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Effect of EMS induction on some morphological traits of Okra (*Abelmoschus esculentus* L)

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Abstract

Okra (*Abelmoschus esculentus* L.) is the only vegetable crop of significance in the *Malvaceae* family and is common in tropical and subtropical regions of the world. Mutation induction has been accepted as a useful tool in plant breeding programs to provide maximum inheritable variability. During this study, the effect of chemical mutagen Ethyl Methane Sulfonate (EMS) at three levels, 0.175%, 0.350% and 0.525% and two soaking period, 18 and 24 h. studied for variation induction on agronomic traits of okra using factorial experiment in a completely randomized design. In the M2 generation, agronomic traits were recorded including plant height, pod number/plant, pod length, pod diameter, stem thickness, seed number/pod, pod weight, number of locules, 100 seed weight and seed yield/plant. Analysis of variance showed that EMS concentration was significant at the 5% level for pod length, stem thickness and seed yield/plant and was highly significant for plant height, number of locules and 100 seed weight at the 1% probability level. The interaction of dose×soaking duration were significant pod number/plant and seed yield/ plant at 5% level and the characteristic of 100 seed weight was significant at 1% probability level. Application of EMS mutation lead to increased plant height, pod number/plant, pod length, pod diameter, stem thickness, seed number/ pod, number of locules, seed yield/plant and decrease of seed weight in okra. These results demonstrating that EMS could greatly affect the most of studied characters in M2 generation which it would increase the efficiency of the breeding objectives.

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Introduction

Abelmoschus esculentus (okra) is widely cultivated in the tropical and subtropical regions of the world. In addition to the use of its immature pods as a vegetable, mature pods are sometimes used as animal feed and as a source of mucilage. Mature seeds are used for oil production and, when ground, as a substitute for coffee. Various plant parts are also used as a thickening or sizing agent in confectionaries. Mays *et al*., (1990) and Anwar *et al*., (2009) have proposed the use of okra (*A. esculentus*) seed oil for fuel/biodiesel production. The plant has been used medicinally in treatment of several disorders. Anticancer, antimicrobial and hypoglycemic activities of plant are reported. The anti-ulcer activity of fresh fruits is recently reported. The crop is generally considered drought resistant and has fewer pests than soybean. Other notable characteristics of this crop including its rapid growth cycle, easy cultivation and high nutritional value.

Because of the nutritional and economic importance of okra, it is imperative that adequate attention be given to ways of producing the seed in such a way that high quality is ensured. There is not much variability in okra in Iran and most of the available varieties give poor yield and are highly susceptible to the yellow vein mosaic virus. Variability is a pre-requisite for any breeding program to evolve high yielding varieties with other desirable attributes. In such a situation, induced mutations can be used to generate useful variation in quantitatively inherited characters.

Induced mutation has been established as an important tool for improvement of certain traits in the existing germplasm. Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base (Micke, 1988). Mutation can be induced by two type of mutagenes including physical and chemical. Various types of chemicals capable of inducing mutation in plants had been found out. They are ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphate (DES), ethylene imine (EI), hydroxyl amine (HA), n-nitroso-n-ethyl urea (NEU), nitrous acid (NA), sulphur mustard, 5-bromouracil, sodium azide (SA) etc. Among these, EMS is a potent mutagen belongs to the category of alkylating agents with a chemical formula C2H5OSO2CH. Its potential of inducing mutations was confirmed in Drosophila, Neurospora, bacteria and in some of higher plants. Jagajanantham *et al*., (2013) reported that EMS was more effective compared other mutagens. Therefore, an attempt has been made to study the effect of EMS mutagenic treatment on morphological characters of okra in M2 generations.

Material and methods

The experiment was conducted at Department of Plant Breeding, Sari University of Agricultural Sciences and Natural Resources during the year 2012.

Plant Material

The Seeds of local variety of okra that cultivated in Mazandaran (N-Iran) were collected.

EMS Induction

Seeds were presoaked in water for 18 hrs and then to induce mutation they put in three different concentrations of EMS solution (0.175%, 0.350%, and 0.525%) for two time period (18 and 24 hrs). The seeds were then washed thoroughly in running tap water for 2 hrs. There were seeds that soaked in water for 18 and 24 hrs as the controls.

Sowing

The treated seeds along with controls were sown immediately after treatments in two factor completely randomized design to grow the M1 generation. M2 generation was raised from selfed seeds of healthy, disease free individual of M1. The data was recorded from four plants selected at random from each treatment and mean per plant was worked out.

Characters

The observations were recorded on various morphological characters, including:

1. Plant height (cm)

2. Pod number per plant
3. Pod length (cm)
4. Pod diameter (mm)
5. Stem thickness (mm)
6. Seed number per pod
7. Pod weight (g)
8. Number of locules
9. 100 seed weight (g)
10. Seed yield per plant (g).

Statistical analysis of recorded data was performed using SAS software.

Results and discussion

The estimate of mean squares of ten studied morphological traits in okra treatments are given in Table 1. Analysis of variance showed that mean squares due to EMS doses were highly significant ($P \le 0.01$) for five characters including plant height, number of locules and 100 seed weight, and were significant ($P \le 0.05$) for pod length, stem thickness and seed yield per plant. On the other hand none of studied characters were affected by soaking duration in EMS. While the interaction effect of EMS doses and soaking duration showed highly significant differences ($P \le 0.01$) for 100 seed weight and significant ($P \le 0.05$) for pod number per plant and seed yield per plant.

Table 1. Mean squares for various characters in induced mutants of Okra.

		Mean Square									
S.O.V	df	PH	PN	PL	PD	ST	SN	PW	NL	SW	SY
D	3	1177.758**	7.875	16.771*	39.931	31.442*	801.031	1.753	11.458**	0.955**	150.209*
SD	1	7.508	4.5	1.125	4.118	4.41	124.031	1.037	0	0.119	64.806
D×SD	3	151.341	9.75	5.812	3.399	9.502	343.865	2.51	0.833	1.29	152.305
E	24	230.518	2.729*	4.047	21.006	7.456	322.281	4.312	1.167	0.161**	45.167*

*, ** - significant at P= 0.005 and P = 0.01 respectively.

Where D: Dose; SD: soaking duration; PH: plant height; PN: pod number per plant; PL: pod length; PD: pod diameter; ST: stem thickness; SN: seed number per pod; PW: pod weight; NL: number of locules; SW: 100 seed weight; SY: seed yield per plant.

The result in Table 2 showed that at least one of mutagenic treatment had significant differences with control for all of studied trait except stem thickness and pod weight. The mean values for yield and yield attributing traits in selected mutant showed positive shift as compared to their respective control. Induction of EMS as mutagen increased plant height, pod number per plant, pod length, pod diameter, stem thickness, seed number per pod, number of locules and seed yield per plant of okra as compared to controls. While significant decrease was recorded for seed weight at 0.525 percent EMS as compared to controls and other doses.

Table 2. Mean performance of different EMS doses in Okra for different characters.

D (ml/100m H_2O)	PH (cm)	PN	PL (cm)	PD (mm)	ST (mm)	SN	PW (g)	NL	SW (g)	SY (g)
Control	31.44 b	2.875 b	14.38 b	21.13 b	11.55 b	74.88 b	12.77 a	5.125 b	6.763 a	11.04 b
0.175	59.38 a	4.125 ab	17.63 a	26.29 a	15.23 a	86 ab	12.71 a	6.875 a	6.738 a	16.01 ab
0.350	52.25 a	5.25 a	16.94 a	22.28 ab	15.95 a	98.5 a	13.72 a	7.375 a	6.733 a	19.11 a
0.525	52.88 a	4.5 ab	15.56 ab	22.62 ab	15.16 a	91.75 ab	12.89 a	7.875 a	6.054 b	20.95 a

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 D: Dose; PH: plant height; PN: pod number per plant; PL: pod length; PD: pod diameter; ST: stem thickness; SN: seed number per pod; PW: pod weight; NL: number of locules; SW: 100 seed weight; SY: seed yield per plant.

The interaction effect of different EMS doses and soaking durations showed in Table 3. The range of the most of studied characters was higher as compared to controls similar to result of the Table 2. There was also significantly decreased seed weight at 0.35 and 0.525 percent EMS with 18 hrs. and 24 hrs. soaking duration respectively. However this result didn't show any significant differences between the treatment and controls for pod diameter, seed number per pod and pod weight. The highest value for plant height, pod number per plant, pod length, stem thickness, number of locules, 100 seed weight and seed yield per plant was achieved at 0.175/18, 0.525/18, 0.175/18, 0.350/18, 0.525/24, 0.350/24 and 0.525/18 (percent /hour) respectively.

Table 3. Mean interaction of different EMS doses and soaking durations in Okra for different characters.

SD (hrs.)	D (ml/100ml H_2O)	PH (cm)	PN	PL (cm)	PD (mm)	ST (mm)	SN	PW (g)	NL	SW (g)	SY (g)
18	Control	33.125^{bc}	3^{bc}	14^{b}	20.96^{a}	11.34^{c}	72.25^{a}	12.76^{a}	5.25^{bc}	6.788^{abc}	10.82^{c}
	0.175	61^{a}	3.75^{bc}	18.5^{a}	26.33^{a}	14.64^{abc}	92^{a}	13.00^{a}	6.75^{ab}	6.51^{bc}	10.16^{c}
	0.350	46.5^{abc}	5^{abc}	15.75^{ab}	23.58^{a}	16.93^{a}	97.25^{a}	13.72^{a}	7.75^{a}	6.24^{c}	15.37^{abc}
	0.525	57.25^{ab}	6.5^{a}	15.5^{ab}	22.87^{a}	13.50^{abc}	81.75^{a}	11.89^{a}	7.5^{a}	6.505^{bc}	25.08^{a}
24	Control	29.75^{c}	2.75^{c}	14.75^{b}	21.29^{a}	11.76^{bc}	77.5^{a}	12.78^{a}	5^{c}	6.737^{abc}	11.27^{bc}
	0.175	57.75^{ab}	4.5^{abc}	16.75^{ab}	26.25^{a}	15.83^{ab}	80^{a}	12.43^{a}	7^{a}	6.965^{ab}	21.86^{ab}
	0.350	58^{ab}	5.5^{ab}	18.13^{a}	20.98^{a}	14.97^{abc}	99.75^{a}	13.71^{a}	7^{a}	7.225^{a}	22.85^{a}
	0.525	48.5^{abc}	2.5^{c}	15.63^{ab}	22.36^{a}	16.82^{a}	101.75^{a}	13.88^{a}	8.25^{a}	5.603^{d}	16.83^{abc}

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 D: Dose; SD: soaking duration; PH: plant height; PN: pod number per plant; PL: pod length; PD: pod diameter; ST: stem thickness; SN: seed number per pod; PW: pod weight; NL: number of locules; SW: 100 seed weight; SY: seed yield per plant.

This results were similar with earlier reports. Biswas *et al.*, (1988) showed that in *Trigonella foenumfraeum* mutagen significantly produced more branches, more buds and number of fruits/plants than the control plants. Similar to our result, Singh *et al.*, (1998) and (2002) were indicated that low doses of mutagens (i.e. 15-30 kR gamma rays and 0.25 and 0.50 percent EMS) increased plant height and number of pods per plant of okra. Warghat *et al.*, (2011) noticed that sodium azide and gamma rays mutagens caused to increase in plant height and number of pods in musk okra (*Abelmoschus moschatus*) as compared to control. Jadhav *et al.*, (2013) showed increase in plant height and pod length of okra induced mutants by EMS. Pushparajan *et al.*, (2014) reported that in the comparison with control, gamma rays produced increased plant height, pod number, pod length, number of seed, number of locules and 100 seed weight in okra. The outcome of these reports were confirmed by our result.

The more altered range of characters arising from occurrence of polygenic mutations with equal frequencies towards positive and negative directions as reported by Singh *et al.*, (1998). It seems that mutagenic treatments were very effective in inducing macromutations for desirable traits which corresponded well with previous studies in okra by Sharma *et al.*, (1991), Kulkarni *et al.*, (1992) and Jadhav *et al.*, (2013). These desirable mutants may be useful and valuable in okra improvement program. Of course it must be noticed that the useful mutant isolated through the present study need to be tested further on a wider scale to establish any changes in chromosome or allele frequency and also to assess its performance in later generations.

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