Effect of chemical mutagens on seed viability and germination in canola (Brassica napus L.)

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Abstract

Mutation induction is considered as an effective way to enrich plant genetic variation, particularly for plant 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 viability,germination and seedling growth characteristics (germination percentage, germination rate, radicle length, and seedling height) in canola (Brassica napus L.). Two pretreatment conditions of soaking in distilled water for 3 hrs and non-soaking, different concentrations of the chemical mutagens [ethyl methanesulfonate(EMS), N-nitroso-N-methylurea (NMU), N-nitroso-N-ethylurea (ENU), and sodium azide], 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. The 0.8 percent EMS for 6 hrs, 12mM ENU and 6mM sodium azide for 8 hrs and 9mM NMU for 4 hrs were considered as optimum treatment conditions in this study.

Key words: Brassica napus, germination, chemical mutagen,seed viability

Introduction

Canola (Brassica napus L.) is one of the most important sources of vegetable oils and protein-rich meals worldwide. With 7% saturated fats, canola oil contains the least amount of saturated fats among the common edible oils. Canola oil is also a good source of vitamins E and K and plant sterols which may keep the heart healthy (6). 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 (11). 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. Mutation breeding in canola has been also used to improve herbicide resistance (10), disease resistance (5), and lower glucosinolate content (3).

Chemical and physical mutagens are available for mutagenic treatment of crop plants. Optimization of the mutation induction conditions in each plant species plays a critical role in the successful employment of the mutagenic events. 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 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 has 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. Mutagen dose, treatment period and their interaction can be considered as the main factors also influenced by pretreatment, temperature, pH, and post-treatment(**9**). LD_{50} (lethal dose 50) is generally used as a criterion to define the optimum mutagenic dose.

Bacelis (2) 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. Patilet al (7) also introduced 0.1-0.2% EMS concentrations as optimum dosages to induce maximum variations in soybean populations.

Germination test is an indication of the potential of a seed lot to emerge under field conditions. However, tetrazolium staining is a timely and accurate method of testing seed viability (1). Therefore, application of both germination and tetrazolium staining tests, rather than by either one alone, provides complementary evidence of seed viability (4).

The objective of the present 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 staining methods.

Materials and Methods

Seeds of spring canola cultivar "RGS003" were exposed to four chemical mutagens which comprised ethyl methane sulfonate(EMS), N-nitroso-N-methylurea (NMU), N-nitroso-N-ethylurea (ENU), and sodium azide. A 4 × 2 × 5 × 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 hrs and non-soaking, five dosages of each mutagen and four treatment periods comprised the experimental factors. For germination percentage, the number of seeds germinated on day 7 was considered. The germination rate index was determined by $\sum(Ni/Di)$ where Ni is the number of seeds germinated between two countings and Di represents the day of counting. Seedling height and radicle length were determined as the mean of 10 seven day-old seedlings per treatment.

Seed viability was tested using a standard tetrazolium staining. To evaluate the effects of different chemical mutagen dosages on seed viability, an experiment was conducted using a factorial experiment (4×5×4) with a completely randomized design replicated three times. Four mutagens, five 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 hrs. They were then incubated in 1% (w/v) solution of 2,3,5-triphenol tetrazolium chloride for 24 hrs at 25 ºC ±1. Seeds with stained embryos were scored as viable.

The analysisof variances (ANOVA), Mean comparisons and linear correlation coefficients(r) were performed using SAS software (8)**.**

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. Pre-treatment significantly affected only germination rate and radicle length. 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.

Mean comparison results showed that average germination percentage, germination rate and radicle length reduced with increasing mutagen concentration and treatment period for all mutagens. Higher concentrations of allmutagens reduced seedling height,as well, except for sodium azide, where the increasing mutagen concentration and treatment period did not affect the seedling height. As expected, control treatment had the highest germination percentage and seed viability in all experiments. The twelve hr treatment with 1.6% EMS induced the least amount of both germination percentage and seed viability. The treatment with 1.6% EMS acting similar to those of 12 hr treatment with different concentrations of this mutagen almost blocked seed germination. 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 hr treatment with this mutagen.

Seed viability in canola was significantly affected by the chemical mutagens (Table 1). Means of seed viability for NMU-treated seeds varied between 91% for control to 36% for the one with 12mM NMU for 8 hrs. These two treatment conditions caused the extreme amounts of germination percentage, as well.

The results of correlation analysis indicated the highly significant positive relationships between germination percentage, on the one side, and germination rate, radicle length, seedling height and seed viability, on the other, in EMS and NMU treated canola seeds.Correlation coefficients between seed viability and other traits were positive for most of the treatments but seed viability was not correlated with germination rate index, radicle length and seedling height under ENU treatment conditions.

Discussion

In this study, inverse relations were found between mutagen concentration and both rate and percentage of M_1 seed germination in canola. In general, EMS-treated seeds produced the lowest values for all traits. 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 hrs 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.

According to LD_{50} criterion, treatment with 0.8% EMS solution for 6 hrs has led to 50% lethality compared to that of control. Nevertheless, this mutagenic treatment may be proposed as the appropriate treatment conditions when one considers overall genomic aberrations caused by a higher mutagenic dose. Compared to the control, treatment with the 12 mM ENU solution for eight hrs and non-soaking pre-treatment induced 50% reduction in germination percentage in canola seeds and this treatment would, hence, be an optimal dose of ENU in mutagenic studies.In the case of NMU, treatments of seeds with the 9mM solution for 8 hrs could be proposed for enhancing the mutagen efficiency. Mean comparisons of the effect of sodium azide treatment revealed that 8 hr non-soaking seed treatment with 6 mM solution of this mutagen induced 50% reduction in germination percentage compared to that of the control treatment. 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. This study was one step toward exploring the most desirable treatment conditions for enhancing mutation efficiency in a canola breeding program. Further research is required to determine the effects of other variables such as genotype, temperature, pH, and posttreatment on mutagen action and M_1 plant survival and reproduction.

Acknowledgments

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

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Table 1. Results of analysis of variance for seed viability in canola influence by mutagen treatments

Source of variation	df	Mean square
Mutagen (M)	3	0.64**
Dosage (D)	4	1.41**
Treatment period (T)	3	0.59**
M× D	12	0.14**
M×T	9	0.09**
D× T	12	0.07**
M× D×T	36	0.05**
Residual	160	0.01
C.V.		9.39

** significant at P≤0.01