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Effect of Ethyl Methanesulfonate (EMS) on the Germination, Growth and Yield of Two Okra (*Abelmoschus esculentus* (L.) Moench) Varieties

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ABSTRACT: The study was conducted to investigate the effect of different concentrations of ethyl methanesulfonate (EMS) on the growth and yield of two okra (*Abelmoschus* esculentus L. Moench) varieties (V35 and Clemson spineless). The experiment was conducted in the screen house and field at Michael Okpara University of Agriculture, Umudike, Abia State. The screen house experiment was set up in a complete randomized design (CRD) with three replications. The field trial was set up in randomized complete block design (RCBD) with three replications. Hundred (100) healthy seeds of each variety were selected in the 6 beakers well labeled. Seeds were pre-soaked in distilled water and allowed to stand for 4 hours. Different concentrations of EMS (0.0 %, 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %) were freshly prepared. The pre-soaked seeds were transferred into the different EMS concentrations and allowed for 4 hours. Fifty (50) seeds were sown in a plastic Petri dish for germination test whereas the remaining seeds were sown in planting bags and kept in the screen house for preliminary investigation. From the result obtained, three concentrations (0.3 %, 0.4 % and 0.5 %) were then chosen for field trial. the result obtained on seed germination showed a concentration-dependent effect with an increase in EMS doses. Germination percentage was the maximum under the untreated seeds (99.00 % and 95.00 %) in both okra varieties. The concentration of EMS (0.5 %) significantly reduced seed germination (57.00 % and 57.00 % respectively. Shoot length and root length were also reduced with increased EMS concentrations. The result obtained on the growth parameters showed that the treatments significantly affected all the growth variables measured. Treatment 0.3 % EMS recorded a significant increase in all the growth traits in V35 whereas 0.4 % EMS enhanced growth in Clemson spineless variety. The result obtained from the yield parameters showed that 0.5 % and 0.4 % EMS positively affected the yield traits in the okra varieties. The result of this study has shown that EMS could be utilized in the mutation breeding of okra with optimum concentration at 0.3 % and 0.5% for improved agronomic qualities. It is therefore recommended that further study should be conducted on the M_2 to observe more variations that could be useful for plant breeders.

Keywords: Okra, mutation, EMS, Seed germination, Growth, Yield

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is one of the popular vegetables known in the tropical and sub-tropical countries of the world. It belongs to the Family Malvaceae and has the typical floral characteristics of that family originating from Africa. It is now widely distributed in the tropics including Nigeria (National Research Council, 2006). It is an important vegetable crop occupying a land area of 277,000 hectares with a production of 731,000 metric tons worldwide and a productivity of 2.63 tha^{-1} in Nigeria (FAO, 2006). The crop is native to West Africa and has become established in the wild in some new world tropical areas. Okra was domesticated in West and Central Africa and known as 'okro' in the Anglophone African countries as a fast-growing common annual vegetable widely consumed in Africa (Schippers, 2000).

It is cultivated for its fibrous fruits or pods and the fruits are harvested when immature and eaten as a vegetable. They are a good source of carbohydrate, protein, fats, vitamins and minerals (Akintoye *et al.*, 2011). Despite its

popular use as a vegetable, it has also been used for several purposes such as coffee additive and paper making (Moekchantuk and Kumar, 2004). The mucilage is also suitable for medicinal and industrial applications (Akinyele and Temikotan, 2007). Its production is constrained by a complex of biotic and abiotic factors at every stage of growth (Anne and Carter, 2004). Unfavourable climatic conditions such as drought, edaphic factors, excess or low light intensity can damage the quality and reduce the yield (Agbogidi and Nweke, 2005).
Mutations are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). It can result in genetic variability which is most desirable in self-pollinated crops such as okra. Several researchers have tried to improve the growth and yield of many crop species by applying the techniques of mutation breeding. Mutagenesis alters the genetic makeup of plants by interference and modification of genes. It is an efficient tool used to create new desirable genetic variability (Koornneef, 2002). Mutation breeding is an important method used for the improvement of crops through the induction of mutations at loci that control economically important traits or by eliminating undesirable genes from elite breeding lines. In several mutations derived varieties, the changed traits have resulted in increasing the yield and quality of the crop, improving the agronomic inputs and consumer acceptance (Ahloowalia *et al.,* 2004). Induced mutagenesis has been successfully utilized in several crop plants such as rice, common beans, soybean, Artemesia and chickpea, suggesting the great potential of this technique for crop improvement (Wani and Anis, 2008). Many agronomical important mutations affecting plant and grain characters have been identified including alteration of grain colour, stem rust resistance and earliness in wheat (Chopra, 2005).

Materials and Methods

Seeds of two okra varieties (Clemson spineless and V35) were collected from National Seed Council, National Root Crop Research Institute, Umudike, Abia State. The experiment was conducted in two phases.
The first phase was a preliminary investigation to choose the optimum concentration at the early stage of development and was set in a complete randomized design (CRD) with three replications in the screen house.
The second phase was carried out in the field in a randomized complete block design (RCBD) with three replications. The seeds were properly arranged and pre-soaked in distilled water for 4 hours. Different concentrations of EMS (0.0 %, 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %) were freshly prepared in buffer 7 according to the method of Mba *et al.* (2010). The pre-soaked seeds were later transferred into the different EMS concentrations and allowed for 6 hours under room temperature. Out of 100 seeds soaked, 50 seeds were sown in a plastic Petri dish for germination study. Germination was monitored for 7 days. The effect of the mutagen on the shoot length and root length was studied in the screen house 21 days after planting. After this, three concentration levels (0.3 %, 0.4 % and 0.5 %) were selected for field trials. The field size was 10 m x 17 m with plot size 2 m × 2 m. The planting distance was 50 cm x 50 cm. The field had a total of 24 plots. A total of 3 seeds were sown per hole and later thinned to one stand per hole. The effect of the mutagen on the germination percentage, shoot length and root length was investigated. Growth parameters such as plant height, number of leaves, lea area and stem girth were measured at different week intervals. The yield parameters measured include days to first flowering, number of pod per plant, pod length, pod diameter and fresh pod weight. The data obtained from the growth and yield parameters were subjected to statistical analysis to assess the extent of induced variations using the analysis of variance (ANOVA) for randomized complete block design (CRD) which was used to compare variable using Genstat software version 20.

Results

Effect of different concentrations of EMS on seed germination, shoot length and root length: Table 1 presents the result of the effect of different concentrations of EMS on seed germination. Germination was monitored for 7 days in the two okra varieties. From the result obtained, it was observed that seed germination was affected by the mutagen concentrations in both varieties of okra. The result obtained in V35 variety showed that the control seeds recorded the highest germination percentage (99.00 %). The lowest concentration of EMS (0.1 %) had 94.00 % whereas the highest EMS concentration (0.5 %) recorded the lowest germination (57.00 %). Similarly, in Clemson spineless, maximum germination was counted under the control seeds with a mean value of 95.00 % treatment 0.1 % EMS recorded 95.00 % while 0.5 % recorded 57.00 %. The effect of the mutagen exhibited a trendy effect with an increase in the concentration.

Figure1 shows the result of different EMS concentrations on the shoot length of V35 and Clemson spineless respectively. In Figure1, the result revealed a gradual decrease in shoot length with an increase in EMS concentration. A higher concentration of EMS negatively affected the shoot length. Similarly, in Figure 2, the highest shoot length was recorded under the control. The treatments exhibited varying degrees of effects with increased EMS concentration. From the result, an increase in EMS concentration also caused a decrease in the shoot length of Clemson spineless. The result obtained on the two okra varieties showed that an increase in EMS concentration caused a gradual decrease in shoot lengths of both okra varieties.

The result of the effect of EMS concentrations on the root length of both okra varieties is presented in Figure 3 and 4 respectively. In V35 variety, the root length was reduced with an increase in EMS concentration. The result showed that the increased plants produced the highest root length. The treatments inhibited the root lengths, particularly at higher concentrations. The result showed that at 0.4 % and 0.5 % EMS, the root length was drastically reduced. Also, in damson spineless, the same result was obtained. Root length was highest under the control plants. An increase in EMS concentration caused a gradual decrease in the root length. The higher concentrations caused a reduction in the root length (Figure 3 and 4).

Table 1: Effect of different concentrations of EMS on seed germination

Variety	EMS conc. (%)	Germination percentage
V35	0	99.00
	0.1	94.00
	0.2	86.00
	0.3	80.00
	0.4	69.00
	0.5	57.00
Clemson spineless	0	95.00
	0.1	89.00
	0.2	80.00
	0.3	76.00
	0.4	64.00
	0.5	57.00

$LSD_{(0.05)}$) treatment 17.9.3^{ns}$LSD_{(0.05)}$) variety 103.5^{ns}$LSD_{(0.05)}$ interaction 253.5^{ns}

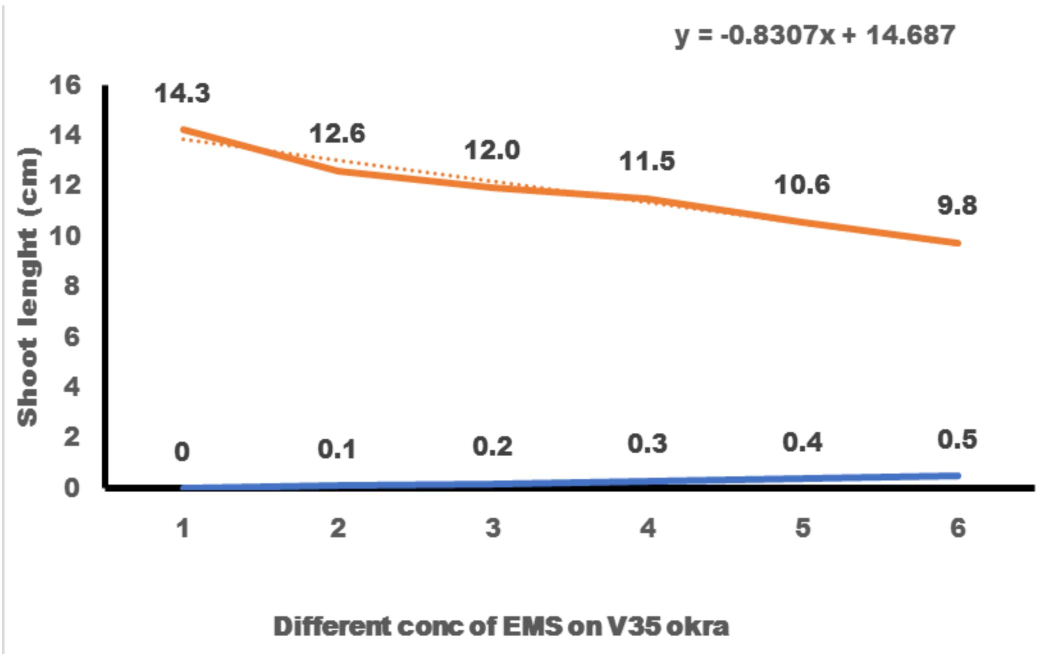

Figure 1: Effect of different concentrations of EMS on the shoot length of V35

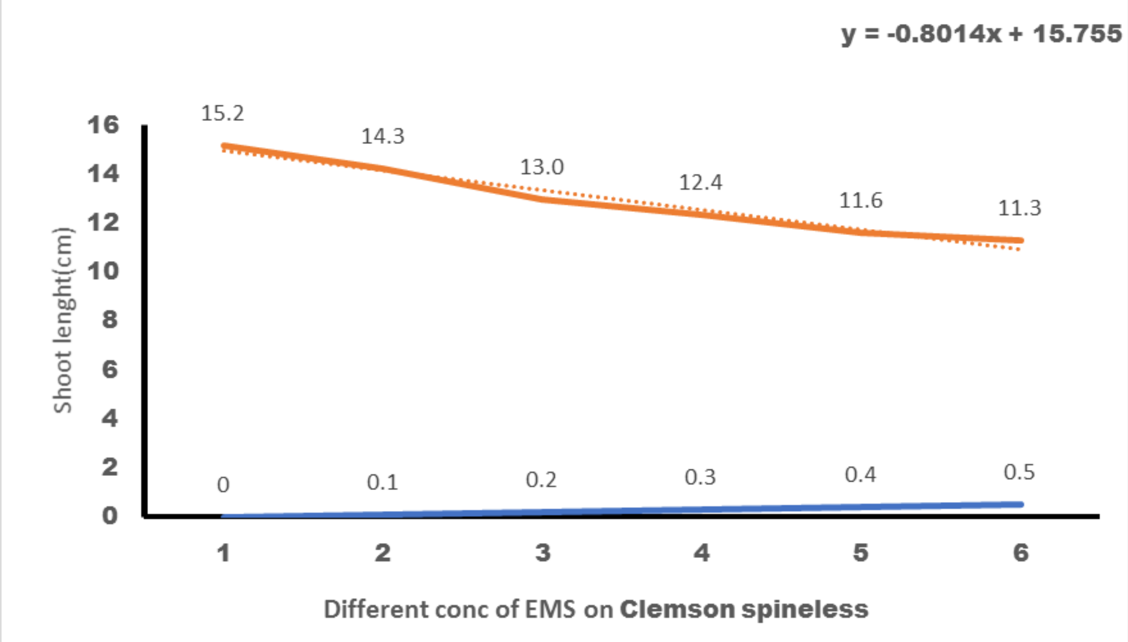

Figure 2: Effect of different concentrations of EMS on the shoot length of Clemson spineless

Effect of EMS concentrations on the vegetative parameters: Table 2 presents the result of the effect of EMS concentrations on the plant height at different weeks after planting. The result obtained showed varying degrees of effect on both okra varieties. In V35 variety, at 2 WAP, 0.4 % EMS produced the tallest plants (5.50 cm) followed by 0.3 % EMS (5.03 cm), treatment 0.5 % produced plants with reduced height (4.91 cm). The control produced plants with 7.67 cm height. At 4 WAP, a similar trend was observed with maximum height recorded under 0.4 % EMS (10.80 cm). Plants with a height of 13.07 cm were produced under the control. Also, at 6 WAP and 8 WAP, the result showed that 0.4 % EMS produced plants with heights of 20.60 cm and 48.67 cm respectively whereas the control had 22.53 cm and 37.33 cm respectively. The result obtained in Clemson spineless also showed that at 2 WAP and 4 WAP, treatment 0.3 % EMS produced the tallest plants with mean heights of 6.93 cm and 16.67 cm whereas 0.5 % EMS recorded 7.77 cm and 14.50 cm respectively. The control recorded 8.88 cm and 14.87. Similarly, at 6 WAP and 8 WAP, the tallest plants were recorded under 0.3 % EMS with heights of 26.27 cm and 41.22cm respectively. The result of the analysis of variance on the plant height showed a highly significant difference ($p \leq 0.05$) in the variety, week, treatment and interaction.

The result presented in Table 3 showed that the mutagen treatments affected the number of leaves when compared to the control. The result obtained in V35 at 2 WAP indicated that 0.4 % of EMS produced the highest number of leaves (5.50) while 0.5 % EMS counted 4.97. At 4 WAP, 0.4 % counted 10.80 followed by 0.5 % (10.00) while 0.3 % recorded 9.47 numbers of leaves. The control plants counted 13.07. Similarly, at 6 WAP and 8 WAP, the result showed that among the different treatment levels, plants treated with 0.4 % EMS counted the highest number of leaves (11.33 and 20.60). The control counted 14.00 and 22.53. However, in Clemson spineless, at 2 WAP, an equal number of leaves was counted for all treatments including the control (4.00) number of leaves. At 4 WAP, the result showed that 0.3 % of EMS counted 7.67 numbers of leaves whereas EMS counted 6.00. Similarly, at 6 WAP and 8WAP, the result showed that a maximum number of leaves was recorded under 0.3 % with mean values of 12.33 and 33.00, 0.5 % EMS counted 8.87 and 17.33. The control had 8.00 and 20.67. The result of the analysis of variance indicated that the effect of the different concentrations of EMS on the number of leaves was highly significant ($p \leq 0.05$) at the various week intervals. Variety and interaction were also highly significant ($p \leq 0.05$).

From the result presented in Table 4, it was observed that the EMS concentration increased the leaf area of the okra varieties at the different weeks. The result obtained in V35 at 2 WAP showed that 0.5 % increased the leaf area (15.12 cm^2) followed by 0.4 % (12.21 cm^2). The untreated plants recorded 21.72 cm^2 leaf area. Also, at 4 WAP, 0.5 % recorded 89.60 cm^2 whereas 0.3 % had 84.99 cm^2. At 6 WAP and 8 WAP, the result showed that 0.3 % EMS also recorded the highest leaf area (420.38 cm^2 and 522.28 cm^2) respectively. The control recorded 334.52 cm^2 and 489.65 $cm^{2.}$ In Clemson spineless, however, the result obtained showed that at 2WAP and 4 WAP, maximum leaf area was recorded under 0.3% EMS (9.17 cm^2 and 71.35cm^2). The control had 8.22cm^2 and 66.66cm^2. Also, at 6 WAP and 8 WAP, 0.3 % recorded the highest leaf area with mean values of 117.20 $cm^2$and 186.95 cm^2respectively. The control leaf area was 153.82$cm^2$and 154.52cm^2.

The result of the stem of different concentrations of EMS on the stem growth of the two okra varieties at the different week interval is shown in Table 5. The control had 0.24 and 0.27 respectively. At 6 WAP and 8 WAP, a similar result was observed. Treatment 0.5 % EMS recorded 0.19 and 0.37 stem girth respectively. At 6 WAP and 8 WAP, a similar result was observed. Treatment 0.5 % EMS recorded 0.94 and 1.52 stem girth. The untreated plants recorded 0.73 and 1.24 respectively. The result obtained in Clemson spineless showed that treatment 0.5 % recorded the highest stem girth (0.28 and 0.60) at 2 WAP and 4 WAP respectively. Also, at 6 WAP and 8 WAP, 0.3 % EMS recorded 1.80 and 1.50 stem girth respectively. The control plants had 0.70 and 1.40 stem girth respective. The result showed that the effect of the various concentrations of EMS with stem girth was very significant ($p<0.05$) when compared to the control.

Table 2: Effect of different concentrations of EMS on the plant height (cm)

Variety	EMS conc. (%)	2 WAP	4 WAP	6 WAP	8 WAP
	0	7.67	13.07	22.53	37.33
V35	0.3	5.03	9.47	17.57	26.23
	0.4	5.50	10.80	20.60	48.67
	0.5	4.97	10.00	18.73	25.80
	Total	5.79	10.83	19.86	34.51
Clemson spineless	0	8.83	14.87	21.79	34.00
	0.3	6.93	16.67	26.27	41.22
	0.4	5.63	12.77	20.42	39.54
	0.5	4.77	14.50	21.69	34.24
	Total	6.54	14.70	22.54	37.25

$LSD_{(0.05)}$ Week 0.601*** $LSD_{(0.05)}$ Week variety 0.850*** $LSD_{(0.05)}$ treatment 0.850***
$LSD_{(0.05)}$interaction 2.405***

Table 3: Effect of different concentrations of EMS on the number of leaves per plant

Variety	EMS conc. (%)	2WAP	4 WAP	6 WAP	8 WAP
	0	7.67	13.07	14.00	22.53
V35	0.3	5.03	9.47	12.67	17.57
	0.4	5.50	10.80	11.33	20.60
	0.5	4.97	10.00	10.00	18.73
	Total	**5.79**	**10.83**	**12.00**	**19.86**
Clemson spineless	0	4.00	7.00	8.00	20.67
	0.3	4.00	7.67	12.33	33.00
	0.4	4.00	6.00	10.00	15.00
	0.5	4.00	6.00	8.67	17.33
	Total	4.00	6.67	9.75	21.50

$LSD_{(0.05)}$ week 0.448***$LSD_{(0.05)}$variety 0.633***$LSD_{(0.05)}$ treatment 0.633***
$LSD_{(0.05)}$ interaction 1.790***

Table 4: Effect of different concentrations of EMS on the leaf area per plant (cm^2)

Variety	EMS conc.(%)	2 WAP	4 WAP	6 WAP	8 WAP
	0	21.72	62.08	334.52	489.65
V35	0.3	9.17	84.99	420.38	522.78
	0.4	12.21	45.03	190.96	197.79
	0.5	15.12	89.60	336.33	316.71
	Total	14.55	70.42	320.55	881.73
Clemson spineless	0	8.22	66.66	153.82	154.52
	0.3	9.17	71.35	184.39	316.20
316.20	0.4	3.30	38.47	117.20	186.95
	0.5	5.52	45.61	170.67	197.86
	Total	6.55	55.52	156.52	21116.38

$LSD_{(0.05)}$Week 10437.7^{NS}$LSD_{(0.05)}$variety14761.1^{NS}$LSD_{(0.05)}$treatment 14761.1^{NS}
$LSD_{(0.05)}$)interaction41750.8^{NS}

Table 5: Effect of different concentrations of EMS on stem girth

Variety	EMS conc. (%)	2 WAP	4 WAP	6 WAP	8 WAP
	0	0.24	0.27	0.73	1.24
V35	0.3	0.22	0.32	0.93	1.51
	0.4	0.13	0.29	0.87	1.24
	0.5	0.19	0.37	0.94	1.52
	Total	0.20	0.31	0.87	1.38
Clemson spineless	0	0.19	0.34	0.70	1.40
	0.3	0.20	0.30	1.80	1.50
	0.4	0.17	0.35	0.99	1.13
	0.5	0.28	0.60	1.23	1.37
	Total	0.21	0.40	1.00	1.38

$LSD_{(0.05)}$ week 0.083^{ns} $LSD_{(0.05)}$ variety 0.1166*** $LSD_{(0.05)}$ treatment 0.1166***
$LSD_{(0.05)}$ interaction 0.1649^{ns}

Effect of concentrations of EMS on the yield parameters: The result of the effect of different concentrations of EMS on the yield parameters is presented in Table 6. The result showed that the effect of the mutagen treatments on the yield parameters was very significant ($p \leq 0.05$) on the two okra varieties. Application of 0.5 % EMS in V35 enhanced days to first flowering (57 days) followed by 0.3 % (58 days). The control flowered at 60.33 days. Similarly, in Clemson spineless, the lowest concentration (0.3 %) induced early flowering (42.67 days) while the increase in the concentration (0.5 %) caused a delay in flowering (47.00 days). The highest number of pods per plant was counted in treatment 0.5 % and 0.4 % EMS respectively in the two okra varieties. Pod length and pod diameter were enhanced by 0.5 % in both varieties. Fresh pod weight was also improved by treatment 0.5 % (17.05 kg) in V35 where 0.4 % measured 112.00 kg under 0.4 % in Clemson spineless. The result statistically showed that variety and treatment effects were highly significant ($p \leq 0.05$) in all the yield traits while the interaction effect was not significantly different ($p \geq 0.05$) for pod length, pod diameter and fresh pod weight (Table 6).

Table6: Effect of different concentrations of EMS on the yield parameters

Variety	EMS conc.	Days tofirst flowering	No. of pods per plant	Pod length	Pod diameter	Fresh pod weight (g)
	0	60.33	5.00	3.14	1.29	9.37
V35	0.3	58.00	5.33	3.57	1.37	7.80
	0.4	60.00	7.00	4.37	1.63	13.72
	0.5	57.00	8.50	4.75	2.35	17.05
	Total	59.00	6.46	3.96	1.66	11.99
Clemsonspinesless	0	45.00	6.67	9.33	2.27	51.00
	0.3	42.67	9.33	10.00	2.13	74.67
	0.4	43.00	10.33	12.00	2.50	112.00
	0.5	47.00	8.33	13.23	2.57	58.67
	Total	44.42	8.67	11.14	2.32	74.08
$LSD_{(0.05)}$variety		0.283***	0.334***	1.099***	0.334***	7.650**
$LSD_{(0.05)}$treatment		0.400***	0.473***	1.555***	0.473***	10.820*
$LSD_{(0.05)}$interaction		0.565***	0.668***	2.198^{ns}	1.555^{ns}	15.310^{ns}

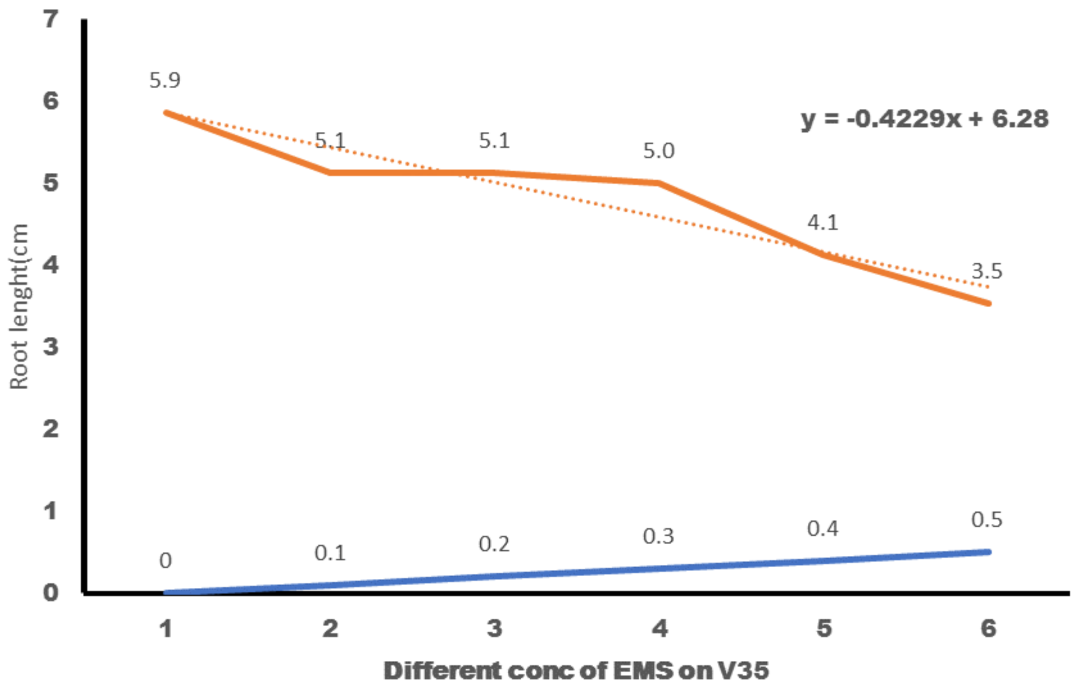

Figure 3: Effect of different concentrations of EMS on the root length of V35

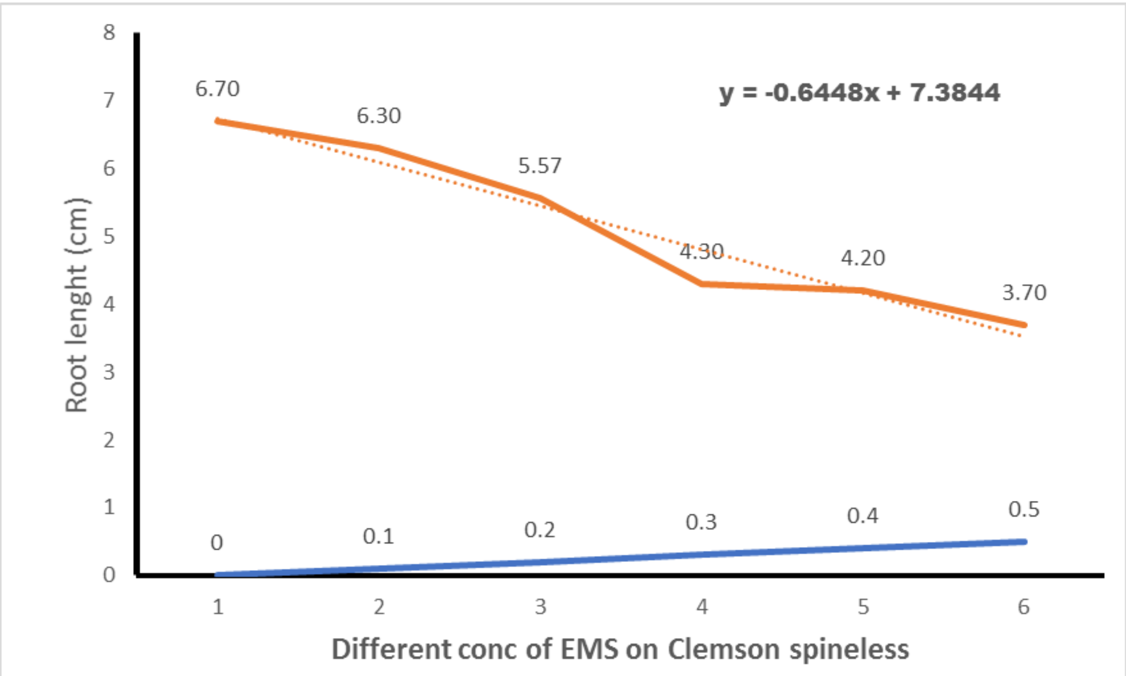

Figure 4: Effect of different concentrations of EMS on the root length of Clemson spineless

Discussion

Chemical mutagens have been applied in improving the quality, yield and growth of many crop species. The seed is used in mutagenesis because it has high regenerative potential and it is advantageous for mutation induction. EMS is a powerful chemical used in inducing mutation in crops which results to create genetic variability. The mutagenic effect of mutagens on seed germination has been reported by many workers. Many researchers have reported a negative effect of chemical mutagen on the germination of seeds. The present study has further added to the available information on the effect of mutagens on seed germination. The result of this study revealed that EMS treatment of seeds of the two okra varieties reduced the germination percentage. The mutagenic treatments revealed a gradually decreasing trend in germination from the lower to the higher concentration of EMS. Similar observations were made by Ariraman *et al.* (2014) on pigeon pea treated with EMS. The result obtained conforms to the report of Baghery *et al.*(2015) on okra seeds treated with different concentrations of EMS. The reduction in germination observed in the two okra varieties might be due to the action of EMS on the seed metabolic process leading to disturbances at the physiological and cytogenetic makeup of the germinating seeds. A similar observation was made by Kumar and Mishra (2004) in okra.

Mutagens have been reported to cause reductions on the shoot and root lengths of many crop species. The result obtained in this study showed that EMS concentrations significantly affected the shoot and root lengths of the okra varieties. The result showed a progressive decrease in the shoot and root lengths with an increase in EMS concentrations. The probable reason for this decrease/reduction could be attributed to the inhibition of cell division and chromosomal damages caused by the EMS concentrations. The result of this finding is in line with the report of Adamu and Aliyu (2007) who reported a decrease in the shoot and root lengths of tomato cultivars treated with sodium azide concentrations.

The result of this study showed that EMS treatments improved the growth parameters of the two okra varieties. Plant height, the number of leaves, leaf area and stem girth increased by application of 0.4% EMS in V35 variety. However, in Clemson spineless 0.3 % EMS improved the growth characters. The increase in plant height, the number of leaves and leaf area under EMS treatments in the okra varieties might be due to chromosomal damages that tend to produce an increase in growth traits. A similar observation was reported by Khan *et al.* (2006) in soybean using radiation with the effect being dose dependent. Also, Zaka *et al.* (2004) reported that the simulating effect might be due to the activation of growth hormones such as auxins. The result of this study however disagrees with the report of Baghery *et al.* (2015) who reported a decrease in all growth parameters of okra treated with different concentrations of EMS. Flowering is an important stage in the reproductive phase of plants as it precedes fruiting. The yield of the okra varieties was significantly affected by the mutagen treatment. The result obtained in this study showed that 0.5 % and 0.3 % EMS induced early flowering in V35 and Clemson spineless when compared to the control plants. This observation agrees with the report of Abraham *et al.* (2013) who reported early flowering at 0.02 % sodium azide concentration in *Sesame indicum.* The number of pods per plant, pod length and pod diameter showed that the higher EMS concentration (0.5 %) improved these traits. This observation agrees with the report of Daudu and Falusi (2012) who reported an increase in the yield parameters of capsicum species at higher mutagenic doses. Similarly, Animausun *et al.* (2014) reported increased yield traits of two groundnut varieties treated with sodium azide concentrations with enhanced performances recorded for 50 mM.

Conclusion

The result of the present study showed that seed germination reduced with increasing concentrations of EMS. Shoot length and root length also showed a decreasing effect with an increase in the mutagen concentrations. The result also showed that EMS is very effective in improving the vegetative and yield traits of the okra varieties. Plant height, number of leaves, leaf area and stem girth were significantly affected by 0.4 % EMS on V35 and 0.3 % EMS on Clemson spineless. Also, yield variables were significantly enhanced by the EMS treatments. Therefore, the study concluded that EMS could be employed by plant breeders in improving the agronomic qualities of okra.

References

Abraham B, Manikandan M, Mebeasillassie A: Effect of chemical mutation by sodium azide on quantitative traits variations in *Sesame indicum* L. Asian J Biol Sci10: 1996-3351. 2013.

Adamu AK, Aliyu H: Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). Sci World J 2: 9-12.2007.

Agbogidi OM, Nweke FU: Effects of crude oil polluted soil on the performance of okra (*Abelmoschus esculentus* L. Moench) in Delta State. Afr J Nat Sci 8: 31-35.2005.

Ahloowalia BS, Maluszynski M, Nichterlin K: Global impact of mutation derived varieties. Euphytica 135: 187.2004.

Akintoye HA, Adebayo AG, Aina OO: Growth and yield response of okra intercropped with live mulches. Asian J Agric Res 5(2):146-153.2011.

Akinyele BO, Temikotan T: Effect of variation in soil texture on the vegetative and pod characteristics of okra (*Abelmoschus esculentus* L. Moench). Int J Agric Res 2(2):165-169. 2007.

Animasaun DA, Oyedeji S, Azeez MA, Onasanya AO: Alkylating efficiency of sodium azide on pod yield, nut size and nutrition composition of samnut 10 and samnut 20 varieties of groundnut (*Arachis hypogea* L.). Afr J Food Agric Nutri 14(7):1684-5374. 2014.

Anne FO, Carter OM: The response of okra to varying levels of poultry manure and plant population density under sole cropping. J Food Agric Environ, 3(3/4):68-74. 2004.

Ariramana M, Gnanamurthy S, Dhanavelb D, Bharathi T, Murugan S: Mutagenic effect on seed germination, seedling growth and seedling survival of pigeon pea (*Cajanus cajan* (L.) Millsp*).* Int Lett Nat Sci, 21:41-49.2014.

Baghery MA, Kazemitabar SK, Kenari RE: Effect of EMS on germination and survival of okra (*Abelmoschus esculentus* L.). Int J Biol 24: 982–989. 2015.

Chopra VL: Mutagenesis: Investigating the process and processing the outcome for crop improvement. Curr Sci 89(2):1-7. 2005.

Daudu K, Falusi M: Effect of Neutron irradiation of agronomic traits of Nigerian pepper (*Capsicum annuum* L.). Eur J Hortic Sci 77(1): 41-45. 2012.

FAO: World reference base for soil resources. World Soil Resources Report 84. Food and Agriculture Organization of the United Nation, Rome. pp.88.2006.

Koorneef M: Classical mutagenesis in higher plants, pp 1-11, in Philip Gelmartin, M. and Chris Bowlar, Eds. Molecular Plant Biology, Oxford University Press. pp. 56-89. 2002.

Kumar A, Mishra MN: Gamma ray's irradiation under dry, pre and post soaked condition on yield and its attributes in M_2populations of Urdbean (*Vigna mungo* L. Hepper). Adv Plant Sci 17: 475-478. 2004.

Mba C, Afza R, Souleymane B, Mohan SM: Induced Mutagenesis in Plants Using Physical and Chemical Agents. Plant Cell Culture: Essential Methods. Pp. 112-129. 2010.

Moekchantuk T, Kumar P: Export okra production in Thailand. Inter-country programme for vegetable IPM in South and SE Asia phase II. Food and Agriculture Organization of the United Nations, Bangkok, Thailand. 2004.

National Research Council: Okra: Lost Crops of Africa: Volume II: Vegetables. Lost Crops of Africa. National Academies Press, Pp.7-15. 2006.

Schippers RR: African indigenous vegetables: An overview of the cultivated species. Natural Resource Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham.UK Science and Biotechnology 74(2):206-212. 2000.

Wani AA, Anis M: Gamma rays and EMS induced bold seeded high yielding mutants in chickpea (*Cicer arietinum* L.). Turk J Biol 32: 161-166.2008.

Zaka RC, Chenal H, and Misset MT: Effect of low doses of short-term gamma radiation on growth and development through two generations of *Pisum sativum*. Sci Total Environ 320: 121-129. 2004.