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Effect of EMS on germination and survival of okra (*Abelmoschus esculentus* L.)

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Abstract. Okra (*Abelmoschus esculentus* L.) is an annual vegetable crop and a member of family Malvaceae that widely cultivated in tropical and subtropical regions of the world. In the current study, the effects of EMS mutagenesis on germination, survival and seedling characteristics of okra were evaluated. Okra seeds were treated with different concentrations of EMS, including 0.175%, 0.350%, 0.525%, 0.700%, 0.875% and 1.050%. The effect of mutagen was observed based on the seed germination, germination speed and plant survival rate (%) and the seedling parameters such as length of seedling and vigor index. The results indicated that values of all parameters were decreased by increasing concentrations of EMS. The LD_{50} values estimated based on the 50% reduction of germination and survival percentage were at 1.050% and 0.525% doses of EMS, respectively.

Key words: EMS, LD_{50}, okra, plant survival, seed germination, seedling parameters.

Introduction

Okra is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. It is suitable for cultivation on a large commercial farm. It is grown commercially in many countries such as India, Turkey, Iran, Bangladesh, Pakistan, Brazil, Ghana, Ethiopia, the Southern United States and etc. Depending on the region, this crop has several uses. Fresh green pods and leaves are used in cooking for seasoning and flavoring or are eaten as a vegetable. Immature pods are steamed, fried, pickled or canned. Fresh okra pod is an excellent source of vitamins A and C, and calcium (NARP 1993). It also contains carbohydrates, potassium, magnesium and other vitamins at a significant level. (Norman 1992). The seeds of okra are a good source of protein and oil. The protein content of okra seeds is up to 45% after oil extraction (Oyenuga 1968) and its oil can be a good substitute for the cottonseed oil (Aminigo and Akingbala 2004). There were also reports about anticancer (Tseng et al. 2004; Sengkhamparn et al. 2009; Vayssade et al. 2010), antimicrobial (De Carvalho et al. 2011), antiulcer (Atodariya et al. 2013), antihyperlipidemic (Sabitha et al. 2011) and hypoglycemic activities (Tomoda et al. 1989, Sabitha et al. 2011) of okra. This plant has a rapid growth cycle, easy cultivation, resistance to pests and high nutritional value. However, there is not much variation in okra and most of the available varieties have low yield and also are sensitive to the diseases such as yellow vein mosaic virus, *Cercospora* leaf spot, fusarium wilt, etc.

A mutation is a sudden heritable change in the DNA of living cell, which neither caused by genetic segregation nor genetic recombination (Van Harten 1998). Spontaneous mutations frequently occur in nature. Drake et al. (1998) reported that in eukaryotic genome, a mutation occurs every 10-8 base pair per generation. Mutation could be artificially induced by specific physical and chemical agents called mutagens. To improve the certain traits in existing germplasm, the induced mutation as an important tool has been established. Following the discovery of mutagenic effects of X-ray on the fly by Muller (1927), induced mutations are being used in the plant breeding. Ethyl Methane Sulphonate (EMS) is a popular mutagenic and carginogenic organic compound that generates random mutations in genetic content through nucleotide substitution. This mutagenic chemical compound is one of the most effective and powerful mutagens (Minocha & Arnason 1962; Hajra 1979) that only produces point mutations (Okagaki et al. 1991).

The frequency and type of produced mutations depends on the plant species or varieties, the dosage of mutagen, the situation of plant before, after and during of the induction. So, it is very important to have knowledge about the plant response or seedling behavior to obtain successful variation through mutation. The LD_{50} used by most of the researcher to determine the lethal dose of mutagens (Warghat et al. 2011; Talebi et al. 2012; Anbarasan et al. 2013). In each mutation breeding program initially LD_{50} is determined, which is used as an optimal concentration for induction. By ignoring this step, mutagen dose can either be high or low resulting mutation frequency.

The present work aimed to investigate the effect of different doses of EMS (0.175, 0.350, 0.525, 0.700, 0.875 and 1.050 ml/100 ml H_2O) on germination and seedling growth of okra.

Material and Methods

The experiment was conducted in a completely randomized design with three replications at Department of Plant Breeding, Sari University of Agricultural Sciences and Natural Resources during the year 2013.

Plant Material

The seeds of local variety of okra that is cultivated in Mazandaran (N-Iran) were collected and cataloged in the herbarium of the Sari Agriculture Sciences and Natural Resources University under the number OPB-43335.

EMS Induction

Seeds were presoaked in water for 18 hrs and then to induce mutation they were transferred to 0.175%, 0.350%, 0.525%, 0.700%, 0.875% and 1.050% (v/v) concentrations of EMS solution for 18 hrs. The seeds were then washed thoroughly in running tap water for 2 hrs. Untreated seeds were presoaked in water for 18 hrs and used as the controls. Based on the EMS-induced mutagenesis, 90 seeds of each treatment (30 seeds per petri dishes) were evaluated for germination besides untreated control. The germinated seeds were sown in soil at the greenhouse following completely randomized design in three replicates.

Germination and seedling growth

Seed germination (SG) percentage for each treatment was recorded on 6th day as under:

$$SG\ \% = \frac{\text{Number of seeds germinated}}{\text{Total number of seed}} \times 100$$

The germination speed (GS) and the vigor index (VI) calculated as described by Maguire (1962) and Abdul-Baki & Anderson (1973) respectively:

$$GS = \sum_{1}^{j} \frac{n_i}{D_i} \qquad VI = \frac{SL_{(mm)} \times GS\ \%}{100}$$

n_i is the number of seeds germinated on i^{th} day and D_i is the number of days from the start of the experiment.

Seedling length (SL) was measured on 10th day after sowing from randomly selected seedlings and expressed in cm.

Plant survival

The plant survival (PS) data were recorded after 30 days of sowing and percent survival was calculated as follow:

$$PS\ \% = \frac{\text{Number of seeds survived}}{\text{Total number of seed sown}} \times 100$$

Statistical analysis

Data were statistically analyzed using ANOVA of SAS software. The significance of differences among means was carried out using the Duncan Test at p= 0.05.

Results and Discussions

The analysis of variance (Table 1) showed that the different doses of EMS significantly affected the studied characters including seed germination percentage, plant survival percentage, seedling length, vigor index ($P<0.01$) and germination speed ($P<0.05$). The germination percentage decreased with increasing the concentrations of EMS as compared to control except 0.525% dose (Table 2 & Fig. 1-a). The highest germination percentage was observed in control and 0.525% dose of EMS (97.67% and 97.33%, respectively), while it was lowest in 1.050% EMS (55.67%). With increasing the dose of EMS, significant decrease was recorded in plant survival percentage was reduced (Table 2 & Fig. 1-b). The highest value for survival percentage was observed in control (93.50%), while the highest mortality was recorded at 1.050% dose of EMS in which survival rate was zero.

Table 1. Mean squares for germination parameters in induced mutants of okra.

		Mean Square				
S.O.V	df	SG	PS	SL	GS	VI
Treatment	6	797.206**	3090.131**	14.397**	13.012*	25.489**
Error	14	1.571	0.357	0.498	2.900	0.335
CV%	-	1.525	1.035	6.157	13.628	6.023

* $P<0.05$ and ** $P<0.01$

Where SG: seed germination percentage; PS: plant survival percentage; SL: seedling length; GS: germination speed; VI: vigor index.

Similar results about the effect of mutagens have been reported in different crops, including cluster bean (Velu et al. 2007), maize (Gnanamurthy et al. 2011), rice (Talebi et al. 2012), soybean (Satpute & Fultambkar 2012), sesame (Anba-

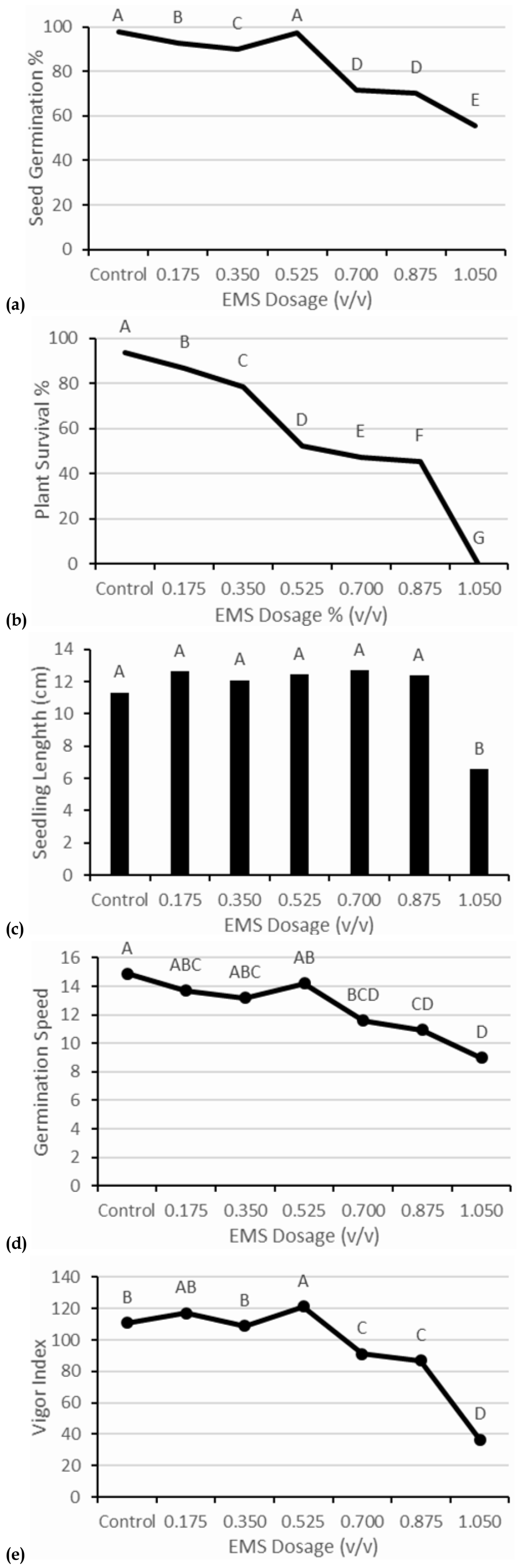

Figure 1. Effect of EMS doses on different parameters of okra, including: a) seed germination percentage, b) plant survival percentage, c) seedling length, d) germination speed, e) vigor index.

Table 2. Effects of various doses of EMS on germination parameters.

D (ml/100ml H_2O)	SG %	PS %	SL (cm)	GS	VI
Control	97.67 ^{a}	93.50 ^{a}	11.33 ^{a}	14.87 ^{a}	110.69 ^{b}
0.175	92.67 ^{b}	87.00 ^{b}	12.63 ^{a}	13.70 ^{abc}	117.09 ^{ab}
0.350	90.00 ^{c}	78.33 ^{c}	12.10 ^{a}	13.19 ^{abc}	108.94 ^{b}
0.525	97.33 ^{a}	52.33 ^{d}	12.47 ^{a}	14.21 ^{ab}	121.33 ^{a}
0.700	71.67 ^{d}	47.33 ^{e}	12.70 ^{a}	11.58 ^{bcd}	90.97 ^{c}
0.875	70.33 ^{d}	45.50 ^{f}	12.37 ^{a}	10.93 ^{cd}	86.98 ^{c}
1.050	55.67 ^{e}	0.00 ^{g}	6.60 ^{b}	9.00 ^{d}	36.66 ^{d}

Note: Means followed by the same letter (s) in each column are not significantly different according to the Duncan's multiple range test (probability level of 5%).

Where SG: seed germination percentage; PS: plant survival percentage; SL: seedling length; GS: germination speed; VI: vigor index.

rasan et al. 2013), cowpea (Gnanamurthy et al. 2013), pearl millet (Ambli & Mullainathan 2014) and pigeon pea (Ariraman et al. 2014). Similarly, Warghat et al. (2011) revealed that sodium azide and gamma rays mutagens decreased the germination and survival percentage of musk okra (*Abelmoschus moschatus*) as compared to control. Jadhav et al. (2012) reported reduction in germination of the okra seed that treated with EMS and gamma rays mutagens. They also reported increase in mortality percentage. Jagajanantham et al. (2013) also noticed that application of EMS and DES mutagens decreased the germination and survival of okra seeds.

It was observed that the seedling length slightly increased in lower dose but with increasing the concentration of EMS, it was showed significant reduce at 1.050% dose as compared to control (Table 2 & Fig. 1-c). Recent researches on rice (Talebi et al. 2012), sesame (Anbarasan et al. 2013) and pearl millet (Ambli & Mullainathan 2014) showed that seedling length was reduced due to EMS mutagenic effect. Similarly, Ariraman et al. (2014) noticed reduction in seedling length of the pigeon pea induced by EMS and gamma rays. Jagajanantham et al. (2013) also found decrease in seedling length of okra due to EMS.

As an effect of EMS, the germination speed and vigor index of okra decreased significantly at 0.700%, 0.875% and 1.050% EMS doses, in the comparison with control (Table 2 & Fig. 1-d; 1-e). Among of the treatments, the highest value for germination speed and vigor index achieved by 0.525% dose. Similar to this result Ariraman et al. (2014) showed that seedling vigor index of pigeon pea decreased due to mutagenesis effect of EMS and gamma rays.

The EMS cause random point mutations as Sikora et al. (2011) expressed. As much as the concentration of EMS rises, the probability of point mutation induction would be increased. This mutations may lead to defects in the synthesis of essential compounds for the plant. The higher doses probably would be caused to more genetic injuries on treated plants which may explain why survival rates are lower among of them.

In spite of the fact that 50 percent of germination was observed at 1.05% dose, limiting the study to this data might cause a mistake in conclusion. Because, the deadly disturbance induced by genetic damages would be appeared on treated seedling a while after germination and emergence stage. So due to the survival rate, it was recommended that the 0.525% dose (with 50% survival rate) be used as optimum dose (LD_{50}) specially in the field researches.

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