TAS Trends in Agricultural Sciences

OPEN ACCESS

Qualitative Mutations Induced by Ethyl Methane Sulphonate in *Phaseolus lunatus* L.

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ABSTRACT

Background and Objective: *Phaseolus lunatus* commonly known as lima bean is an underutilized crop in Nigeria, which possess the potential to serve as an alternative source of protein supply but yet no considerable efforts have been made in time past towards the genetic improvement of this crop. This study was undertaken to assess the efficiency of ethyl methane sulphonate in inducing morphological and physiological mutations in lima bean, thus increasing the genetic variation available in the gene pool which can serve as an important source of variant genes for further breeding. **Materials and Methods:** Landraces of *Phaseolus lunatus* were obtained, presoaked in distilled water for 6 hrs and treated with freshly prepared concentrations of ethyl methane sulphonate (0.1, 0.2, 0.3 and 0.4%) for 6 hrs, a control experiment was also set up. Treated seeds and control were sown in polythene bags filled with top garden soil and arranged using a randomized complete block design to raise the M_1 and M_2 generation. **Results:** Morphological mutants: Invaginated leaf margin, bifurcated leaf apex, biapex leaf and enlarged leaf were observed in M_2 mutants treated with 0.2, 0.3 and 0.4% of the mutagen. Physiological mutants: A change in seed coat color from brown to black with off-white patches and a change in flower color from yellow to purple was observed in M_2 mutants treated with 0.4% ethyl methane sulphonate. The percentage frequency of M_2 progenies segregating for seed coat and flower color change was 92.31%. **Conclusion:** However, this research concludes that 0.3 and 0.4% ethyl methane sulphonate are more effective at inducing morphological and physiological mutations in *Phaseolus lunatus*.

KEYWORDS

Ethyl methane sulphonate, *Phaseolus lunatus*, morphological mutants, physiological mutants

INTRODUCTION

Pulses are the chief components of the agricultural system, effectively boosting food and nutrition, revenue and environment across the globe and hence assumed ideal for acquiring food security in the developing world including Nigeria. *Phaseolus lunatus* belong to the Family Fabaceae[1]. It is commonly known as lima bean in English and Papala in Yoruba[2]. Lima bean seeds are considered to be a good source of nutrients[3], such as valuable proteins with content from 14.24 to 24.92%[4] and rich in essential amino acids[5]. They provide complex carbohydrates, mainly starch and dietary fiber, vitamins (B complex) and minerals (zinc, iron and calcium)[6]. The most abundant proteins are storage proteins, primarily globulin and albumin[7]. In addition to their good macronutrient composition, lima bean is considered beneficial for health because of the low glycemic index due to the presence of slow-release carbohydrates[8].

ISSN: 2957-9449 (Online)
ISSN: 2957-9430 (Print)
https://doi.org/10.17311/tas.2023.88.92

Received: 08 Sep. 2022
Accepted: 02 Mar. 2023
Published: 31 Mar. 2023

It is an underutilized crop in Nigeria, which possess the potential to serve as an alternative source of protein supply but yet no considerable efforts have been made in time past towards the genetic improvement of this crop. Plant scientists in Nigeria are currently using mutagens as a tool to broaden the genetic diversity of this crop to produce improved cultivars.

Seed coat colour is an important agronomic trait that determines the marketability of a dry bean variety. People in a location have a specific preference for certain colours of beans. A genetic understanding of lima bean seed coat colour inheritance is very important in cultivar development breeding and market acceptability. It is possible to induce a change in seed coat colour through mutation[9,10].

Mutation breeding is an alternative to conventional plant breeding and is a source of increasing variability, conferring specific improvement without significantly altering its acceptable phenotype[11].

Induced mutation breeding, which is recognized as a valuable supplement to conventional breeding in crop improvement, has been least applied in grain legumes[12]. Among available mutagens, ethyl methyl sulphonate (EMS) is a potent and popular chemical mutagen that has been effectively used to induce a high density of random irreversible point mutations uniformly distributed in the genome[13]. Hence, this study was undertaken to assess the efficiency of ethyl methane sulphonate in inducing morphological and physiological mutations in lima bean, thus increasing the genetic variation available in the gene pool which can serve as an important source of variant genes for further breeding.

MATERIALS AND METHODS

Study area: The research was carried out in the research experimental field of the Department of Botany, Ahmadu Bello University, Zaria during the 2016 and 2017 planting seasons (June-November).

Research protocol: Landraces of *Phaseolus lunatus* were obtained from local farmers at Sabon Gari local government area of Kaduna State, Nigeria.

The seeds were presoaked in distilled water for 6 hrs and later transferred into freshly prepared concentrations of EMS (0.1, 0.2, 0.3 and 0.4) for 6 hrs. After treatment, the seeds were washed thoroughly under a running tap for 5 min to remove any residual effect of the mutagen. Treated seeds along with the control seeds were sown in triplicates in one hundred and fifty polythene bags (51.5×38.3 cm), each filled with sterilized top garden soil and arranged using a Randomized Complete Block Design (RCBD), to raise the first mutagenic (M_1) and second mutagenic (M_2) generation along with the control.

After the maturation of M_1 plants, pods were harvested from each treatment and threshed to obtain M_1 seeds which were sown to raise the M_2 generation. After flowering and maturation of the M_2 plants, pods were harvested and threshed and M_2 seeds were obtained. Leaves, flowers and seeds of M_2 plants were observed for morphological and physiological mutations.

Data collection: The number of plants showing morphological and physiological mutations was counted and percentages were calculated and presented in tables. The number of plant showing morphological variations in their leaves was presented in a bar chat.

RESULTS AND DISCUSSION

Morphological mutations such as invaginated leaf margin were observed in plants treated with 0.3% EMS, bifurcated leaf apex in plants treated with 0.2 and 0.3% EMS, biapex leaves were observed as a result of treatment with 0.3 and 0.4% ethyl methane sulphonate and enlarged leaf size was observed at the M_2 generation in plants treated with 0.3 and 0.4% EMS as represented on Fig. 1 and shown in Fig. 2. Morphological mutations that occurred may be a result of a change in the genes that code for the shape of the leaves due to treatment with the mutagens. This report corroborates findings in dolichos bean[14] and tomato[15].

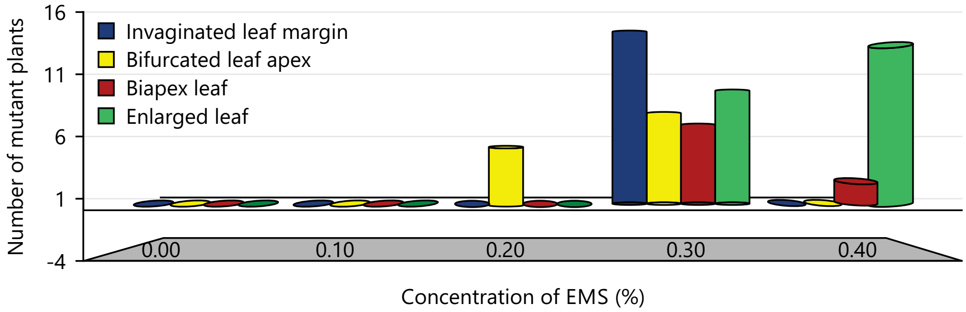

Fig. 1: Leaf morphology mutant in M_2 progenies

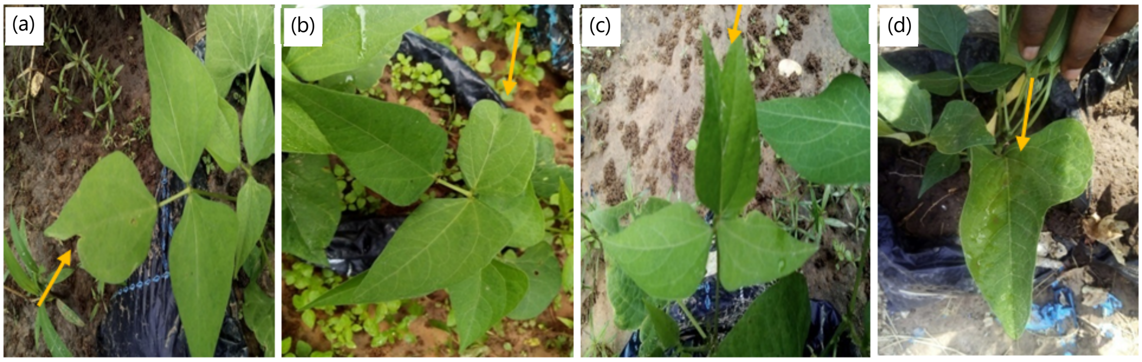

Fig. 2(a-d): Morphological mutants induced by ethyl methane sulphonate, (a) Invaginated leaf margin, (b) Bifurcated leaf apex, (c) Biapex leaf and (d) Enlarge leaf size

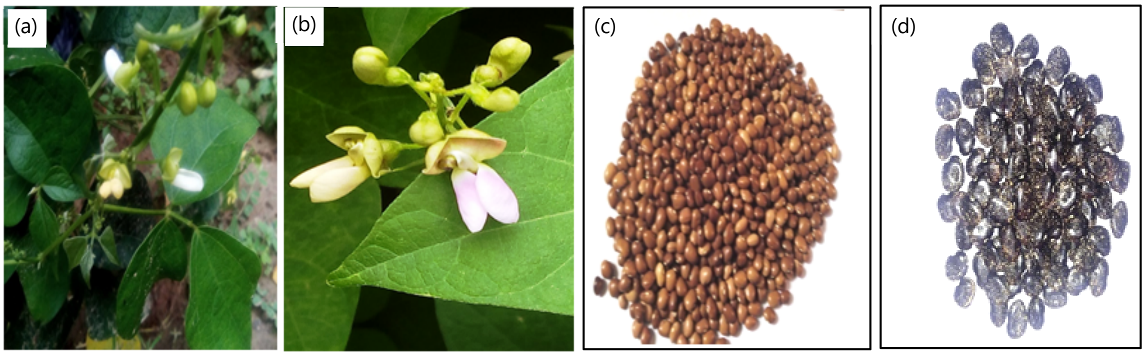

Fig. 3(a-d): Physiological mutants induced by ethyl methane sulphonate, (a) Control flower, (b) Mutant flower, (c) Control seed and (d) Mutant seed

Physiological mutants were observed in M_2 plants treated with 0.4% ethyl methane sulphonate (Table 1). The plants showed a purple and white colored flower which varied greatly from the control plants with a yellow and white flower. More so, the seed coat colour of M_2 seeds, treated with 0.4% ethyl methane sulphonate was black with off-white patches and larger in size compared to the control seed which had a brown seed coat colour and a smaller size (Fig. 3). The physiological spectrum of mutation which occurred in this study in the flower and seed coat colour could be a result of the occurrence of substitution mutation on the genes controlling these traits thereby leading to the replacement of the nucleotide of a triplet codon by another nucleotide resulting in the production of a protein with single amino acid and consequently altering the phenotype of the flower and seed. This conforms to the report of 0.1% EMS inducing a change in flower colour of grasspea[16] and the change in the seed coat colour of cowpea exposed to 30KR of gamma rays[17].

Table 1: Seed coat and flower colour phenotype in M_2 progenies

Concentration (%)	M_2 progenies segregating seed coat colour	Mutant seed coat colour	Mutant flower colour	Number of plants
0.00	00	Nil	Nil	00
0.10	00	Nil	Nil	00
0.20	00	Nil	Nil	00
0.30	00	Nil	Nil	00
0.40	48	Black with off white patches	Purple	48

Table 2: Frequency of M_2 progenies segregating for seed coat/flower colour

Concentration (%)	Number of treated seeds	Number of plants observed	M_2 segregating seed coat/flower colour	Frequency (%)
0.00	60	59	00	00.00
0.10	60	57	00	00.00
0.20	60	55	00	00.00
0.30	60	54	00	00.00
0.40	60	52	48	92.31

The highest percentage frequency (92.31%) of M_2 progenies segregating for seed coat colour and flower colour was observed at the 0.4% treatment as shown in Table 2. This is similar to the report on the effect of 15KR gamma irradiation on groundnut[12].

CONCLUSION

This research concludes that ethyl methane sulphonate is effective at inducing morphological and physiological mutations in *Phaseolus lunatus* at 0.3 and 0.4% concentrations. Morphological mutants such as invaginated leaf margin, bifurcated leaf apex and bi apex leaf were induced at 0.3% and enlarged leaves at 0.4% concentration at M_2 generation. An enlarged leaf increases the surface area which can as well increase the rate of photosynthesis and provide more foliage for animals. A change in seed coat colour from brown to black with off white patches increased seed size and a change in colour of flower from white to purple was also observed in mutants treated with 0.4% ethyl methane sulphonate at M_2 generation. The percentage frequency of M_2 progenies segregating for seed coat and flower colour change was 92.31%. The change in seed coat colour and increase in seed size has increased the genetic variability of the germplasm for further breeding and could also enhance its acceptability by farmers and final consumers.

SIGNIFICANCE STATEMENT

This study discovered that 0.3 and 0.4% EMS are efficient at inducing viable morphological and physiological mutations in *Phaseolus lunatus* that can be beneficial to breeders who hope to improve the available germplasm thus providing better varieties. This study will help researchers uncover critical areas in mutation breeding of *Phaseolus lunatus* that many researchers are yet to explore especially in Nigeria. Thus, a new theory on creating genetic variability and broadening the gene pool for further breeding of *Phaseolus lunatus* can be arrived at.

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