EMS) and combined mutagen treatments in soybean [*Glycine max* (L.) Merrill]. Soybean Genet. Newslett. Available online at http://www.soygenetics.org/articleFiles/40inducedvariationinquantativetraitsduetophysica,chemi%5B1%5D.pdf/. Vol. 31.
Ramulu SK (1972). A comparison of mutagenic effectiveness and efficiency of NMU and MNG in Sorghum. Theor. Appl. Genet. 42: 101-106.
Rowland GG (1991). An EMS-induced low linoleic acid mutant in McGregor flax (*Linum usitatissimum* L.). Can. J. Plant Sci. 71: 393-396.
Sala CA, Bulos M, Echarte AM (2008). Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Sci. 48: 1817-1822.
SAS Institute Inc (2008). SAS/STAT ® 9.2 User's Guide. Cary, NC: SAS Institute Inc.
Schnurbush T, Mollers C, Becker HC (2000). A mutant of *Brassica napus* with increased palmitic acid content. Plant Breed. 119: 141-144.
Singh R, Kole CR (2005). Effect of mutagenic treatments with EMS on germination and some seedling parameters in mungbean. Crop Res. 30: 236-240.
Snustad DP, Simmons MJ (2006). Principles of Genetics. 4th ed. Wiley, Hoboken, NJ.
Spasibionek S (2006). New mutants of winter rapeseed (*Brassica napus* L.) with changed fatty acid composition. Plant Breed., 125: 259-267.
Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics, A Biometrical Approach, 2nd Edition. McGraw-Hill. New York.
Stephenson P, Baker D, Girin T, Perez A, Amoah S, King GJ, Østergaard L (2010). A rich TILLING resource for studying gene function in *Brassica rapa*. Plant Biol. 10: p. 62.
Swanson EB, Coumans MP, Brown GL, Patel JD, Beversdorf WD (1988). The characterization of herbicide tolerant plants in *Brassica napus* L. after in vitro selection of microspores and protoplasts. Plant Cell Rep., 7: 83-87.
Swanson EB, Herrgesell MJ, Arnoldo M, Sippell DW, Wong RSC (1989). Microspore mutagenesis and selection: canola plants with field tolerance to the imidazolinones. Theor. Appl. Genet. 78: 525-530.
Szarejko I, Forster BP (2007). Doubled haploidy and induced mutation. Euphytica, 158: 359-370.
Velasco L, Fernandez-martinez JM, De Haro A (2008). Inheritance of reduced linolenic acid content in the Ethiopian mustard mutant N2-4961. Plant Breed. 121: 263-265.
Wong RSC, Swanson E (1991). Genetic modification of canola oil: high oleic acid canola.. *In* Haberstroh C, Morris CE (eds.), Fat and Cholesterol Reduced Food. Gulf, Houston, Texas, pp. 154-164.