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Induced Mutagenesis of Flowering, Phenology and Yield in M1 Generation of Bambara Groundnut (*Vigna Subterrnea* (L.) Verdc)

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ABSTRACT

A highly cherished Bambara groundnut variety, 'Caro' was treated with different concentrations of ethyl methane sulphonate (EMS) for different durations to raise an M1 generation. In the M1 generation, the mutagenic effect of EMS on seed germination, growth and seed yield at different doses (0.01%, 0.1%, 0.25% and 0.5%) and for different durations (6 hours, 12 hours and 24 hours) were studied. Characters like days to first flowering, days to 50% flowering, number of flowers per plant, number of leaves, number of nodes per plant, number of stems per plant, plant spread, plant height, number of branches per plant, pod length, pod width, number of pods per plant, number of seeds per plant, seed length, seed width and seed yield were measured. Data collected were subjected to analysis of variance (ANOVA) and means were separated using least significant difference (LSD). The result showed that there was significant effect ($p<0.05$) of ethyl methane sulphonate concentrations in all the parameters except on number of days to maturity. The concentration, 0.1% and soaking duration of 6 hours was more promising in creating variability. Apparently, could Ethyl methane sulphonate significantly improved the performance and create genetic variability of Bambara groundnut and therefore can be employed in breeding programme for Bambara groundnut.

KEYWORDS: *Vignasubterranea, ethyl methane sulphonate, Induced mutation*

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INTRODUCTION

Bambara groundnut (*Vignasubterranea* (L.)Verdc.) is a legume belonging to the family, Fabaceae. Its seed contains significant amounts of protein. In spite of its nutritional qualities, the crop has received limited research attention. Its improvement through conventional hybridization has not been very successful to date. This has been attributed to several inherent factors that are peculiar to the crop,but particularly the very small and delicate ground-level flowers.

In order to enhance the cultivational and nutritional qualities, modern horticultural technologies including mutation breeding could be employed (Banu*et al.*, 2005). Mutation breeding has contributed significantly to plant improvement, resulting in release of at least 2250 varieties of different crops. In India, at least 300 cultivars have been developed in at least 55 plant species (Kharkwal*et al.,* 2004).

Mutation is a heritable change in the amount, arrangement, or structure of the DNA (Baydar*et al.*, 1999). If mutations are artificially induced, it can create profound effects on quality and productivity of crops (Bedigian, 2004) and can be used for human benefit through selective breeding. Utilizing natural or induced genetic variation is a proven strategy in crop improvement (Ganesan, 1995). There are many agents both chemical and physical for inducing mutation (Ganesan, (1998). The agents that induce mutation are called mutagens.

Mutagens are not only beneficial to create genetic variability in a crop species, but also useful for the effective control of pests during post-harvest storage (Chaudhuri, 2002). EMS (Ethyl Methane Sulphonate) is a powerful mutagenic and carcinogenic organic compound. It produces only point mutation (Okagaki*et al.*, 1991). Ethyl methane sulphonate is considered a valuable tool, from which varieties could be developed that is economically important. (Jayamary*et al.*, 1995). Therefore, the present investigation was undertaken with the objective to study the effects of chemical mutagen ethyl methane sulphonatein creating variability that can be exploited to an advantage.

MATERIALS AND METHODS

Study area

The study was conducted at the Teaching and Research Farm of the Department of Crop Science, University of Nigeria, Nsukka Nigeria. It is located at Latitude 06° 52`N; Longitude 07°24`E, and altitude of 447.2 above sea level. Nsukka is in the derived savannah agro-ecological zone with vegetation predominantly of grasses interspersed with trees.

The experimental material is a popular Bambara groundnut landrace, 'Caro' which is highly cherished in Nigeria

Experimental design

The experiment was laid out in a 5 x 3 factorial in Randomized Complete Block Design (RCBD) in three replications. The factors were ethyl methane sulphonate concentrations; 0.01%, 0.1%,0.25 %, 0.5% and soaking duration; 6, 12 and 24 hours. The treatment combinations were: T_0 = control, T_1 = 0.01% 6 h, T_2 = 0.1% 6 h, T_3 = 0.25% 6 h, T_4 = 0.5% 6 h, T_5 = 0.01% 12 h and T_6 = 0.1% 12 h, T_7=0.25%12 h, T_8=0.5%12 h, T_9=0.01%24 h, T_{10}=0.1% 24 h, T_{11}=0.25% 24 h and T_{12}=0.5% 24 h). The seedlings were sown directly at a spacing of 0.5m by 1m. The following parameters were recorded: days to first flowering, days to 50% flowering, number of flowers per plant, number of leaves, number of nodes per plant, number of stems per plant, plant spread, plant height, number of branches per plant, pod length, pod width, number of pods per plant, number of seeds per plant, seed length, seed width and seed yield were measured.

Data Analysis

The data collected were subjected to Analysis of variance (ANOVA) using GenStat statistical package 12th edition. Significant differences means were separated using Least Significant Difference (LSD) at 5% level of probability.

RESULTS

Six (6) qualitative morphological traits as outlined in the descriptors for Bambara groundnut developed by the International Bambara Groundnut Network (IPGRI, IITA, BAMNET, 2000) were used to characterize the landrace *Caro*Bambara groundnut. The results as displayed in figure 1 below showing that these qualitative morphological descriptors showed a varying degree of differentiation among the Bambara groundnut land race evaluated. In fact a reasonable number of the qualitative characters showed high variability among the treated *Caro* studied. The eye pattern of the Caro showed variability of the morphological trait. The predominant ground colour of eye was the white hilum of eye with black tinge.

Leaves were all compound leaves with three leaflets (trifoliate). They were attached to the stem by the petiole which was long, erect, grooved and thickened at the base. However, leaf colour differed among the treated and ranged from light to dark green, likewise leaf veins were pigmented red or whole green. Leaves were pinnately trifoliate. Two stipels were subtend to the terminal leaflet, while only one was assigned to each of the two lateral leaflets. The oval leaflets were attached to the ranchis with marked pulvini. Four and five leaflets were observed in this study. The terminal leaflets was larger than the lateral leaflets and were observed to have slight differences in shapes; oval, lanceolate, elliptic and round.

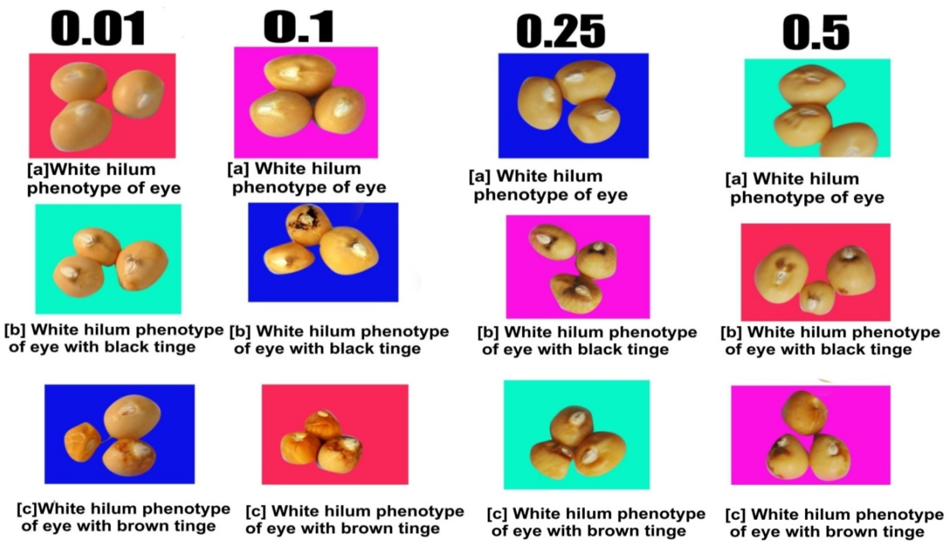
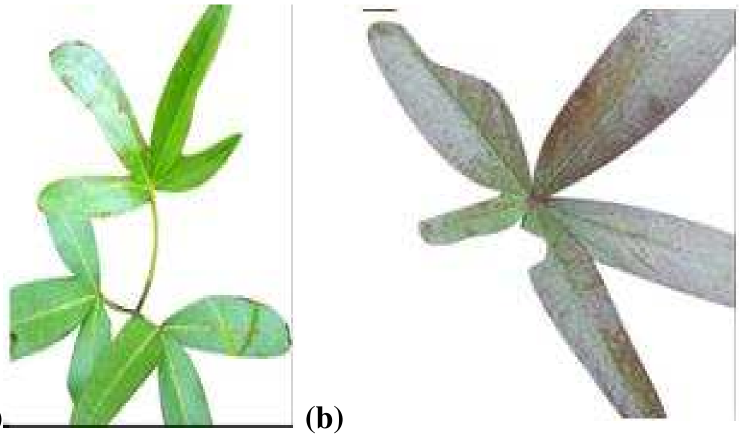


Figure 1: Testa colours of Bambara groundnuts showing variabilities



(A) (b)

Figure 2: Examples of different leaflets formed at different concentrations in EMS-induced bambara groundnut population. Selected mutant leaflets were presented to illustrate the diversity mutations observed from this Bambara groundnut mutagenized population.
A = Four leaflets B = Five leaflets

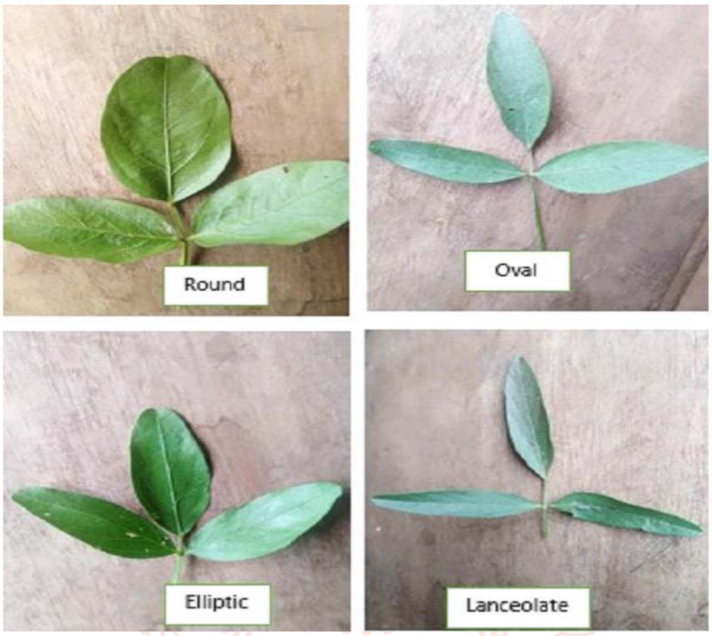


Figure 3: Mutants of terminal leaflet shape induced by ethyl methane sulphonate.
A = Round B = Oval C = Elliptic D = Lanceolate

Result presented in Table 1 showed that ethyl methane sulphonate concentrations had significant effect ($p<0.05$) on the days to first flowering, 50% flowering, number of flowers per plant, number of pods and reproductive efficiency. However there were no significant differences ($p>0.05$) on number of aborted pods. 0.5% concentration of EMS had the highest number of flowers (16) per plant and least number of aborted pods (1) per plant. Similarly 0.25% concentration of EMS recorded the highest reproductive efficiency (82). However in other parameters (days to first flowering, days to 50% flowering and number of pods per plant) control had the best result of 40 days, 34 days and 7 in that order.

Table1: Effect of different concentrations of ethyl methane sulphonate in M_1 generation on first flowering, 50% flowering, number of flowers per plant, number of pods per plant, number of aborted pods and reproductive efficiency

Concentrations of mutagen	Days to first flowering	Days to 50% flowering	Number of flowers per plant	Number of pods per plant	Number of aborted pods	Reproductive efficiency
0.01	42.00	38.16	9.08	8.49	0.59	93.50
0.1	46.05	41.82	7.15	4.63	2.52	64.76
0.25	50.04	45.08	7.33	6.02	1.31	82.13
0.5	52.40	41.40	16.20	2.27	13.93	14.01
Control	33.93	34.20	11.20	6.67	4.53	59.55
LSD(0.05)	0.61	0.55	0.52	0.35	19.26	62.79

EMS concentrations had significant effect ($p<0.05$) on number of branches, number of leaves, nodes, plant height, plant spread, number of stems, number of pods per plant, pod length and pod width, (Table 2). Concentration 0.01% caused highest number of leaves while concentration 0.5% caused the least number of leaves. 0.1% concentration of EMS resulted to the highest number of nodes per plant while 0.25% concentration resulted to the least number of nodes per plant. Control obtained the highest number of stems per plant while 0.5% concentration resulted to the least number of stems per plant. Concentration 0.01% had the highest plant spread and highest plant height while 0.5% concentration recorded the least plant spread and least plant height. Concentration 0.01% triggered the highest number of branches per plant and highest pod width while 0.25% concentration produced the least number of branches per plant and the least pod width.

Table 2: Effect of ethyl methane sulphonate concentrations in M_1 generation on the number of branches per plant, number of leaves, number of nodes per plant, plant height, plant spread and number of stems per plant, pod length and pod width

Concentrations of mutagen	Number of branches per plant	N umber of leaves per plant	Number of nodes per plant	Plant heigh t (cm)	Plant spread (cm)	Number of stems per plant	Pod length (cm)	Pod width (cm)
0.01	5.03	41.74	5.22	25.31	16.00	23.42	2.15	0.62
0.1	4.24	15.88	6.19	22.07	6.80	18.69	2.15	0.57
0.25	4.15	27.42	5.14	20.34	8.21	20.13	1.54	0.54
0.5	4.53	15.83	5.20	15.13	6.02	15.47	1.58	0.56
Control	4.87	40.60	5.93	24.64	12.15	30.00	2.17	0.58
LSD (0.05)	0.35	0.67	0.33	0.18	0.06	0.65	0..03	0.02

Table 3 showed that the different concentrations of EMS showed significant ($p<0.05$) effect on seed length, seed width and seed yield but no significant ($p>0.05$) effect on number of seeds per plant. The highest number of seeds was observed for 0.5% concentration and control. Concentration 0.1% caused the least number of seeds. Control had the longest seed length while concentration 0.1% had the shortest seed length. Concentration 0.5% caused the highest seed width while 0.1% concentration and control had the least seed width. While 0.1% concentration of EMS caused the highest seed yield, the least yield was observed for 0.25% concentration.

Table 3: Effect of ethyl methane sulphonate concentrations in M_1 generation on the number of seeds per plant, seed length, seed width and seed yield

Concentrations of mutagen	Number of seeds per plant	Seed length (cm)	Seed width (cm)	Seed yield/(g)
0.01	1.16	1.70	0.68	9.41
0.1	0.97	1.53	0.59	43.54
0.25	1.10	1.54	0.63	6.33
0.5	1.33	1.61	0.69	10.51
control	1.33	1.71	0.59	9.15
LSD (0.05)	ns	0.04	0.04	0.08

ns = not significant

The result in Table 4 showed that soaking duration in ethyl methane sulphonate had significant effect ($p<0.05$) on days to first flowering, 50% flowering, number of flowers per plant, number of pods, number of aborted pods and reproductive efficiency. Soaking duration of 6 hours triggered early flowering in Bambara groundnut while soaking duration of 12 hours resulted in late flowering. Similarly 12 hours soaking duration caused the longest days to 50% flowering while soaking duration of 24 hours caused the shortest days to 50% flowering. The soaking duration that recorded the highest number of flowers per plant was 24 hours while the least was obtained for 12 hours soaking durations. Soaking duration of 6 hours caused the highest number of pods per plant while soaking duration of 12 hours caused the least number of pods per plant. Similarly soaking duration of 24 hours produced the highest number of aborted pods while 12 hours soaking duration had the least. The highest reproductive efficiency was obtained for 6 hours soaking duration while soaking duration of 24 hours caused the least reproductive efficiency.

Table 4: Effect of soaking durations in ethyl methane sulphonate on the days to first flowering, 50% flowering, number of flowers per plant, number of pods per plant, number of aborted pods per plant and Reproductive efficiency

Soaking durations of mutagen	Days to first flowering	Days to 50% flowering	Number of flowers per plant	Number of pods per plant	Number of aborted pods	Reproductive efficiency
6	37.84	38.00	7.00	4.84	2.16	144.62
12	49.14	50.13	4.34	3.26	1.08	133.12
24	47.68	32.27	22.48	5.50	16.98	24.46
LSD(0.05)	0.47	0.42	0.40	0.27	8.90	100.73

Soaking durations had significant effect ($p<0.05$) on number of leaves, nodes per plant, plant spread, plant height and number of branches, pod length, pod width and number of pods per plant (Table 5). Soaking duration of 6 hours caused the highest number of leaves while the least number of leaves was obtained for soaking duration of 24 hours. The highest number of nodes and the highest number of stems per plant was observed for 24 hours soaking duration and 6 hours soaking duration respectively. Soaking duration of 12 hours caused the least number of stems per plant. Soaking duration of 12 hours resulted to the highest plant spread while soaking duration of 24 hours caused the least plant spread. Soaking duration of 6 hours produced highest plant height while soaking duration of 24 hours caused the least plant height. The highest and the least number of branches were obtained for 24 and 12 hours of soaking duration respectively. Similarly soaking duration of 6 hours and 24 hours resulted in the highest pod length and the least pod length respectively. Soaking duration of 12 hours caused highest pod width while soaking duration of 6 hours caused the least pod width.

Table 5: Effect of soaking duration in ethyl methane sulphonate on the number of branches, number of leaves, number of nodes, plant height, plant spread, number of stems, number of pods, pod length and pod width

Soaking durations of mutagen	Number of branches per plant	Number of leaves per plant	Number of nodes per plant	Plant height (cm)	Plant spread (cm)	Number of stems per plant	Pod length (cm)	Pod width (cm)
6	4.52	34.92	5.28	26.44	10.31	23.24	1.93	0.54
12	4.31	25.28	5.49	23.24	11.40	19.87	1.92	0.63
24	4.85	24.69	5.84	14.80	7.80	21.51	1.90	0.55
LSD(0.05)	0.27	0.52	0.25	0.14	0.05	0.50	0.03	0.02

Significant differences ($p<0.05$) were observed on the effect of soaking durations on seed length, seed width and seed yield. On the contrary no significant ($p>0.05$) effect was observed on number of seeds per plant (Table 6). Soaking duration of 6 hours caused the highest number of seeds per plant while soaking duration of 12 hours caused the least number of seeds per plant. Similarly 12 hours soaking duration had the highest seed length as against 6 hours soaking duration that had the least seed length. The highest and the least seed width were observed for soaking duration of 12 and 6 hours respectively Soaking duration of 6 hours resulted to the highest seed yield while 12 hours of soaking caused the least seed yield.

Table 6: Effect of soaking duration in ethyl methane sulphonate on the number of seeds per plant, seed length, seed width and seed yield

Soaking durations of mutagen	Number of seeds per plant	Seed length (cm)	Seed width (cm)	Seed yield/(g)
6	1.24	1.59	0.54	25.66
12	1.13	1.65	0.69	10.83
24	1.17	1.61	0.68	10.88
LSD (0.05)	ns	0.03	0.03	0.07

ns = not significant

DISCUSSION

Seeds treated with ethyl methane sulphonate displayed a variety of potential mutations in this study. This may be due to the pleiotropic impact of mutated genes (Muhammad *et al*., 2021).

Out of the nine qualitative morphological descriptors used in this study, the highest variability was observed for hilum phenotype of eye. Other qualitative morphological features that showed some variation among the Bambara groundnut Caro were leaflet shape. These results have some implication in breeding and selection programmes for crop improvement in Bambara groundnut. It has been reported that selection is effective only when significant genetic variability exits in high frequency among the genotypes (Adebisi*et al*., 2001; Nwakuche*et al*., 2019). Several other works have reported substantial level of variation in qualitative morphological characters in Bambara groundnut (Mohammed, 2014; Siise and Massawe, 2013; Nwakuche*et al*., 2019).

In this study the result on number of days to first flowering ranged from 33 to 52. These figures of days to first flowering were within the range of the result of days to flowering without treating the seed with ethyl methane sulphonate. This is contrary to the report of a previous study (Ouedraogo*et al*., 2008).they reported that flowering in Bambara groundnut ranged from 32 to 42 days after sowing. Seemingly these values were similar, only that in the present investigation higher number of days to first flowering was recorded. The reason the number of days to flowering was between 33 to 52 days is because EMS enhances genetic variability. The variation also of the days to flowering among the mutant might be due to genotypic factor as well as the environmental conditions that prevailed during the crop growth period (Shegro*et al.,*2013). Swanevelder (1997) also reported several variations in days to flowering among Bambara groundnut accessions. Similar result was reported in Sesame, Cowpea and Mungbean (Menash*et al*., (2007), Pavadai and Dhanavel (2004), Khan and Wani (2005).

Concentrations had significant effect on number of leaves, nodes, plant spread, plant height and number of branches, number of pods per plant, pod length and pod width. The reason concentration of ethyl methane sulphonate produced better result than control is because Ethyl methane sulphonate significantly improved the performance and create variability of the Bambara groundnut. This is in agreement with the results reported by other workers in Sesame (Prabhakar, 1998; Rajathi, 2008) and in Cowpea (Banu*et al*., 2005). Similar results were also observed in Soybean (Cheng and Chandlee, 1999) and in other plants like black gram.

The number of pods obtained in this study is higher in some other studies and similar in some other studies. The reason for higher number of pods in this study was due to lower concentration and soaking duration of EMS. From this result obtained, plant height treated with lower concentration of EMS recorded significant increase. EMS has been shown to be the most potent of the chemical mutagen and alkylating agent (Muhammad *et al*., 2021). This finding agrees with Mensah*et al*., (1992) working on the mutagenic effects of hydroxylamine and streptomycin on growth and seed yield of cowpea (*Vignaunguiculata* (L.) Who reported that, the lower concentration had higher shoot length than higher concentration had higher shoot-length. This also agrees with work done by Ajayi*et al.,* (2010) who reported reduced variability as the level of EMS increases.

Number of pods per plant reported is contrary to the values reported by Masindeni (2006). The observation is similar with the past results from Stoilova*et al*. (2013).

Concentrations obtained significant effect on seed length, width and seed yield but no significant ($p>0.05$) effect on number of seeds per plant. The reason concentration of ethyl methane sulphonate produced better result than control is because Ethyl methane sulphonate significantly improvedthe performance and create variability of the Bambara groundnut. Such observations were reported by previous workers in soybean (Dhole *et al*., 2003; Pavadai and Dhanavel, 2004 and 2005; Pavadai, 2006). Similar observations were also made in other plants like black gram (Arulbalachandran, 2006), Cowpea (Odeigah*et al*., 1998) and Sesame (Sengupta and Datta, 2004).

The result on number of seeds per plant was low and in the same range with other studies because higher dosage and treatment combinations for the EMS treated plants showed higher death. The result on number of seeds per plant was similar to others such as Berchie*et al*. (2012) and Mabhaudi*et al*. (2013) which all reported reduced seed yield in Bambara groundnut landraces in response to limited water availability under field conditions. On the contrary seed yield result of this study was contrary to report of Nura*et al*. (2013) on the mutagenic effect of colchicine on Pigeon peas (*Sesamumindicum*L.). In the EMS-treated plants, higher dose and treatment period combinations resulted in higher death and lower yield in the plant attributes. Similar findings were made with EMS-treated fenugreek seeds, where no callus cultures developed when treated with EMS concentrations greater than 1% (Muhammad *et al*., 2021).

Soaking durations had significant effect on the number of days to first flowering, 50% flowering, number of flowers per plant, internode length, petiole length, peduncle length, terminal leafletlengthand terminal leaflet width and no significant effect ($p>0.05$) days to maturity. The reason for the significant is because the shorter the soaking duration, the more genetic variability of ethyl methane sulphonate on the parameter measured. This is in agreement with Adebayo (2014), who reported increase in protein, ash, fat content of lima bean that were soaked for 12hr, 36hr and 24hr respectively. This finding implies that the mineral contents of the samples decreased as the soaking time progressed.

Soaking duration had significant effect ($p<0.05$) on number of leaves, nodes, plant spread, plant height and number of branches, number of pods per plant, pod length and pod width. The reason is that the lower the soaking duration, the more variability obtained on parameter measured which is in agreement with Adebayo (2014) who observed significant difference in the moisture content of all the samples studied, the moisture content of samples soaked for 6hr. The result of the effect of soaking time decreased with increasing soaking time was reported by (Obizoba and Atii, 1991) in sorghum and (Adebayo, 2014) lima bean respectively on the parameter measured. This finding was not surprising because the lower the soaking time, the more variation. Obasi and Wogu (2008) reported similar finding in a work they carried out on maize. Such observations were also reported by previous workers in Soybean (Dhole *et al*., 2003; Pavadai, 2006) and sesame (Sengupta and Datta, 2004). Results reported in Sesame (Mensah*et al*., 2007) and *Trigonellafoenumfraeum*(Datta and Biswa, 1988) showed significant difference in the number of leaves per plant. The same researches on Sesame (Mensah*et al*., 2007) and *Trigonellafoenumfraeum*in number of branches per plant showed significant difference. Number of pods per plant reported was contrary to the values reported by Masindeni (2006).

Soaking duration obtained significant ($p<0.05$) effect on seed length, width and seed yield but no significant ($p>0.05$) effect on number of seeds per plant. This revealed that variations existed among the treated Caro in respect to morphological traits studied might be due to ethyl methane sulphonate used.

The number of seeds per plant result is contrary to that of Nura*et al*. (2013) who studied the mutagenic effect of colchicine on pigeon peas (*Sesamumindicum*L.). These results are similar to others such as Berchie*et al*. (2012) and Mabhaudi*et al*. (2013) which all reported reduced seed yield in Bambara groundnut landraces in response to limited water availability under field conditions. Seed yield results is also contrary to the reported result by Rangaswamy, 1973 in sesame; soybean in Pavadai and Dhanavel, 2004; cowpea in Banu*et al*., 2005.

CONCLUSION

The use of chemical mutagen in crop improvements has been reported in a number of species (Ojomo and Chheda1972, Biswas andDutta 1988). In this study, Ethyl methane sulphonate were observed to cause variation in most traits in the *Caro* landrace Bambara groundnut studied. Concentration 0.1%- and 6-hours' time of exposure to EMS performed best for most traits. In general, the dose of a chemical mutagenic treatment comprises several parameters, of which the most important are concentrations, duration of treatments and temperature during treatment (Udensi and Ontui, 2013).

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