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Preliminary Study on the Effect of Different Concentrations of EMS on Two Pigeon Pea (*Cajanus cajan* L. Millsp.) Accessions

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ABSTRACT: In the present study, seeds of Pigeon pea (*Cajanus cajan* L. Millspaugh) accessions (Brown and Cream colours) were treated with different concentrations of Ethyl methane sulphonate (0.0 %, 0.01 %, 0.02 %, 0.03 %, 0.04 %, 0.05 %, 0.06 % and 0.07 %). The seeds were sown and monitored for 30 days. Data obtained showed a trendy effect. Data obtained showed that germination percentage was decreased with an increase in the mutagen concentration when compared with the control. The result also showed that the survival percentage of the plants reduced with an increase in the concentration of the mutagen. The lethal dose (LD_{50}) was determined upon germination and survival of the pigeon pea accessions. The result showed that 50 % germination was recorded under treatment 0.05 % EMS in the brown colour accession and 0.04 % EMS in the Cream colour. These concentrations are therefore considered as the LD_{50} values.

Keywords: Pigeon pea, EMS, Germination, Survival, Lethal dose

Introduction

Pigeon pea (*Cajanus cajan* Linn.) has been ranked by FAOSAT (2021) as the sixth most important legume crop globally. The crop has been reported to be cultivated in some countries of Africa, Asia and the Caribbean. Though the pigeon pea is a cultivated crop in West Africa, it, however, plays a crucial role in the livelihood of subsistence farmers in Ghana and Nigeria (Dansi *et al*., 2012, Ayenan *et al*., 2017). Odeny (2007) has reported the high tolerance of the crop to drought stress. It also has high biomass productivity and provides needed nutrients and moisture to the soil. Pigeon pea is self-pollinating, therefore natural outcrossing is limited and the existing gene pool is not enough for the improvement of the agronomic traits of the crop.

With the global climate changing and increased incidence of drought, *Cajanus cajan* offers resilience to cropping systems (Khoury *et al*., 2015). This is expected to expand the cultivation of the crop to new areas. However, pigeon pea has tremendous untapped potential for the improvement of the quantity and quality of production in Africa. Changes in climatic patterns are becoming more unpredictable, there is, therefore, a need for resistant and adaptive varieties to be developed regularly for sustainable production. An induced mutation is important for developing genetic diversity in breeding programs. The rate of occurrence of spontaneous mutation is very low and the variations resulting from these are not enough to be exploited for breeding Therefore, artificial mutations are induced using chemical or physical mutagens (Chaudhary *et al*., 2019).

Kharkwal and Shu (2010) have stressed that induced mutations play an important role in enhancing world food security. The mutagenic effect of EMS has been extensively studied in many plants. Applying different concentrations of EMS, the frequency and saturation of mutations can be regulated. Mutagenic agents can cause an induction by inducing different extensions of genomic lesions which range from base mutations to insertions or deletion of larger fragments (Menda *et al.*, 2004, Kim *et al*., 2006). A dose-response curve needs to be established when the optimal dose of a chemical mutagen for a specific crop or target tissue has not earlier been established. This study, therefore, is aimed at establishing the concentrations of EMS needed for mutation induction in two pigeon pea accessions found in Eastern Nigeria.

Materials and Methods

The study was at the screen house of Michael Okpara University of Agriculture, Umudike. Umudike lies on latitude 05^o 291 N and longitude 07^o 331 E in the rainforest area of the South-East agricultural zone of Nigeria. The area covers about 100,000 m^2 and lies about 8 to 10 kilometres east of Umuahia, the Abia State capital. It has a humid tropical climate with marked wet and dry seasons. The rainy season spans eight months (from March to October) and the dry season starts from November to February. The average annual rainfall for Umudike ranges from 1568.4 mm to 2601.3 mm within a ten (10) years period. The rainy season has its peaks occurring irregularly between June and October (Agroclimatic Data, 2007). Two landraces of pigeon pea were collected from Nsukka, Enugu State. The seeds were identified at the taxonomic unit of the Department of Plant Science and Biotechnology, MOUAU. The identification was done based on the colour of the seeds.

Topsoil was collected within the University premises and was sieved using a mesh to remove dirt and other particles which might inhibit the plant growth. A total of 48 planting bags were filled with the top and arranged with proper tagging according to the different treatment levels. The bags were perforated for draining water during irrigation. The experiment was set up in a complete randomized design (CRD) with three replications.

Seed treatments with EMS were done at the postgraduate laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. A total of 700 healthy seeds were selected in each of the pigeon pea accessions. One hundred seeds were selected into seven groups and put into a 250 ml beaker. The first group served as the control whereas the six other groups represented the various sodium azide concentrations. Distilled water (200 ml) was added to the beakers containing the seeds, respectively. Pre-soaking of the seed was done for 4 hours. The seeds were removed from the water and could air dry for 20 minutes.

Glass beakers of 500 ml were selected and washed under running tap water. The beakers were properly labelled and arranged. Different concentrations (0.00 %, 0.01 %, 0.02 %, 0.03 %, 0.04 %, 0.05 %, 0.06 % and 0.07 % w/v) were freshly prepared in buffer 7 according to the method of Mba *et al.* (2010). After the preparations, the pre-soaked seeds were soaked into the freshly prepared concentrations for 6 hours with intermittent shaken to ensure uniformity. After the treatment time, the seeds were removed from the solutions and washed thoroughly in running tap water five times to remove the residual chemicals on the seeds. The seeds were air-dried for 30 minutes before sowing. Sowing was done immediately.

Data collection

Germination percentage: Germination was observed in all the treatments for 14 days. The number of germinated plants in each treatment was counted after 14 days for the germination percentage. It was calculated by the total number of germinated seeds divided by the total number of seeds sown. Germination percentage was calculated using the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Survival percentage: The number of surviving plants in each treatment was counted at 30 days after sowing for the survival percentage. It was calculated by the total number of survival plants by the total number of seeds sown.

$$\text{Survival percentage (\%)} = \frac{\text{Total number of survival seeds}}{\text{Total number seeds sown}} \times 100$$

Lethal Dose (LD_{50}): The number of surviving plants in each treatment was counted 30 days after sowing for LD50. It was calculated by the total number of germinating seeds by the total number of survival seeds.

$$LD_{50}\ (\%) = \frac{\text{Total number of survival plants}}{\text{Total number of germinated seeds}} \times 100$$

Results

Effect of different concentrations of EMS on the germination percentage: Table 1 shows the result of the effect of the different concentrations of EMS on the germination percentage of the two pigeon pea accessions. The result obtained showed that the mutagen concentrations affected the germination percentages of the two pigeon pea accessions. The result showed a gradual decrease in germination with an increase in the concentration of the mutagen. Regarding treatments, germination percentage varied among the pigeon pea accessions. Data obtained on the Brown colour accession showed that germination percentage ranged from 93.3 % to 37.3 %. The maximum germination percentage was counted in the control (100.0 %). Treatment 0.01 % EMS counted 93.3 % followed by 0.02 % (81.0 %) while 0.03 % counted 69.7 %. The minimum germination percentage was counted in the highest concentration (0.07 % EMS) with 37.3 % germination.
The result obtained on the Cream colour also followed a similar trend. The maximum germination was recorded in the control (98.3 %). Treatment 0.01 % recorded 91.3 %. 0.02 % counted 83.7 %, 0.03 % recorded 72.3 % while 0.04 % counted 56.3 5 germination, respectively. The minimum germination percentage was counted in 0.07 % EMS (37.0 %). The result obtained revealed a decreasing effect of the mutagen concentrations on the germination percentage of the pigeon pea accessions. Analysis of variance showed that the effect of the mutagen concentrations on the germination percentage of the pigeon pea accession was highly significant ($p>0.05$). The accession was very significant ($p>0.05$) while the interaction was not significantly different ($p>0.05$) as shown in Table 1.

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

Accession	Conc. (%)	Germination (%)
Brown colour	0.00	100.0
	0.01	93.3
	0.02	81.0
	0.03	69.7
	0.04	58.7
	0.05	50.7
	0.06	45.7
	0.07	37.3
	Total	67.0
Cream colour	0.0	98.3
	0.01	91.3
	0.02	83.7
	0.03	72.3
	0.04	56.3
	0.05	49.0
	0. 06	44.0
	0.07	37.0
	Total	66.5
$LSD_{(0.05)}$ Accession		**
$LSD_{(0.05)}$ Conc.		***
$LSD_{(0.05)}$ inter.		NS

Effect of different concentrations of EMS on the survival percentage of the two pigeon pea accessions: The result of the effect of the different concentrations of EMS on the survival percentage is presented in Table 2. Plant survival was monitored for 30 days in each of the accessions and the average percentage was recorded. The result obtained indicated a decrease in survival percentage with increased mutagen concentrations. The survival of the seedlings was dependent on the concentration of the mutagen in the two pigeon pea accessions. Among the various treatment levels, the control showed the highest survival percentage. In the Brown colour accessions, the control had the maximum survival percentage (99.7 %) followed by the lowest mutagen concentration (0.01 %) with an 81.0 % survival percentage. Treatment 0.02 %, 0.03 % and 0.04 % counted 63.7 %, 44.0 % and 34.7 % respectively. The minimum survival percentage was 0.07 % (14.0 %). The result showed that the survival percentage was drastically reduced with an increase in the mutagen concentration.
Similarly, in the cream colour accession, the control recorded the highest survival percentage (97.7 %) followed by the lowest mutagen concentration (0.01 % EMS) with 79.9 % survival, the treatment 0.02 % recorded 68.3 % while 0.03 % was 42.3 % respectively. The result also showed that the highest mutagen concentration (0.07 %) recorded the minimum survival percentage (15.0 %). The result clearly showed that EMS concentrations

significantly reduced the survival percentages of the pigeon pea accessions. The effect of the mutagen concentrations revealed a gradual decrease with an increase in concentration. The result of the analysis of variance showed that survival percentage was significantly different ($p>0.05$) with accessions. A highly significant difference ($p>0.05$) was observed in the concentration while interaction (accession and concentration) was highly significantly different ($p>0.05$)

Table 2: Effect of different concentrations of EMS on survival percentage

Accession	Conc. (%)	Survival (%)
Brown	0.0	99.7
	0.01	81.0
	0.02	63.7
	0.03	44.0
	0.04	34.7
	0.05	25.0
	0.06	21.0
	0.07	14.0
	Total	47.9
Cream	0.0	97.7
	0.01	79.7
	0.02	68.3
	0.03	42.3
	0.04	30.3
	0.05	22.0
	0.06	19.0
	0.07	15.0
	Total	46.8
LSD(0.05) Accession		***
LSD(0.05) Conc.		***
LSD(0.05) inter.		***

Effect of different concentrations of EMS on the lethal dose (LD_{50}): Data on the effect of EMS concentrations on the LD_{50} in the pigeon pea accessions are presented in Table 3, Fig 1 and Fig 2. The data revealed that LD_{50} differed significantly with the pigeon pea accessions by mutagen treatments. The interaction effect between pigeon pea accessions and treatments was observed non-significant. From the result obtained, lethality increased with an increase in the mutagen concentration (Fig. 1 and 2). Data obtained showed that minimum lethality was recorded in the control. The result recorded in the Brown colour showed that the control recorded 100.0 % lethality as minimum lethality followed by treatment 0.01 % EMS (86.8 %) and 0.02 % EMS (78.6 %) while 0.03 %, 0.04 % and 0.05 % recorded 63.2 %, 59.9 % and 50.8 % mortality, respectively. Maximum mortality was recorded in treatment 0.07 % (37.6 %). The optimum dose was recorded between 0.01 % to 0.05 % EMS in the Brown colour accession. The treatment above 0.05 % recorded very poor germination percentage and observed more mortality. Hence, the LD_{50} in the Brown colour was recorded in 0.05 % EMS (Fig 1).

The Cream colour also recorded very high mortality with an increase in the mutagen concentration. The minimum mortality was recorded in the control (99.3 %). Treatment 0.01 % recorded 87.2 % mortality followed by 0.02 % EMS (79.9 %). Mortality was recorded maximum in 0.07 % EMS (38.8 %). The result showed that optimum dose was recorded between treatments 0.01 % to 0.04 % EMS. From the result, it was observed that treatments above 0.04 % increased mortality.

Table 3: Effect of different concentrations of EMS on the lethal dose (LD_{50})

Accession	Conc. (%)	LD_{50} (%)
Brown colour	0.0	100.0
	0.01	86.8
	0.02	78.6
	0.03	63.2
	0.04	59.9
	0.05	50.8
	0.06	46.0
	0.07	37.6
	Total	65.4
Cream colour	0.0	99.3
	0.01	87.2
	0.02	79.9
	0.03	58.6
	0.04	54.0
	0.05	46.3
	0.06	41.9
	0.07	38.8
	Total	63.3
LSD(0.05) Acc.		**
LSD(0.05) Conc.		***
LSD(0.05)Inter.		NS

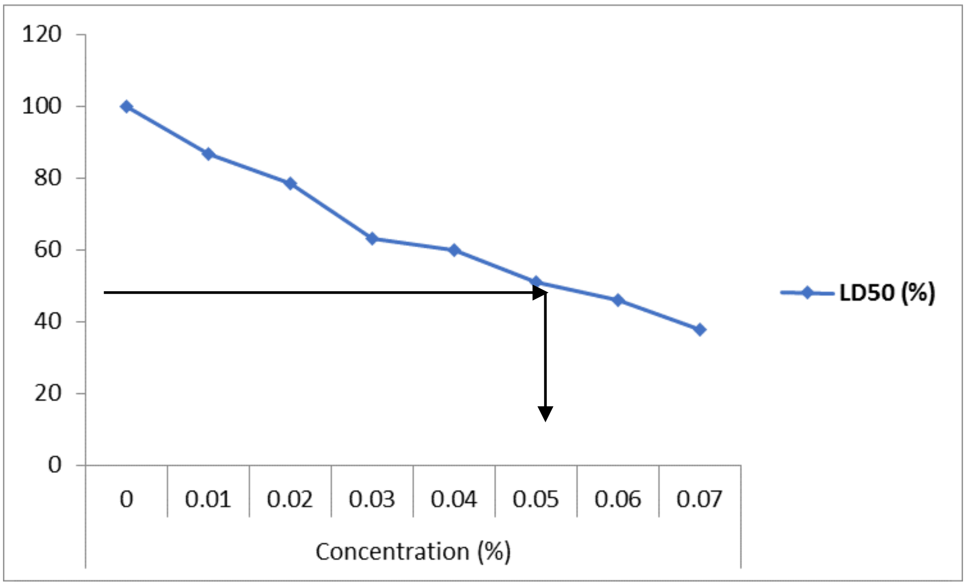

Figure 1: Effect of different concentrations of EMS on the LD_{50} of Brown colour accession

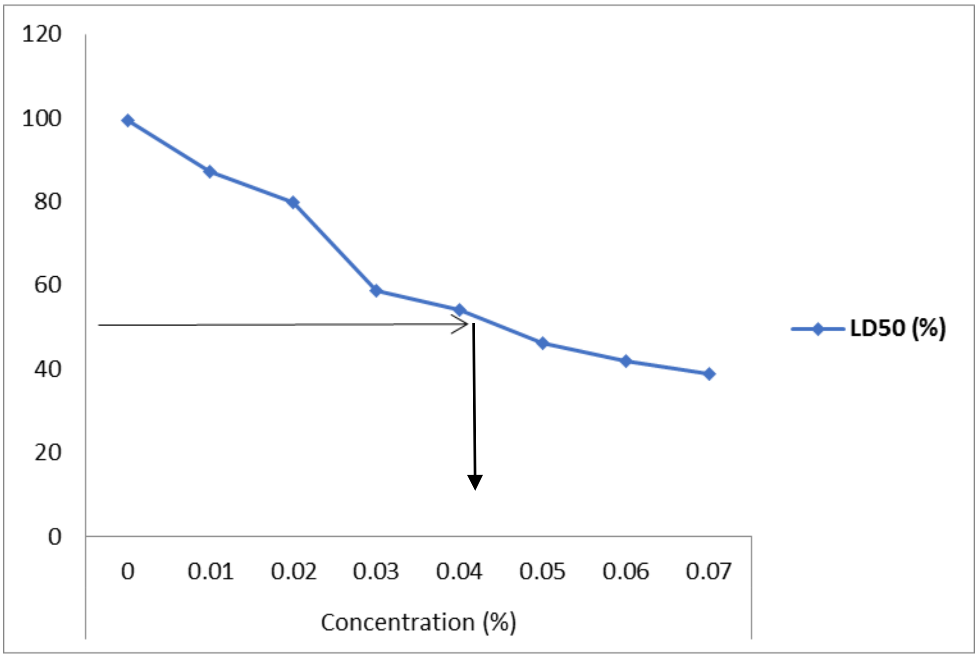

Figure 2: Effect of different concentrations of EMS on the LD_{50} of Cream colour accession.

Discussion

The mutagenic effect of chemical mutagens on seed germination and survival percentages is well documented. Many workers have reported a negative effect of chemical mutagens on the germination and survival of crop plants. The findings from this study have further added to the existing literature on the effect of mutagen on seed germination as well as the survival percentage of crop plants. The result from the study revealed that the mutagen concentrations gradually decreased the germination and survival percentages with an increase in concentrations. The result however showed a trendy effect, higher concentrations of the mutagen caused a severe reduction in seed germination and survival of the plants. These observations conform to the findings of Ariraman *et al*. (2014) who reported a decrease in germination and survival percentages of pigeon pea treated with different EMS concentrations. Similarly, the findings of the study are further supported by the report of Baghery *et al.* (2015) who reported a gradual decrease in seed germination and survival percentage of okra seeds treated with EMS concentrations. The seed germination exhibits a sharp-concentration relationship which decreased with an increase in the mutagen concentration. The reduction in germination and survival observed in the study could be attributed to the action of the mutagen on the seed metabolic processes which resulted in disturbances at the physiological and cellular levels of the seeds. Markeen *et al*. (2013) opined that the inhibition of respiratory disruption of enzyme formation and utilization of enzymes are common abilities of mutagens. This probably might explain the reduction in seed germination and survival percentages observed in the pigeon peas with increased concentrations of the mutagen. Liamngee *et al*. (2017) reported that with an increase in sodium azide concentration, the mutagen was able to inhibit the energy supply and utilization, disrupt enzymatic activities thus preventing essential processes that can result in the death of the embryo. The decrease in seed germination and survival percentages observed in this study is further supported by the work of Gunasekaran and Pavada (2015) in groundnut; Raina *et al*. (2018) in cowpea varieties; Anbarasan *et al*. (2013) in *Sesamum*; Kumar and Mishra (2004) in okra seeds.

The determination of dose for chemical and physical mutagens is often made by varying the concentration and duration of treatment as well as maintaining the pH of the solution. To obtain a mutant, the dose of the mutagen should be sufficiently high to increase the possibility of inducing mutation, however, the concentration should not be so high as to cause damage to the cells and tissues which might result in lethality. LD_{50} generally varies with plant species, the type and status of the plant material and the stage at which lethality is measured. This is evident from the result of this study.

The lethal dose (LD_{50}) in this study was determined based upon germination and survival percentages. The result from the study showed that mortality increased with an increased in mutagen concentration. Observations from this study also support the reports on the effect of mutagens on the mortality curves of plant species as the

LD_{50} of the pigeon peas studied varied respectively. The estimation of LD_{50} values showed accession lethality towards mutagen concentration increase in both pigeon peas. The lethal dose values observed were found to be below 50 % from 0.05 % EMS and 0.04 % EMS in both pigeon pea accessions respectively. These concentrations were considered as the LD_{50} of the pigeon peas. This observation agrees with the report of Ariraman *et al*. (2014) who reported an LD_{50} value of 25 mM in pigeon pea treated with different concentrations of EMS. Also, Gunasekaran and Pavadai (2015) reported that the LD_{50} value was observed in 0.5 % EMS and 0.4 % DES respectively in groundnut varieties. The difference in response of the pigeon peas to the mutagen concentrations observed in this study is in strong agreement with the report of Laskar *et al.* (2017) who posited that the LD_{50} value varies according to crop species, varieties, seeds or other plant materials, nature of the treatment, method of raising, cultural practices and other parameters.
Since the mutagen concentration interacted differently with the pigeon pea accessions, it is pertinent to keep in view the appropriate LD_{50} value as it could be useful in inducing a wide range of desirable mutations in the agronomic traits/qualities of crops.

Conclusion

One of the most major requirements for mutation induction is the selection of an efficient dose of the mutagenic agent for mutating the starting material. Generally, an increase in the concentration of EMS results in enhancing mutation but causes proportionally even greater seedling damage which results in a decrease in survival. The study showed that seed germination percentage was inhibited with the increase in the concentrations of mutagen. The survival rate was highly reduced with increasing the concentration of mutagen. The result of this study has added to the available literature and could serve as a guide to future researchers.

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