EFFECTS OF ETHYL METHANE SULPHONATE ON VEGETATIVE TRAITS OF SELECTED GENOYTPES OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

Abdullahi, A., Daudu O. A. Y., Falusi, O. A., Abubakar A. and Audu, M. A. I.
Federal University of Technology, Minna-Nigeria.
Correspondence email: Ameenahabdul495@gmail.com
Mobile: 07068130094

Abstract
Groundnut (*Arachis hypogaea* L.) is a multi-purpose legume crop widely cultivated in Sub-Saharan Africa (SSA). However, several species of groundnut suffer substantial yield losses as a result of narrow genetic base and insect-pest attack. The present study aimed to investigate the effect of EMS on morphological traits of four (4) groundnut (*Arachis hypogaea* L.) genotypes viz Samnut 24, Samnut 25, Samnut 26 and ICG4412. Field experiment was laid out in Complete Randomized Design (CRD) with three (3) replicates. The seeds of the four (4) groundnut accessions were treated with various concentration of EMS (0.0 %, 0.1 %, 0.2 %, 0.3 % and 0.4 %) for 6 hours, they were then removed and rinsed with distilled water to remove the trace of mutagen sticking to the seed coat. Data on vegetative parameters were collected following standard procedures. Results obtained showed that 0.1 % EMS concentration had the highest plant height (46.79 cm) at week 12 but was not significantly different from 0.3 % and 0.4 % concentrations (43.37 and 43.28 cm) respectively. The significantly least plant height at week 12 after planting was recorded at 0.2 % concentration (35.28 cm). No significant difference was observed among the varieties in terms of plant height. EMS concentration 0.1 % showed the significant highest number of leaves per plant at week 12 (297) while the least was recorded at 0.4 % concentration (177). Among the varieties, ICG 4412 had the significant highest number of leaves (289.20) at week 12 after planting and the least value was obtained in variety SAMNUT 26 (158.93). EMS concentration 0.1 % had the significant highest number of branches at maturity (13.25) while the least was recorded at 0.4 % EMS concentration (8.92). In terms of varietal performance, Samnut 26 had the significantly highest number of branches per plants (12.47), but was not significantly different from ICG 4412 (12.07). SAMNUT 25 was observed the have the significantly least number of branches per plant (9.13). The results obtained showed significant amount of variability in morphological parameters of the genotypes as a result of exposure to EMS which may essentially affect their productivity. This study revealed that EMS concentration 0.1 % Is the optimum concentration for inducing genetic variability in groundnut plant.

Keywords: Accessions, ethyl methyl sulfonate (EMS), genotypes, groundnut and mutagens.

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as earthnut, monkeynut and peanut is a native of South America belonging to the family Leguminosea (Fabaceae) genus *Arachis* (Tillman and Stalker, 2009). It is an annual or perennial plant that is distinguished from most other species by producing aerial flowers,fruiting below the soil level and it only domesticated species in the genus is *Arachis hypogaea* L. (Tillman and Stalker, 2009). Groundnut is grown in diverse environments throughout the world between 40 °N and 40 °S (Food and Agriculture Organization, 2013). Groundnut seeds are a rich source of oil (35–56%), protein (25–30%), carbohydrates (9.5–19.0%), minerals (P, Ca, Mg and K) and

vitamins (E, K and B) (Gulluoglu *et al*., 2016). The shells are also used for fuel by some local oil factories or they are sometimes spread on the field as a soil amendment (Ahmed *et al*., 2010).

2.0 Literature Review

Suradkar (2013) studied improvement of groundnut (*Arachis hypogaea* L.) through chemical mutagen Ethyl Methane Sulfonate (EMS) conducted a study to check the chemical mutagen (EMS) sensitivity on groundnut and to find out suitable concentration of EMS (0.05%, 0.10%, 0.15% and 0.20%) on two varieties of groundnuts TAG-24 and AK159. He concluded that 0.05% concentration of EMS induces good genetic variability in both varieties.

Mayor *et al*. (2018) studied induced chemical mutagenesis in groundnut (*Arachis hypogaea* L.) they treated groundnut variety (LGN-1) with EMS at different concentrations (0.2%, 0.4%, 0.6%, 0.8% and0.1 2%) to study it effect on various morphological traits when compared with control. Significant result were observed in seed germination, plant height, number of leaves and number of branches after treating the seed with the mutagen. They concluded that, lower concentration appear to be better effective treatment for inducing variability as compared with other concentrations (0.4%, to 0.12%).

It has also been found that boiled groundnuts have two and four fold increase in biochanin A, isoflavone antioxidants and genistein content, respectively (Craft *et al*., 2010).

3.0 Materials and methods

3.1 Experimental site

The study was conducted at the experimental garden of the Department of plant Biology, Federal University of Technology Minna Niger State, Nigeria. Minna is located in the north central geopolitical zone of Nigeria found within latitude 9°36′ north and longitude 6°34′ east. Minna covers a land area of 88 square kilometers with an estimated human population of 488,788 (Niger State MAAH Bulletine, 2008). Temperature ranges between 35.0 °C and 37.5 °C while relative humidity varies from 40 to 80 % (Adeboye *et al*., 2011). The area has two seasons: raining season between May to October and dry season between November and April each year. It has a low humid soil type with favourable climatic condition for planting which make it easy for groundnut crop to grow successfully and express all it traits.

3.2 Experimental material

The groundnut varieties used for the experiment were collected from the Institute for Agricultural Research (IAR), Samaru, Zaria, Kaduna State, Nigeria.

3.3 Mutagenic Treatments

Mutagenic treatment was conducted in the laboratory of the Department of plant Biology, Federal University of Technology Minna. Groundnut seeds were presoaked in distilled water for 4 hours. This allows the mutagen to diffuse more rapidly to the tissues of interest (Foster and Shu 2011). The seeds were then soaked for 4 hours at different concentrations of Ethyl Methane Sulphonate (0.0, 0.1, 0.2, 0.3, and 0.4%) (Adeeba *et al*., 2018). The treated seeds were thoroughly washed in running tap water to remove the residual effects of the mutagen if any.

3.4 Morphological Parameters

The morphological parameters were taken using the method of (Ghalmi *et al*., 2010) and (Falusi *et al*., 2012).

3.4.1 Plant height
The height of each plant were taking using meter rule from the base of the plant to the lowest leaf

3.4.2 Number of Leaves
The numbers of leave were counted manually

3.4.3 Leaf Area
The leaf area of the groundnut plants were calculated using the formular LXW (Length x width)

3.5 Statistical Analysis

The morphological data was subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS 20) version 20 to determine the level of significance among the treatment. Duncan Multiple Range Test (DMRT) was used to separate the means where there are significant differences.

4.0 Results and Discussion

4.1. Effect of EMS on Plant Height of the Groundnut Genotypes

Notable variations were observed in plant height at different concentration of ethyl methane sulphonate (EMS) across different weeks. At 2 and 4 weeks after planting, the highest plant height was recorded at 0.1% concentration (19.03±1.49 cm and 26.73±1.55 cm) respectively, but was not significantly different ($p>0.05$) from plant height at 0.00% concentration (17.09±0.95 and 22.68±1.27 cm), 0.3% (17.23±1.76 cm and 22.16±1.94 cm) and 0.4% (18.20±1.69 and 24.60±1.91 cm). The significantly least plant height at week 2 and 4 were observed in 0.2% EMS concentration (12.13±1.74 and 16.86±2.26 cm respectively).

In terms of varietal performance, SAMNUT 24 had the significantly highest plant height at week 2 (19.81±0.94) cm while the least was recorded in SAMNUT 26 (13.91±1.23 cm). there is no significant difference($p>0.05$) in varietal performance of SAMNUT 25 and ICG 4412 (16.06±1.79 and 17.16±1.54) cm with that of SAMNUT 24 and SAMNUT 26 at week 2. No significant difference were observed in PH at week 4, 6, 8 10 and 12 (table 4.4.1).

Table 4.1.1: Effect of EMS on Plant Height of the Groundnut Genotypes

Parameters	PH2	PH4	PH6	PH8	PH10	PH12
0.0	17.09±0.95^{b}	22.68±1.27^{b}	28.03±1.12^{b}	31.91±1.26^{ab}	35.78±1.13^{ab}	40.16±1.34^{ab}
0.1	19.03±1.49^{b}	26.73±1.55^{b}	31.48±1.51^{b}	35.52±1.44^{b}	40.61±1.61^{b}	46.79±2.12^{c}
0.2	12.13±1.74^{a}	16.86±2.26^{a}	22.49±2.50a	27.88±2.60^{a}	31.14±2.74^{a}	35.28±2.99^{a}
0.3	17.23±1.76^{b}	22.16±1.94^{b}	27.65±2.16^{ab}	33.33±2.08^{b}	37.48±1.84^{b}	43.37±1.28^{bc}
0.4	18.20±1.69^{b}	24.60±1.91^{b}	29.57±1.54^{b}	34.20±1.34^{b}	38.30±1.01^{b}	43.28±1.05^{bc}
Varieties						
Samnut26	13.91±1.23^{a}	21.68±1.75^{a}	28.20±1.52^{a}	34.04±1.23^{a}	38.21±1.21^{a}	43.74±1.24^{a}
Samnut24	19.81±0.94^{b}	25.47±1.23^{a}	30.77±1.18^{a}	34.94±1.28^{a}	39.11±1.28^{a}	44.10±1.63^{a}
Samnut25	16.06±1.79^{ab}	21.73±2.13^{a}	26.22±2.11^{a}	30.45±2.03^{a}	34.21±2.09^{a}	38.41±2.33^{a}
ICG4412	17.16±1.54^{ab}	21.53±1.89^{a}	26.19±2.00^{a}	30.83±2.01^{a}	35.12±2.03^{a}	40.85±2.09^{a}

Means followed with same letter(s) along each column are not significantly different at 5% probability level.

PH (plant height)

4.2 Effect of EMS on Number of Leaves of the Groundnut Genotypes

For number of leaves, EMS concentration 0.1 showed the significantly highest number of leaves per plant at week 2 (20.17±1.64) but was not significantly different ($p>0.05$) from 0.3 % concentration (19.33±1.54). Statistically, concentration 0.0 % was observed to have the least number of leaves at week 2 and was significantly different from other concentrations. Although, no significant difference was observed in number of leaves per plants at week 4, concentration 0.1 % recorded the significantly highest number of leaves at week 10 and 12 (231.67±30.23 and 297.33±36.80, respectively) while the least was recorded at 0.4 % concentration (149.67±14.81 and 177.00±16.91, respectively).

In terms of varietal performance, SAMNUT 24 was observed to have the significantly highest number of leaves at week 2 (20.27±1.78) while the least was recorded in SAMNUT 25 (15.07±1.05). The significantly highest number of leaves at week 4, 6, 8, 10 and 12 were recorded in ICG 4412(56.27±2.47, 157.47±12.53, 205.07±13.72, 251.73±15.43, 289.20±17.97) respectively, while the significant least were recorded in SAMNUT 26 (26.27±0.64, 43.07±2.93, 72.27±7.08, 106.53±9.52, 158.93±16.46, respectively).

Table 4.2.1: Effect of EMS on number of leaves of the Groundnut Genotypes

Treatments	NL2	NL4	NL6	NL8	NL10	NL12
0.0	13.00±0.30^{a}	31.67±4.62^{a}	83.50±23.63^{a}	128.17±27.49^{a}	174.67±30.63^{ab}	216.08±33.11^{abc}
0.1	20.17±1.64^{c}	41.33±4.29^{a}	91.33±15.45^{a}	145.67±18.40^{a}	231.67±30.23^{b}	297.33±36.80^{c}
0.2	17.17±1.62^{bc}	36.00±3.45^{a}	86.50±15.62^{a}	121.83±16.40^{a}	167.67±21.14^{ab}	206.75±21.56^{ab}
0.3	19.33±1.54^{c}	43.17±4.65^{a}	89.50±9.21^{a}	143.33±13.01^{a}	214.33±21.40^{ab}	280.08±23.89^{bc}
0.4	14.33±0.69^{ab}	37.00±3.97^{a}	74.50±12.75^{a}	113.00±13.29^{a}	149.67±14.81^{a}	177.00±16.91^{a}
Varieties						
Samnut26	16.13±1.16^{a}	26.27±0.64^{a}	43.07±2.93^{a}	72.27±7.08^{a}	106.53±9.52^{a}	158.93±16.46^{a}
Samnut24	20.27±1.78^{b}	39.20±3.73^{b}	84.80±9.13^{b}	128.53±12.63^{b}	211.87±27.75^{bc}	262.73±35.03^{b}
Samnut25	15.07±1.05^{a}	29.60±1.24^{a}	54.93±2.37^{a}	115.73±8.10^{b}	180.27±15.15^{b}	230.93±21.61^{b}
ICG4412	15.73±0.78^{a}	56.27±2.47^{c}	157.47±12.53^{c}	205.07±13.72^{c}	251.73±15.43^{c}	289.20±17.97^{b}

Means followed with same letter(s) along each column are not significantly different at 5% probability level.

NL (number of leaves)

4.3 Effect of EMS on Leaf Area and Number of Branches at Maturity

No significant differences was observed in leaf area at week 2 across all EMS concentrations and across varieties. The significant highest leaf area was observed at 0.1 % EMS concentration at week 4, 6, 8,

10 and 12 (15.71±1.02, 17.56±0.93, 19.95±0.78, 21.76±0.83, and 24.12±0.85, respectively) while the least leaf area across the weeks was observed in SAMNUT 26.

EMS concentration at 0.1 % had the significantly highest ($p<0.05$) number of branches per plant (13.25±1.02) but was not significantly different from EMS concentration 0.3 % (13.17±1.35), concentration 0.4% was observed to have the significantly least number of branches (8.92±0.56).

In terms of varietal performance, SAMNUT 26 had the significant highest number of branches per plant (12.47±0.89) but was not significantly different from ICG 4412 (12.07±0.90) while the least was observed in SAMNUT 25 (9.13±0.87), Table (4.3.1)

Table 4.3.1: Effect of EMS on Leaf Area and Number of Branches at Maturity

Treatments	LA2	LA4	LA6	LA8	LA10	LA12	NB
0.0	5.39±0.31^{a}	10.59±0.63^{a}	13.08±0.49^{a}	15.11±0.49^{a}	17.11±0.59^{a}	19.64±0.59^{a}	10.±58±1.18^{ab}
0.1	6.14±0.39^{a}	15.71±1.02^{c}	17.56±0.93^{c}	19.95±0.78^{c}	21.76±0.83^{b}	24.12±0.85^{b}	13.25±1.02^{b}
0.2	5.05±0.59^{a}	10.92±1.12^{ab}	13.09±1.14^{a}	15.21±1.14^{a}	17.72±1.25^{a}	19.96±1.32^{a}	10.25±0.91^{ab}
0.3	6.16±0.78^{a}	13.75±0.88^{bc}	16.21±0.72^{bc}	18.69±0.64^{bc}	21.09±0.71^{b}	23.30±0.79^{b}	13.17±1.35^{b}
0.4	6.28±0.60^{a}	12.56±1.27^{ab}	14.76±1.19^{ab}	17.34±1.13^{ab}	19.67±1.08^{ab}	21.78±1.02^{ab}	8.92±0.56^{a}
Varieties							
Samnut26	6.31±0.60^{a}	9.37±0.55^{a}	12.40±0.51^{a}	15.46±0.52^{a}	17.58±0.55^{a}	19.87±0.58^{a}	12.47±0.89^{b}
Samnut24	6.05±0.51^{a}	15.68±0.75^{c}	17.55±0.78^{c}	19.60±0.77^{b}	22.07±0.83^{b}	24.68±0.84^{b}	11.27±1.17^{a}
Samnut25	5.63±0.56^{a}	13.22±1.07^{b}	14.87±1.03^{b}	16.68±1.09^{a}	18.54±1.09^{a}	20.41±1.02^{a}	9.13±0.87^{a}
ICG4412	5.23±0.25^{a}	12.57±0.86^{b}	14.94±0.86^{b}	17.29±0.86^{ab}	19.68±0.81^{a}	22.07±0.79^{a}	12.07±0.90^{b}

Means followed with same letter(s) along each column are not significantly different at 5% probability level.

LA (leaf area), NB (number of branches)

Discussion

Highly efficient mutagenesis is vital for breeding programs. The success of Ethyl methyl sulfonate (EMS) mutagenesis depend on many factors such as the treatment duration, EMS concentration and temperature. (Arisha *et al*., 2014; Gnanmurthy *et al*., 2014; Asif *et al*., 2019). Decrease in plant height observed in some mutant line due increase in EMS concentration might be due to physiological or cell damage. High concentration of EMS have also been reported to result in damage to cell constituents, molecules (Chowdhury and Tah, 2011) and growth regulators (Salim *et al*., 2009). The present result

confirm these earlier reports in groundnut (Gwnasakaran and Paradai 2015); mung bean (khan and wani 2006); soybean (pavadai *et al*., 2009).

In this study significant increase in plant height of some of the EMS concentration (0.1, 0.3 and 0.4%) over the control might be due to the effect of EMS which induces point mutation in the mutant line. This result is not in line with that of mayur *et al*. (2018), who recorded highest plant height in the control than the treatments in groundnut genotypes. This might be due to differences in EMS doses. The differences in plant height among the varieties might be attributed to differences in genotypes. Similarly, previous studies reported that sensitivity to chemical mutagens differs with genotype (Kumar *et al*., 2015; Ali *et al*., 2010).

In addition, Olorunmaiye *et al*. (2019) reported significant correlation between plant height and number of pod per plant in EMS mutant line of groundnut genotype. Therefore, plant height is a good character for selection in breeding programs. The number of leaves per plant in response to different concentrations of EMS showed that lower concentration had more effect on the plant than higher concentration over the control.

Sharma *et al*. (2005) also reported that lower doses of mutagens were more efficient than the higher doses in urdbean. However, the ability of concentration range of 0.1-0.4% EMS to induce better growth and higher biological yield compared to the 0.00% treatment showed the concentrations were able to activate optimally the phyto-hormones and growth regulators in the plant.

Number of leaves associated positively with number of pod per plant. Similar observation was made by Olorunmaiye *et al*., (2019).

The larger leaf area induced by the treatment in the present study translated to higher photosynthetic surface and ultimately better growth and higher biomass yield. Growth enhancement by application of moderate concentrations of EMS was demonstrated in *Vigna spp* (Khan *et al*., 2004) and sodium azide in groundnut on Samnut 10 and 20 (Animasaun *et al*., 2014). In terms of number of branches, some of the mutant lines showed higher number of branches (13) than the control (10), this might be due to the mutagenic effect of EMS on the mutant line.

5. Conclusion

The present work showed the utility of 0.1-0.4% concentrations of EMS to significantly improve growth performance and that 0.1 % is the optimum concentration for inducing genetic variability in the groundnut genotypes.

REFERNCES

Ahmed, S., Rafay, A., Singh, R.K. & Verma, U.K. (2010). Response of groundnut varieties to spacing. *Indian Journal of Agronomy*, 31(3),248-251

Ali, H., Shah, T.M., Iqbal, N., Atta, B.M. & Haq, M.A. (2010). Mutagenic induction of double-podding trait in different genotypes of chickpea and their characterization by STMS marker. *Plant Breeding*, *129*, 116–119.

Animasaun, D. A., Oyedeji, S., Azeez, M. A. & Onasanya, O. A. (2014). 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.). *African Journal of Food, Agriculture, Nutrition and Development,* 14(7), 9498-9510.

Chowdhury, R. & Tah, J. (2011). Assessment of chemical mutagenic effects in mutation breeding programme for M1generation of carnation (*Dianthus caryophyllus*). *Research in Plant Biology,* 1(4), 23- 32.

Craft, B.D., Hargrove, J.L., Greenspan, P., Hartle, D.K., Amarowicz. R. & Pegg, R.B. (2010). Recent Advances in food and flavor chemistry. Food flavor and encapsulation, health benefits, analytical methods, and molecular biology of functional foods, Cambridge, UK: R. *Society of Chemistry* 283-296.

FAO (Food and Agriculture Organization). (2013). Groundnut Statistics. Rome: FAO: Food and Agriculture Organization of the United Nations.http://www.faostat.fao.org.

Gulluoglu, L., Basal, H., Onat, B., Kurt, C. & Arioglu, H. (2016). The effect of harvesting on some agronomic and quality characteristics of peanut grown in the Mediterranean region of Turkey. *Field Crops Resource* 21:224–232.

Gunasekaran, L. & Pavadai, P. (2015). Studies on induced physical and chemical mutagenesis in groundnut (*Arachis hypogia*) *International Letters of Natural Sciences*, 35, 25-35

Khan, S., Wani, M.R. & Parveen, K. (2004). Induced genetic variability for quantitative traits in *Vignaradiata. Pakistan Journal of Botany*, 36, 845-850

Khan, S. & Wani, M.R. (2006). MMS and SA induced genetic variability for quantitative traits in mungbean. *Indian Journal of Pulses Research*, 19(1), 50–52.

Kumar, A., Kumar, V., Lal, S.K., Jolly, M. & Sachdev, A. (2015). Influence of gamma rays and ethyl methane sulphonate (EMS) on the levels of phytic acid, raffinose family oligosaccharides and antioxidants in soybean seeds of different genotypes. *Journal Plant Biochemistry Biotechnology, 24*,204–209.

Mayur, V., Jadhav., Rahul, K., Zote. & Amol, D. (2018). Studies on Induced Chemical Mutagenesis in Groundnut (*Arachis hypogaea* L.). *International Journal of Current Microbio logy and Applied Sciences* 7(10):

Olorunmaiye, K., Stephen, G., Joseph, G., Animasaun, D. A. & Oyedeji, S. ((2019). Mutagenic Com ponents and Dosage Effects of Ethyl Methanesulphonate *Ife Journal of Science,* 21, (2)

Pavadai, P., Girija, M. & Dhanavel, D. M. (2009). Effectiveness and efficiency and biochemical content of physical and chemical mutagens in soybean (Glycine max (L.) Merr.). *Journal of Phytology*, 1 (6), 444-447.

Salim, K., Fahad, A. & Firoz, A. (2009). Sodium azide a chemical mutag en for enhancement of agronomic trait of crops plants. *Journal of Science and Technology*, 4, 1-21

Sharma, S.K., Sood, R. & Pandey, D.P. (2005). Studies on mutagen sensitivity, effectiveness and efficiency in urdbean [*Vigna mungo* (L.) Hepper]. The *Indian Journal of Genetics & Plant Br eeding,* 65(1), 20–22.

Tillman, B. L. & Stalker, H. T. (2009). Peanut: In Johann Vollmann and Istvan Rajcan Handbook of Plant Breeding, *Oil Crops* 4: 287 – 316.