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Research Article

Effect of Mutagens on the Proximate, Mineral and Anti-nutrient Composition of *Phaseolus lunatus* L. Seeds

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Abstract

Background and Objective: Protein deficiency is considered as the commonest form of malnutrition in the developing countries. *Phaseolus lunatus* seed is an important source of vegetable protein and is highly underutilized in the country despite its great potential. Proximate, mineral and anti-nutrient composition of *Phaseolus lunatus* seeds, treated with varying concentration of mutagens were investigated. **Materials and Methods:** Seeds were treated with fast neutron irradiation from Americium Beryllium source (²⁴¹Am/Be), colchicine by soaking in freshly prepared concentration of Colchicine 0.2 mM for 6 h and ethyl methane sulphonate by soaking in freshly diluted concentration of EMS (0.1%) for 6 h. Treated seeds were sown and harvested after two months. Harvested seeds were analyzed and tested for their proximate, minerals and anti-nutrient composition following standard procedures. **Results:** The mutagens significantly ($p < 0.05$) affected all proximate composition except crude fibre and ash contents. Anti-nutrients varied significantly ($p < 0.05$) except tannin which ranges from 2.08-2.84 mg g⁻¹. All mineral contents also varied significantly ($p < 0.05$) except potassium. **Conclusion:** *Phaseolus lunatus* seeds can serve as an alternative to common legumes for protein supplement and mutagens can be used to improve their nutrient quality.

Key words: Fast neutrons, colchicine, ethyl methane sulphonate, anti-nutrients, *Phaseolus lunatus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phaseolus lunatus belongs to the family Fabaceae¹. It is commonly called lima bean in English and “waken rumpa” in Hausa². In Nigeria, Lima bean is cultivated mainly for the dry seeds. Like other grain legumes, it is an important source of vegetable protein and it also improves soil fertility. Despite the great potential of this crop, it is highly underutilized in the country and it has not received much attention in terms of utilization as a crop improvement. The state of nutrition in Nigeria is still characterized by inadequate calorie and protein supplies³. Experts considered protein deficiency as the commonest form of malnutrition in the developing countries, especially in regions where diets are mainly based on roots and tuber crops⁴.

Fast neutrons are densely ionizing radiations used in crop breeding to induce mutation which could lead to beneficial improvements in traits of crops such as their nutritional composition. Fast neutrons irradiation is considered as a valuable tool for developing varieties that are economically and agriculturally important and have high productivity potential⁵. Fast neutrons have also been credited with increase in some of amino acids in irradiated *Arachis hypogaea* and *Phaseolus vulgaris* seeds⁶.

Colchicine and ethyl methane sulphonate are chemical mutagens used for improving the yield and nutritional composition of crops. Colchicine has been used to induce useful mutations in several leguminous crops such as *Vigna unguiculata*⁷, *Glycine max*⁸ and *Phaseolus lunatus*⁹. Ethyl methane sulphonate has also been used to induce mutations on *Phaseolus lunatus*².

In view of the urgent need for new or unusual sources of food, especially high-quality protein, this research was carried out to evaluate the effect of fast neutrons, colchicine and ethyl methane sulphonate at low dose/concentration on the chemical composition of *P. lunatus*.

MATERIALS AND METHODS

Sourcing of seeds: About 30 kg of healthy landrace of *Phaseolus lunatus* was obtained from local farmers in Zaria, Kaduna state Nigeria and identified in the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria Nigeria.

Mutagenic treatment of seeds: Treatment with fast neutrons was conducted according to the recommended methods¹⁰.

Healthy dry seeds of *Phaseolus lunatus* were exposed to fast neutrons from Americium Beryllium source (²⁴¹Am/Be) with a flux of $1.5 \times 10^4 \text{ n cm}^{-2} \text{ sec}^{-1}$ at Center for Energy Research and Training, Ahmadu Bello University, Zaria for 2 h corresponding to 0.16 Sv dose. Seed treatment with Colchicine was conducted as described by Essel *et al.*⁷. Healthy dry seeds of *Phaseolus lunatus* were presoaked in distilled water for 6 h, after which the seeds were soaked in freshly prepared concentration of Colchicine 0.2 mM for 6 h. Seeds exposure to EMS was conducted as described by Borkar and More¹¹. Healthy dry seeds of *Phaseolus lunatus* were presoaked in distilled water for 6 h, after which the seeds were soaked in freshly diluted concentration of EMS (0.1%) for 6 h.

Experimental design and sowing of seeds: Treated seeds were sown in polythene bags (51.5 cm × 38.3 cm) filled with sterilized top soil at the Botanical garden of Ahmadu Bello University, Zaria located on Lat 11°14'N; Long 7°65'E in July, 2016. The pods were harvested at maturity and pods were broken to obtain the dried seeds, harvested seeds were ground using domestic grinder, passed through a 2 mm mesh sieve and then oven dried and kept in a sealed plastic container till it was ready for analysis at Institute of Agricultural Research, Ahmadu Bello University, Zaria, located on Lat 11°16'N; Long 7°63'E in September, 2018.

Proximate, minerals and anti-nutrient analyses of seeds:

The proximate analysis was determined using recommended methods of the Association of Official Analytical Chemist¹². Determination of α -Galactosidase, Protease Inhibitor and Lectin was carried out according to the recommendations of Garro *et al.*¹³, Adeyemo and Onilude¹⁴ and Burbano *et al.*¹⁵, respectively. The phosphorus content of the seed was determined using recommended methods of the Association of Official Analytical Chemist¹². The other elemental concentrations were determined after wet digestion of sample ash with a mixture of nitric and perchloric acids (1:1 v/v), using Atomic Absorption Spectrophotometer (AAS, Buck Model 20A, Buck Scientific, East Norwalk, CT06855, USA).

Data analyses: One-way Analysis of Variance (ANOVA) was adopted to determine if there was significant difference ($p \leq 0.05$) between the proximate, anti-nutrients and mineral composition of treated seeds. Duncan's Multiple Range Test (DMRT) was used to rank means where significant. All analyses were carried out using SAS 9.1.3 software package.

RESULTS

The result for the proximate composition of treated *P. lunatus* seeds is shown in Table 1. Ethyl methane sulphonate (0.1%) treated seeds had the highest crude protein (23.75%), lipid (2.50%) and nitrogen free extract (64.99%) contents. Fast neutron irradiation (0.16Sv) treated seeds had the highest crude fibre (5.12%) and ash content (4.09%), while colchicine (0.6 mM) had the highest dry matter (91.15%). Mutagenesis significantly varied the dry matter, crude protein, lipid and nitrogen free extracts contents of the seeds.

The results of anti-nutrient composition of treated *P. lunatus* seed is presented in Table 2. There was significant difference ($p < 0.05$) in tannin content of the treated seeds, other anti-nutritional content did not vary significantly ($p > 0.05$). However, fast neutron irradiation (0.16Sv) treated seeds had the highest of all the anti-nutritional contents (2.84, 0.42, 0.08 and 0.62 mg g⁻¹ for tannin, α -galactosidase, protease inhibitor and lectin contents, respectively) compared to all other treated seeds.

The results for the mineral contents of treated *P. lunatus* seeds is represented in Table 3. All mineral contents varied significantly except for potassium, which also had the least range of values (0.30-0.37 g kg⁻¹). Ethyl methane sulphonate (0.1%) treated seeds had the highest calcium (15.15 g kg⁻¹) and Magnesium (0.81 g kg⁻¹) contents while fast neutron irradiation (0.16Sv) treated seeds had the highest, potassium (0.37 g kg⁻¹) and phosphorus (3.48 g kg⁻¹) contents.

DISCUSSION

The insignificant difference in some proximate composition of *Phaseolus lunatus* seeds treated with the mutagens maybe due to the inability of the mutagens to induce valuable change in the nutritional properties of the seeds of *Phaseolus lunatus*. However, the increase in the crude fat content in treated seeds could be as a result of favourable mutations that must have occurred in the genes responsible for the determination of the fat content of the seeds, this finding is contrary to the findings of Ezeagu and Ibegbu¹⁶, who reported a crude fat value of 0.21% and Heuze *et al.*¹⁷, who reported a value of 2.1% in untreated seeds of *Phaseolus lunatus*.

The anti-nutrients of the treated seeds were also significantly low, which suggests that mutagens have a reducing effect on the anti-nutrients of *P. lunatus*. Al-Kaisey *et al.*¹⁸ reported gamma irradiation (Cobalt-60) to significantly reduce trypsin inhibitors, phytic acid and oligosaccharide flatulence of broad bean as the dose of radiation increased. Legume lectins are carbohydrate-binding proteins of non-immune origin with anti-nutritional factors that can cause intestinal disorders, which makes them to be of grave concern for human health and safety. They however have some health benefits which include anti-cancer; anti-human immunodeficiency virus (anti-HIV) and anti-microbial infection potentials and prevention of mucosal atrophy¹⁹. Therefore, its presence in low concentration can be beneficial to animals.

Table 1: Proximate composition of *Phaseolus lunatus* seeds treated with the mutagens

Variables	Dry matter (%)	Crude protein (%)	Crude fibre (%)	Lipid (%)	Ash (%)	NFE (%)
FNI (0.16Sv)	89.90±0.10 ^b	22.69±0.03 ^b	5.12±0.04 ^a	2.11±0.02 ^c	4.09±0.01 ^a	59.99±0.01 ^b
Colc (0.6 mM)	91.15±0.02 ^a	22.50±0.02 ^c	4.95±0.05 ^a	2.30±0.04 ^b	3.84±0.03 ^a	61.41±1.41 ^{ab}
EMS (0.1%)	89.25±0.05 ^c	23.75±0.04 ^a	5.06±0.05 ^a	2.50±0.05 ^a	3.70±0.20 ^a	64.99±0.01 ^a
p-value	0.001	0.000	0.170	0.013	0.202	0.047

Means with the same superscript along rows do not vary significantly ($p > 0.05$). FNI: Fast neutron irradiation, Colc: Colchicine, EMS: Ethyl methane sulphonate

Table 2: Anti-nutrients of *Phaseolus lunatus* seeds treated with the mutagens

Variables	Tannin (mg g ⁻¹)	α -Galactosidase (mg g ⁻¹)	Protease inhibitor (mg g ⁻¹)	Lectin (mg g ⁻¹)
FN (0.16Sv)	2.84±0.01 ^a	0.42±0.03 ^a	0.08±0.01 ^a	0.62±0.02 ^a
Colc (0.6 mM)	2.36±0.04 ^b	0.38±0.01 ^a	0.08±0.00 ^a	0.58±0.02 ^a
EMS (0.1%)	2.08±0.02 ^c	0.30±0.07 ^a	0.06±0.01 ^a	0.55±0.04 ^a
p-value	0.001	0.293	0.281	0.346

Means with the same superscript along rows do not vary significantly ($p > 0.05$). FNI: Fast neutron irradiation, Colc: Colchicine, EMS: Ethyl methane sulphonate

Table 3: Mineral composition of *Phaseolus lunatus* seeds treated with the mutagens

Variables	Calcium (g kg ⁻¹)	Phosphorus (g kg ⁻¹)	Potassium (g kg ⁻¹)	Magnesium (g kg ⁻¹)
FN (0.16Sv)	9.93±0.13 ^b	3.48±0.02 ^a	0.37±0.01 ^a	0.59±0.08 ^b
Colc (0.6 mM)	1.11±0.02 ^c	2.53±0.02 ^c	0.32±0.02 ^a	0.27±0.03 ^c
EMS (0.1%)	15.15±0.06 ^a	3.03±0.13 ^b	0.30±0.01 ^a	0.81±0.01 ^a
p-value	0.000	0.010	0.062	0.008

Means with the same superscript along rows do not vary significantly ($p > 0.05$). FNI: Fast neutron irradiation, Colc: Colchicine, EMS: Ethyl methane sulphonate

Mineral contents of the treated seeds are higher than that reported by Heuze *et al.*¹⁷ for the raw seeds of *P. lunatus*, except for phosphorus (3.03-3.48 and 5.50 g kg⁻¹) and potassium (0.30-0.37 and 17.90 g kg⁻¹). The results for proximate and minerals composition obtained in this study supports the claim of Hassan *et al.*²⁰, who reported that gamma radiation does not significantly affect the nutritional quality of plants.

Other researchers could explore the possibilities of using a different type mutagen to reduce the anti-nutritional component of lima bean and improve the mineral and proximate composition. This will help researchers know the best mutagen that can adequately be used for improvement of the nutritional value of lima bean.

CONCLUSION

This work showed that fast neutron irradiation, colchicine and ethyl methane sulphonate significantly affects the nutritional qualities of *P. lunatus* and the mutagens positively affected the anti-nutrients of the seeds. The proximate composition is still considerable high and can serve its purpose of a supplementary source of protein for animal consumption.

SIGNIFICANCE STATEMENT

This study discovered that the proximate, mineral and anti-nutritional composition of lima bean was significantly improved by mutagenic treatment; this can be beneficial for improving the nutritional composition of unconventional leguminous crops. This study will help the researchers to uncover the critical areas of the impact of mutagenesis on improvement of nutritional composition of lima bean that many researchers in Nigeria, have not explored fully.

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