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Determining ethyl methane sulfonate-mediated (EMS) mutagenesis protocol for inducing high biomass yield in fodder barley (*Hordeum vulgare* L.)

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Abstract

Barley (*Hordeum vulgare* L.) has narrow genetic base for selection of promising ideotypes for the following traits: high biomass yield for livestock feed, enhanced agronomic and nutritional traits, and resistance to biotic and abiotic stresses. Ethyl methane sulfonate (EMS) mutagenesis offers opportunities for inducing genetic variation for key traits for development of feed barley ideotypes. The objective of this study was to determine optimal EMS dosage and exposure time to induce genetic variation for selection of high biomass yield six-row feed barley mutants. Five EMS dosages (i.e. 0.1%, 0.3%, 0.5%, 0.7% and 0.9% v/v) and five exposure times (i.e. 0.5 hr, 1 hr, 1.5 hr, 2 hrs and 2.5 hrs) were used on a six-row fodder barley using a 5 x 5 factorial treatment structure in a complete randomized design with three replications. Non-treated seed were used as a comparative control. Data was recorded for percent germination, seedling survival, shoot height, root height, shoot and root biomass. Significant ($p < 0.05$) EMS dosage x exposure time was observed for assessed traits indicating their influence on phenotypic variation in feed barley. Overall, a declining trend was observed for assessed traits with increased EMS dosage and exposure time. The LD_{50} value of 0.64% (v/v) EMS dosage was identified as an optimal dose for large-scale mutagenesis protocol to select fodder barley mutants with high biomass yield.

Keywords: Chemical mutagenesis, EMS, feed barley, lethal dose, optimal dose.
Abbreviations: EMS_Ethyl methane sulfonate; LD_{50}_50% lethal dosage; DMSO_ dimethyl sulfoxide; %G_ Percent germination; %SS_ Percent seedling survival; SVI_ Seedling vigor index; RB_ Root biomass; SB_ Shoot biomass; SL_ Shoot length; RL_ Root length.

Introduction

Barley (*Hordeum vulgare* L., 2n=2x=14) is an important crop serving as human food and livestock feed (Giraldo et al., 2019). Six-row barley has higher protein content (~13%), starch (60%), fat (1.9%) and high fiber (5%); whereas two-row barley has a higher sugar content (68%) suitable for the malting induatry (Gupta et al., 2010). Barely grain provides essential vitamins (i.e., E and B-complex), minerals (i.e. Ca, P, K, Mg and Na), antioxidants and phytochemicals that provide excellent nutrition and health benefits (Baik and Ullrich, 2008; Sullivan et al., 2013).

Six-rowed barley has a narrow genetic base due to its inherent self-pollinating nature (Martin et al.,1991; Rasmusson and Phillips, 1997; Matus and Hayes, 2002). This has resulted in limited genetic variation for selection of promising ideotypes for high biomass yield for livestock feed, enhanced agronomic and nutritional traits, and resistance to biotic and abiotic stresses. (Muñoz-Amatriaín et al., 2014; Nice et al., 2017; Gao et al., 2018). Therefore, there is need to widen the genetic base of barley to improve key agronomic traits including biomass and grain yield and component traits, nutritional composition, biotic and abiotic stress resistance.

Global barley production is estimated at 138 million tonnes from approximately 80 million hectares of land (FAOSTAT, 2019). European countries produce approximately 60% of the world's barley, whereas Asia and the Americas produce 15 and 13%, respectively. Sub-Saharan African countries produce ~ 2.6 million tonnes of barley. For instance, in Kenya, the estimated barley production is 50 000 tonnes and contribute to 0.1% of the global production (FAOSTAT, 2019). Low barely production in sub-Saharan Africa (SSA) including Kenya is partly attributed to limited efforts to develop feed barley varieties that suits to the changing agro-climatic conditions. The Beef Research Institute (BRI) of the Kenya Agricultural and Livestock Research Organization (KALRO) focuses on improving productivity of beef

cattle by generating appropriate, sustainable pasture and fodder management technologies through innovative research to support livestock production. Therefore, there is need to develop "climate smart" feed barley varieties with high biomass and grain yield to increase livestock feed production and curb the acute shortage of livestock feed (Lukuyu et al., 2011).

Mutation breeding using chemical mutagenesis is widely used procedure in improvement programs to create genetic variation and select "new" mutants possessing suitable agronomic, food and feed related nutritional traits (Krishna et al., 2016; Monica and Seetharaman, 2016). Ethyl methanesulfonate (EMS) is the most effective chemical mutagen commonly used for inducing genetic variation for quantitative and qualitative traits in crop plants (Ke et al., 2019; Devi and Mullainathan, 2012; Aruldoss et al., 2015). EMS alters the DNA structure directly by alkylating guanine (G) bases, causing mispairing with thiamine (T) instead of cytosine (C), resulting in a transition from G/C to A/T (Rafi et al., 2016). The ease of application to seeds and its detoxification through hydrolysis for disposal makes EMS a recommendable mutagen for improving genetic diversity in crops (Pathirana, 2011; Serrat et al., 2014). In addition, EMS increase point mutations compared to physical mutagens such as gamma radiation (Van Harten, 1998). The most important step for inducing mutations is selection of an appropriate dosage of the mutagen; defined as the concentration of mutagen together with duration of treatment at a specific temperature.

Determining the 50% lethal dosage (LD_{50}) is an important step for initiating EMS mutagenesis (Jain, 2010). LD_{50} refers to the mutation dose that result in 50% reduction in seed germination percentage after seed exposure for a prescribed time period under specific conditions (Mba et al., 2010; Beyaz et al., 2016). In barley, EMS mutagenesis has been previously used to improve genetic variation and develop mutants possessing key agronomic traits such as grain yield; tolerance to abiotic and biotic stresses (Munns et al., 2010). The use of EMS to develop barely mutants with increased biomass yield in six-row fodder barley has not been reported in SSA. Therefore, it is important to induce mutation in fodder barley and select mutants with increased biomass yield for use in livestock production systems to improve livestock productivity. In light of the above background, the objective of this study was to determine optimal EMS dosage and exposure time to induce genetic variation in six-row fodder barley to select novel mutants.

Results

Effect of EMS dosage, exposure time and their interaction effects on assessed traits

Analysis of variance showing mean square values and test of significance for EMS dosage, exposure time and their interaction effects on germination percentage and growth parameters is shown in Table 1. EMS dosage x exposure time interaction effect was highly significant ($p < 0.001$) for all recorded traits indicating that these two factors influenced phenotypic variation of feed barley. EMS dosage showed significant ($p \leq 0.01$) effect on all assessed traits. The duration of exposure time to EMS showed significant ($p \leq 0.01$) effect on SL, RL, SB and RB.

Interactive effect of EMS dose and exposure time on assessed traits

EMS dosage x exposure time interaction effects on germination parameters of feed barley are presented in Table 2. %G decreased with increased EMS dosage and exposure time. EMS dosage of 0.9% v/v for 2.5hrs recorded significantly low %G of 23.4% whereas 0.1% v/v EMS for 1hr improved %G by 71.3%. Untreated seeds (control) recorded the highest %G of 88.6%. The %SS also decreased with increased EMS concentration and exposure times. EMS dosage of 0.1% v/v for 2 hrs recorded the highest %SS of 76.2%, whereas EMS dosage of 0.9%v/v for 2.5hrs resulted in the lowest %SS of 14.3%. The control treatment recorded %SS of 90.5%. Seedling vigor index (SVI) also decreased with increased EMS concentration and exposure times. Seed exposure for 1 hr at EMS dosage of 0.1% v/v led to significantly higher SVI of 3814.4, whereas significantly lower SVI (906.5) was recorded for seeds exposed for 2 hrs at 0.9%v/v EMS dosage.

EMS dosage x exposure time interaction effects on growth parameters of feed barley are presented in Table 3. EMS concentration of 0.1% v/v for exposure time of 1.5hrs resulted in SL of 42.8cm, whereas the lowest SL of 25cm was recorded at 0.9 % v/v at exposure time of 1hr. RL decreased as the concentration of EMS and exposure times increased. The highest RL of 22.5 cm was recorded at the lowest EMS dosage of 0.1% v/v for an exposure time of 2 hrs. Contrastingly, the lowest RL of 10 cm was recorded at the highest EMS dosage of 0.9% v/v and longest exposure time of 2.5hrs. Shoot and root biomass decreased as the concentration of EMS and exposure times increased. The highest SB of 1.27 g was recorded for EMS dosage of 0.3% v/v for exposure time of 1 hr, whereas EMS dosage of 0.9% v/v for the longest exposure time of 2.5 hrs led to the lowest SB of 0.36 g. Exposing the seeds for the shortest time of 0.5 hrs at the lowest EMS dosage of 0.1% v/v led to seedlings with the highest RB (0.68 g). The lowest RB (0.17 g) was recorded for seeds treated with 0.9% v/v EMS dosage for a period of 2hrs.

Determination of LD_{50}

A fitted model for %G and EMS dosage was used to calculate LD_{50} using the linear regression equation of y= -56.02x +85.81 (Figure 1). Generally, increased EMS dosage resulted in reduced %G. According to the fitted model, LD_{50} value of 0.64% (v/v) EMS dosage was the ideal for inducing mutation in fodder barley.

Discussion

Genetic improvement of barley for agronomic and food- and fodder-related nutritional traits, and resistance to biotic and abiotic stress factors is limited due to the low genetic base of the crop. As a result, development and release of "new" barley varieties for the food and feed industry has been limited in sub-Saharan Africa including Kenya. Thus, increasing genetic diversity in barley is useful for developing fodder barley varieties with enhanced biomass yield and key farmer-preferred traits for cultivation and industrial uses. The present study determined an effective EMS protocol for inducing genetic variation for high biomass yield of six-row fodder barley. The study revealed that increased EMS dosage resulted in decreased

Table 1. Analysis of variance showing mean squares and significance tests for EMS dosage, exposure time and their interaction effects for assessed traits in six-row fodder barley.

Source of variation	df	SL	RL	SB	RB	%G	%SS	SVI
Replicates	2	5.27	13.04	0.04	0.05	15.10	143.94	44162.69
Dosage (D)	4	277.01**	132.80**	0.82**	0.09**	2006.53**	1161.79**	9800264.92**
Exposure time (ET)	4	88.94**	6.99*	0.22**	0.08**	46.48ns	76.98ns	350591.43ns
D x ET	16	33.60**	15.68**	0.07**	0.03*	168.15*	430.51*	572030.85**
Error	48	1.43	1.66	0.02	0.01	53.37	167.58	517688.65

df = degrees of freedom, SL = Shoot length, RL = Root length, SB = Shoot biomass, RB = Root biomass, %G = Germination percent, %SS = Seedling survival percent, SVI = Seedling vigor index , * = significant at 5% level of significance, ** = significant at 1% level of significance, ns = non-significance.

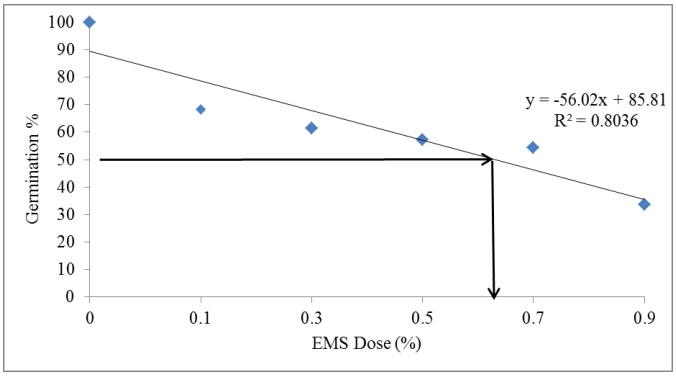

Fig 1. Germination percentage plotted against EMS dosage and used to calculate the lethal dose (LD_{50}) for inducing random mutations in a six-row fodder barley.

Table 2. Mean ± standard error for percentage germination (%G), percent survival (%SS) and seedling vigor index (SVI) of fodder barley assessed under variable EMS dosage and exposure time.

EMS dosage (%)	Exposure time (hr)	%G	%SS	SVI
0.1	0.5	53.4±3.70bcdefg	66.4±5.03b	2751.9±171.02bcdefg
	1	71.3±7.50b	67.9±8.94b	3814.4±362.90b
	1.5	67.8±3.63bc	71.4 ±0.00b	3667.7±154.65bc
	2	61.9±4.77bcd	76.2±4.77b	3468.5±309.58bcd
	2.5	48.2±4.47bcdefg	57.1 ±8.23b	2903.8±209.47bcdef
0.3	0.5	52.4±4.73bcdefg	54.0 ±3.09bc	3134.5±323.04bcde
	1	48.2±4.47bcdefg	52.4±4.73bc	2451.3±301.30cdefgh
	1.5	62.4±4.51bcd	52.4±4.73bc	3094.8±245.51bcde
	2	52.4±4.73bcdefg	59.5±1.48b	2869.2±287.95bcdef
	2.5	56.6±0.50bcdef	74.8 ±2.50b	3078.4±21.43bcde
0.5	0.5	42.9±0.00defgh	67.3±5.15b	2420.6±40.02defgh
	1	56.0±6.73bcdef	55.7±6.78bc	3176.8±402.05bcde
	1.5	46.1±3.17cdefgh	47.1±9.26bc	2753.4±212.07bdcefg
	2	40.8±6.11defgh	51.9±4.50bc	1802.9±260.09fghij
	2.5	55.0±1.03bcdefg	51.9 ±4.50bc	3008.3±165.69bcdef
0.7	0.5	57.6±0.50bcde	51.9 ±4.50bc	3301.6±106.16bcde
	1	46.6±3.73cdefgh	59.5±2.40b	2585.8±207.01cdefgh
	1.5	51.3±4.32bcdefg	59.5±2.40b	2500.6±225.81cdefgh
	2	55.0±1.04bcdef	57.1±8.23b	2278.2±74.01defghi
	2.5	42.8±0.01defgh	49.1±3.35bc	2203.0±33.16efghi
0.9	0.5	36.4±4.18efgh	57.1 ±14.27b	1578.8±144.10ghij
	1	33.4±4.77fgh	56.7 ±14.07b	1078.1±112.04ij
	1.5	31.3±2.70gh	52.4± 9.50bc	925.4±181.65j
	2	23.8±4.77h	37.7±18.84bc	906.5±170.36j
	2.5	23.4±4.73h	14.3 ±0.00c	1418.7±155.16hij
Control		88.6a	90.5a	4284.7a

Means followed by the same letters in a column for each EMS dosage and exposure times are not significantly different.

Table 3. Mean ± standard error for shoot and root traits of fodder barley assessed under varied EMS dosage and exposure time.

EMS dosage (%)	Exposure time (hr)	SL (cm)	RL (cm)	SB (g)	RB (g)
0.1	0.5	34.7±0.30ef	16.9±0.63defg	1.13±0.07abcd	0.68±0.09a
	1	35.1±0.26ef	18.6±0.94bcde	1.11±0.04abcd	0.46±0.04abc
	1.5	33.4±1.12fg	20.8±0.53abc	0.93±0.05abcde	0.39±0.01abc
	2	33.5±0.77fg	22.5±0.33a	0.74±0.06defghi	0.25±0.06bc
	2.5	38.1±0.34cde	22.4±1.97a	0.76±0.13cdefghi	0.33±0.04abc
0.3	0.5	38.0±0.26cde	21.7±1.06ab	0.93±0.04abcde	0.44±0.11abc
	1	33.0±1.38fgh	17.7±0.89cdef	1.27±0.03a	0.59±0.11ab
	1.5	35.4±0.30def	14.1±0.84fgh	1.18±0.01abc	0.46±0.11abc
	2	37.3±1.04cde	17.5±0.76cdef	0.90±0.05abcdef	0.28±0.01bc
	2.5	39.6±0.42abc	14.8±0.54	0.83±0.04bcdefgh	0.25±0.07bc
0.5	0.5	42.7±0.43ab	13.8±0.76ghi	1.22±0.05ab	0.30±0.04bc
	1	40.9±0.30abc	15.8±1.25efgh	1.03±0.26abcd	0.32±0.07bc
	1.5	42.8±0.33a	17.7±0.24cdef	0.68±0.14efghi	0.36±0.10abc
	2	30.1±0.89ghi	14.3±0.47fgh	0.88±0.07abcdefg	0.30±0.08bc
	2.5	37.8±1.41cde	16.8±0.66defg	1.21±0.04ab	0.27±0.07bc
0.7	0.5	42.7±0.74ab	14.7±0.67fgh	1.00±0.13abcd	0.25±0.04bc
	1	39.0±0.58bcd	16.4±0.57efg	0.96±0.11abcde	0.38±0.05bc
	1.5	34.9±0.62ef	13.7±0.45ghi	0.74±0.01defghi	0.33±0.02abc
	2	27.6±0.23ijk	13.8±0.71ghi	0.46±0.04ghi	0.27±0.01bc
	2.5	34.6±0.67ef	16.9±0.28defg	0.90±0.05abcdef	0.22±0.03c
0.9	0.5	29.8±0.62hij	14.5±0.90fgh	0.57±0.05fghi	0.21±0.10c
	1	25.0±0.97k	10.0±0.00j	0.50±0.00fghi	0.32±0.05bc
	1.5	28.4±0.72ijk	10.5±1.20ij	0.46±0.04ghi	0.28±0.10bc
	2	26.1±0.08jk	12.3±1.13hij	0.44±0.06hi	0.17±0.10c
	2.5	28.8±1.40ij	14.1±0.09fgh	0.36±0.07i	0.27±0.06bc
Control		40.3abc	20.8abc	1.10abcd	0.42bc

SL = Shoot length, RL = Root length, SB = Shoot biomass, RB = Root biomass. Means followed by the same letters in a column for each EMS dosage and exposure times are not significantly different.

germination percentage, seedling survival, seedling height, root and shoot biomass (Table 2). A reduced germination percentage could be attributed to disturbances of seed meristematic tissue at cellular level resulting in chromosome damage, disrupting growth promoters due to increased accumulation of growth inhibitors (Jayakumar and Selvaraj, 2003). In rice, EMS treatment significantly decreased germination percentage (Talebi et al., 2012), concurring with the present findings. Earlier studies carried out in barley reported a decrease in germination due to changes in metabolic functioning of cells after EMS treatment (Sharma and Swaminathan, 1969). Hadebe et al. (2017) reported that the highest reduction in germination (10%) was observed in vernonia seeds treated at high EMS dose of 1.1% and long exposure duration of 2hr, which is in agreement with the present study. A study by Espina et al. (2018) in soybean showed that the highest EMS dosage of 0.9%v/v for longest exposure duration of 24hrs, resulted in 0% germination, while 70% germination capacity was obtained at the lowest EMS dose of 0.3% at shortest exposure duration used, in agreement with the result of the present study (12hrs). Generally, EMS dosage of 0.9% v/v and exposure time of 2.5hrs appears effective for inducing genetic variation of the resultant mutant plants. This value is lower than the value reported in the previous studies and could be due to differences in crop species. The highest dose obtained in the present study could be the best dose to induce high mutation frequency in feed barley.

The present study showed that increased exposure duration and EMS dose led to a decline in seedling survival (Table 2). For example, 0.1% v/v EMS dosage for 2hrs gave the highest survival rate of 76.2%; whereas 0.9%v/v EMS dosage at 2.5hrs resulted in the lowest survival rate of 14.3%. This could be because seeds exposed to shorter periods absorb lower quantities of the mutagen, leading to lesser detrimental effect as compared to those exposed for longer durations (Kulkarni, 2011). For example, a study by Shirani et al. (2016) showed that at high concentrations of EMS (3%v/v) and longer exposure duration of 3 hrs resulted in no survival of banana shoot tips. A significant reduction in survival percentage (42.84%) was observed at the highest EMS doses of 0.5% and exposure duration of 5 hrs in *Coriandrum sativum* (Kumar and Pandey, 2019). Decrease in survival rate may be due to physiological disturbances, cytogenetic and chromosomal damage which lead to mitotic arrest and reduced cell division (Khursheed et al., 2008; Girija et al., 2013). This indicates optimum EMS dosage and exposure time determine survival rate of barely seedlings.

EMS is known to affect plant growth and development. In the present study, increased EMS dosage and exposure time caused a decline in shoot length, root length, shoot and root biomass (Table 3). This might be attributed to inactivation of auxin levels, which are plant-growth promoters (Kanakamanay, 2008; Ashok Kumar et al., 2009). Ali et al. (2019) reported a significant decrease in the root length of upland rice using EMS concentration of 2% v/v, much higher than tested concentrations in the present study. Muñoz-Miranda et al. (2019) reported that moderate concentrations of EMS (0.5% v/v) and longer exposure duration of 3hrs resulted in reduced

plant growth. This implies that higher EMS dose and longer exposure duration to EMS caused decreased levels in the assessed growth parameters of barley.

The LD_{50} of the mutagen is useful for determining an optimal dose for mutation induction. The LD_{50} was calculated using seed germination percentage at different doses of EMS (Figure 1). As a result, EMS dosage of 0.64% v/v was recommended as the most effective and efficient for inducing genetic variability and selection of promising six-row barley mutants. The LD_{50} in the present study is much lower than a value of 1.2% reported in banana (Shirani et al., 2016). Olaolorun et al. (2019) reported lethal doses of 1.07% and 1.81% v/v EMS for wheat genotypes LM29 and LM75, respectively. These values are much higher than reported in the present study attributed to differences in crop species, genotypes used and ambient conditions during mutagenesis (Liamngee et al., 2017). For artificially induced mutations either with physical or chemical mutagens, LD_{50} is considered to be an ideal level to achieving high frequency of mutations (Anbarasan et al., 2013). The present study determined the optimum treatment condition of 0.64% v/v EMS concentration. The mutagenesis protocol will be useful to develop recessive and point mutations to aid selection of best individuals involving the M2-M5 mutant families with high fodder biomass yield.

Materials and Methods

Experimental site and plant materials

The study was conducted under glasshouse conditions at the Controlled Environmental Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. A commonly used six-row feed barley landrace variety that is early maturing but less biomass was obtained from Kenya Agricultural and Livestock Research Organization (KALRO) Lanet and used for the study.

Experimental design and treatments

The experiment was conducted as a factorial (5 x 5) treatment structure involving five EMS dosages (i.e., 0.1%, 0.3%, 0.5%, 0.7% and 0.9%) and five levels of exposure periods (i.e. 0.5 hr , 1 hr, 1.5 hr, 2 hrs and 2.5 hrs) using a factorial treatment structure in a completely randomized design (CRD) with three replications. EMS treated seeds were established in seedling trays under a constant temperature of 24°C. Untreated seed were used as a comparative control.

Seed sterilization and pre-soaking

Seed sterilization and pre-soaking was done as described in Mba et al. (2010). Thirty uniform seeds were placed inside a customized 8 cm long and 4 cm wide labelled plastic mesh bags for each treatment combination. The seeds were surface sterilized by soaking the mesh bags in 70% ethanol for 1 min and washing under running water at room temperature for 2 min. The seeds were then soaked in 30% sodium hypochlorite solution for 5 min and washed using running water for 2 min. The seeds were then pre-soaked in distilled water for 24 hrs at room temperature before EMS treatment. Untreated seed were soaked in distilled water for 24hrs at room temperature and then air-dried before planting alongside the EMS treated seeds.

EMS preparation

The EMS solution was prepared following protocol described by Mba et al. (2010). Briefly, a 2% solution of dimethyl sulfoxide (DMSO) was prepared prior to EMS preparation, for use as a carrier agent for EMS treatment. The DMSO was autoclaved at 120 °C and 103.5 kPa for 15 min and set to cool down at room temperature for 5–6 hrs. The EMS solutions at five concentration levels of 0.1, 0.3, 0.5, 0.7 and 0.9% were prepared accordingly by making up 1 L with 2% DMSO solution using a micropipette.

EMS mutagenesis

Seeds were subjected to five EMS dosages (0.1, 0.3, 0.5, 0.7 and 0.9% v/v) at constant temperature of 24°C for five exposure periods (0.5 hr, 1hr, 1.5hrs, 2hrs and 2.5hrs). The mesh bags containing the seeds were immersed in EMS solution in a beaker. The beakers were placed in a water bath maintained at 24°C for the different time durations. After each treatment condition, excess EMS was washed off using running water for 3hrs. The mesh bags were placed on paper towels afterwards overnight to drain the excess EMS solution. The seeds were planted the following morning as described below.

Trial establishment

EMS-treated and untreated seeds (comparative control) were planted at 1 cm depth in seedling trays under glasshouse condition using pine bark growth medium and one seed was planted per hole. The seedlings were watered four times daily using a mist irrigation system. The relative humidity in the glasshouse was set at ~63% and controlled using a fogger system.

Data collection

Data were collected on the following parameters: percent germination (%G) recorded as the proportion of germinated seeds per total number of seeds sown at eight days after sowing. Seedling survival (%SS) was calculated as the proportion of number of survived seedlings per total number of germinated seeds. Destructive sampling was done 21 days after planting and data on shoot height, root height, shoot biomass and root biomass was collected. Shoot length (SL, in cm) was measured as the length from the base of the plant to the tip of the flag leaf, whereas root length (RL, in cm) was measured from the base of the plant to the tip of the longest root. Seedling vigour index (SVI) was estimated using a formula described by Abdul-Baki and Anderson (1973) as follows:

$$\mathrm{SVI} = \mathrm{Germination}(\%) \times \mathrm{Total\ seedling\ length\ (cm)}$$

Data analyses

Data collected was subjected to analysis of variance (ANOVA) using PROC GLM procedure of the SAS package version 9.3. Treatment means were separated using Tukey's test procedure at 5% level of significance. The lethal dose (LD_{50}) was estimated using a linear regression model as follows: $y = a + bx$ where *y* is the dependent variable (i.e., germination percentage), *x* is the independent variable (EMS dosage), and *a* and *b* are the constant and slope, respectively.

Conclusions

The treatment combinations that yielded optimum treatment conditions in this study will be utilized to induce large-scale mutation in barley to select novel mutants varieties. The present study determined the optimum treatment condition for inducing genetic variation in feed barley. Results revealed that EMS dosage of 0.64%v/v of EMS can be used to increase genetic variability for key traits in barley.

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