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Evaluation of Proximate and Mineral Composition of Mutant Dolichos Lablab (*Lablab purpureus L.*) Accessions in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKK designed the study, wrote the protocol and the first draft of the manuscript. Author MGK provided the germplasms author KCP reviewed the experimental design and all drafts of the manuscript. Author EC managed the analyses of the study. All authors read and approved the final manuscript

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ABSTRACT

Plant breeding through induced mutation technology is a potent method to creating new variants of food crops with of desirable phenotypic, genetic and biochemical functions. It is a catalyst in developing improved crop varieties where classical hybridization or selection have limitations. It has been used to improve nutrition quality and higher yield in a number of legumes. Dolichos Lablab (*Lablab purpureus L*) is multipurpose legume that has not been exploited extensively for food nutritional properties through breeding. The purpose of the study was, therefore, to generate awareness that nutritional status of D. *Lablab* could be improved through mutation induction and be a good source of food components essential for good health. Twenty-four dolichos Lablab germplasms including 20 mutant accessions and 4 commercial genotypes were evaluated for proximate values and mineral contents in Kenya in 2021 based on Association of Official Analytical Chemists (AOAC). Data analysis was based on least significant difference (*P* = .05).The revealed percent moisture, ash crude fat, crude fibre, crude proteins and total starch content ranged :

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7.35% -11.84%, 1.09 -2.90%, 3.35% - 8.05% , 8.86 - 12.70% , 20.03% - 28.87% and 25.50% - 39.00% .The mineral content of phosphorous (P), potassium(K) and calcium (Ca) ranged from 27mg - 57mg, 132mg -297mg and 7mg - 19mg. A significant positive correlation between ash levels to P and K concentration and on P to K concentration. The result of analysis ranked 9 (WT026, WT018 ,MT110 , BT188 , BT032, BT114, MT076 BT137,GT09) different accessions that positively contributed to the nutritional content of the investigated dolichos lablab accessions. Further research on the superior accessions can be done on yield potential, resistance to biotic and abiotic constrains, sensory preferences or used in bio fortification of existing genotypes.

Keywords: Orphaned crops; Legume; diversity; nutrition; food security.

1. INTRODUCTION

There is a necessity to enhance mineral elements and amino acids essential for human and animals, alteration of protein and fatty acids profiles for nutritional and health purposes [1]. Dolichos lablab is an important legume in Kenya in the daily livelihoods amongst communities that utilized it as a vegetable, pulse, forage, cover and green manure crop [2]. It is classified as an 'underutilized or orphaned crop' in Kenya which has contributed to limited research towards its improvement for nutritional and yield potential compared to main stream dry beans or common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). Dolichos is more drought tolerant to common beans (*Phaseolus vulgaris*), soybean (*Glycine max*) [3,4] It also yields more grains compared to cowpea (*Vigna unguiculata*) [5].In Kenya dolichos lablab beans more market price from 2.5USD to 4USD per 2kg across markets compared to common which costs range from 0.8to 2.5USD. Therefore, Lablab purpureus can effectively substitute other legumes in Kenya [3] and [6].

The Sustainable Development Goal (SDG) Number 2, demands for alternative sources of food to realize zero hunger and ending all forms of malnutrition by 2030 [7]. Improvement of orphaned crops such as Lablab beans for nutritional values and yield will enable realization of the food and nutrition security in Kenya. Induced mutations have played a great role in increasing quality and nutrition components of a crop. Of the 3,000 mutant varieties developed globally, 776 mutants have been induced for nutritional quality [1,8]. Several mutant genes have been successfully introduced into commercial crop varieties that significantly enhance the nutritional value of those crops. Previous research on dolichos accessions in Kenya and other parts of the world major on hybridization studies, morphological and genetic diversities or anatomy of D. lablab [9] .Research on the nutritional content of D. lablab have involved a limited number of accessions and /or also with a limited number of parameters analyzed. For that reason, this research investigates the differences of percent ash, crude fat, crude fiber, crude protein, and mineral contents (Phosphorous, potassium and calcium) among dolichos accessions bred through mutation induction in Kenya.]

2. MATERIALS AND METHODS

2.1 Germplasms Accessions

The accessions used in the current study comprised 20 M3 mutant lines selected at University of Eldoret based on earliness and yield potentials following mutation induction of gamma irradiation at 300gy and 400gy doses respectively. The mutation on dolichos seed were from a cobalt 60 (^{60}Co) facility at the plant genetics and breeding laboratory in Seibersdorf, Vienna, Austria in 2018. Four parental genotypes coded (W, M, B, G) in the experiment were included as checks. The accessions are as described in Table 1.

2.1.1 Experimental site

The proximate and mineral content analysis in the dolichos accessions were accomplished in Egerton University animal science department and at University of Eldoret biotechnology laboratories based [10].

2.2 Proximate Component Analysis

The dry beans used in the study and must were finely ground into flour and kept at 4–6°C in sealed polyethylene bags until analysis. Moisture, ash, crude fat, crude protein, and total starch were analysed according to [10] methods as described below. Samples were analyzed in duplicates.

Table 1. Description of the accessions used in the study

Entry	Accession	Status of accession	Seed colour	Seed size
1	MT076	Mutant	Cream	Small
2	MF015	Mutant	Dotted (brown)	Medium
3	BF137	Mutant	Brown with grey dots	Small
4	MF048	Mutant	Black	Small
5	GT076	Mutant	Black	Medium
6	MT049	Mutant	Cream	Large
7	BT183	Mutant	Brown with grey dots	Medium
8	BT039	Mutant	Dotted	Medium
9	MT110	Mutant	Cream	Small
10	G	Released variety	Black	Medium
11	BT046	Mutant	Dotted (brown)	Large
12	BT188	Mutant	Dotted (black)	Medium
13	B	Released variety	Brown with black dots)	Large
14	BF032	Mutant	Dotted (brown)	Medium
15	M	Released variety	Cream	Medium
16	BF105	Mutant	Brown with black dots	Large
17	GT095	Mutant	Black	Small
18	BT114	Mutant	Brown with black dots	Medium
19	BT154	Mutant	Brown with black dots	Medium
20	BT166	Mutant	Dotted (red)	Medium
21	GT032	Mutant	Black (white)	Medium
22	W	Released variety	Black	large
23	WT018	Mutant	Cream	Large
24	WT026	Mutant	Brown	Large

2.2.1 Determination of moisture

Water content in the dolichos genotypes were determined by removing moisture and then by measuring weight loss; 3g of bean powder were accurately weighed in a pre-weighed petri-dish and dried in a hot air oven for 6-12h at 100±2ºC. The dish with the sample was then cooled in desiccators and weighed. This exercise was repeated until the difference in weight between two successive weighing becomes constant. From the weight loss during drying, amount of moisture was calculated using the following formula $Moisture\ (\%) = \frac{W1-W2}{W}100$

W1 = Weight of sample with Petri dish before drying
W2= Weight of sample with Petridish after drying
W = Weight of sample

2.2.2 Determination of ash content

1g of dried sample was accurately weighed into pre-weighed, clean crucible. The crucible was heated to the point of charring of the sample on a hot plate. The crucible with the carbon residue obtained as a result of ignition, was placed in muffle furnace at temperature of 650°C until the carbon residue disappeared. The sample was allowed to cool and then weighed. From the difference in weight obtained the ash content was calculated using the formula:

$$Total\ ash\ (\%) = \frac{Weight\ of\ crucible\ with\ Ash(g)}{Weight\ of\ crucible\ with\ sample}100$$

2.2.3 Crude fat estimation

10g of sample in a thimble were taken and plugged the top of the thimble with a wad of fat-free cotton. The thimble was dropped the into the fat extraction tube of a Soxhlet apparatus. The bottom of the extraction tube was attached to a Soxhlet flask. 75mL of hexane was poured through the sample in the tube into the flask. The top of fat extraction tube was attached to the condenser and the sample extracted for 6h on a heating mantle at 40°C. At the end of the extraction period the thimble from the apparatus was removed and concentrated the extract at rotavapor at 40°C.It was then dried at 100ºC for 1h, cooled and weighed. The difference in weights gave the ether soluble material present in the sample.

$$Crude\ fat\ (\%) = \frac{Weight\ of\ hexane\ soluble\ material}{Weight\ of\ sample} x100$$

2.2.4 Determination of crude fiber

1gram of milled sample was taken into the beaker and 60ml of boiling sulfuric acid added. It was then connected to the digestion apparatus. The sample was then boiled for exactly 30minutes, then filtered through filtering cloth and was washed with hot water until it was free from acid. The residue was then transferred on the cloth into the flask with 200ml of boiling sodium hydroxide solution. Immediately it was connected to the flask with the digestion apparatus and boiled further for exactly 30minutes. The flask was removed and immediately filtered through Gooch crucible. It was washed with hot water until it was free from alkali and then with 10ml of alcohol. It was then dried at 105-110°C in an air oven for about 2hours. Cooling was done at room temperature in desiccator and weighed. The process was repeated 30minute after drying, cooling and weighing until the difference between two successive weightings is less than 1mg. The lowest weight was noted as the weight of crucible and contents after drying.

The contents were then incinerated in the crucible in the electric muffle furnace at 620°C for 30minutes. It was then cooled to room temperature in desiccator and weighed.

The process of 30minute incineration was repeated, cooling and weighing until the difference between two successive weightings was less than 1mg. The lowest weight which was noted was considered as the weight of crucible and ash after incinerating. The difference between the two weightings was the weight of crude fiber.

$$Crude\ fibre\ ,(\%)\ by\ weight = \frac{(W1 - W2)}{W} x100$$

Where, W is weight of sample, g
W1 is weight of crucible and contents after drying, g
W2 is weight of crucible and ash after incinerating, g

2.2.5 Determination of crude proteins

The test for protein measurement was based on the nitrogen content (Kjeldahl method). 0.5g of sample and digestion mixture (copper sulphate + potassium sulphate) was weighed into a Kjeldahl flask and 10mL of concentrated H2S04 was added. The Kjeldahl flask was then heated on a mantle (in slanting position) until colour of solution changed to pale blue green. The clear solution was made up to 25mL under cold conditions. The Kjeldahl apparatus was set up for protein estimation. 20mL of 4% boric acid and 1mL of mixed indicator (bromocresol green) was taken in conical flask and placed under condenser. 5mL of sample with 20mL of 40% NaOH and 10mL water were added to distillation tube through funnel. When water started boiling inside the round bottom flask the steam that was produced was then passed into distillation tube. The ammonium (NH3) gas that was evolved in distillation tube was then trapped in boric acid. Upon ammonia evolution, the colour of boric acid changed to blue. For maximum ammonia evolution, the process was continued for 20min. The solution was then titrated with standard HCl (0.01N) until blue colour of the solution disappeared. The amount of nitrogen in the samples were calculated by the following equation

$$\%\ of\ Nitrogen = \frac{14\ XNormality\ of\ HCl\ X\Delta VX100}{Weight\ of\ Sample\ 1000} x100$$

% Protein = % of Nitrogen × 6.24

2.2.6 Determination of total starch

Total starch was found by difference method and expressed as Percentage of total starch.

Total starch (%) = 100 − [Moisture + Ash + Fat + Protein]

2.3 Determination of Mineral Contents (Phosphorous, Potassium and Calcium)

The mineral contents in the dolichos accessions were determined by dry ash method (AOAC, 2000). Five grams of samples were ashed at 550ºC for 8 hours then drops of 6N HCl were added and evaporated. The samples were incinerated further for 1 hour and diluted using 1N nitric acid. The samples were placed in 100 mL volumetric flasks and made to 100 mL using 1N nitric acid. Standards were prepared using the 1N nitric acid and the absorbance read in the atomic absorption spectrophotometer.

The dilution factor for all minerals was 100 the determination of Ca, 1.0ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentrations of minerals were recorded in terms of "ppm" are converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000.

2.4 Data Analysis

Following the completion proximate and mineral content analysis data analysis was performed using SAS 8.0. Both of multivariate and univariate analyses were administered for data analysis processes in which the dolichos lablab accessions were categorized as independent variable; while the proximate and mineral contents were investigated as dependent variables. Testing for significant difference was achieved using Least Significant Difference (LSD) at 0.05 for univariate analysis.

3. RESULTS AND DISCUSSION

3.1 Results

There was significant difference in mean of percent moisture ,ash , crude fat ,crude fibre ,crude protein and total starch and in the mineral concentrations of phosphorous, potassium and calcium $P<0.005$ Table 2. Results also show that there was significant difference In percent moisture content, in MT110, GT076, and GT095 with means of 11.84 ±0.20, 11.71 ± 0.22 and 7.35 ± 0.03. The following accessions had significant difference in percent ash content WT026 ,WT018 and MF015 with means of 2.90±0.09, 2.86±0.07and 1.09±0.01.There was significant difference in crude fat in lines MT110 and W with mean recordings of 8.05±0.71 and 3.35±1.41respectively. The accessions of dolichos lablab with significant differences in percent crude fibre were MT049, WT018 and BT039 with percent means of 12.70±0.01, 12.62±0.37, and 8.86±0.71.The accessions BF032, MT110, BT188 and MT076 had significant difference in percent crude proteins with mean of 28.87±0.18, 28.78±0.30, 28.66±0.14 and 20.03±0.01. In total starch, mutant accession WT026 significantly the highest mean of total starch 39.00 while accessions BF137 had the lowest mean of 25.50±3.54.

In mineral composition the accessions the highest significant difference in phosphorous level were in WT026 and BT188 with mean concentrations of 57±0.00 and 56±0.01.The accession with the lowest significant phosphorous concentration were BT166 with a mean of 27±0.03 and BT039 (27±0.00). In terms of concentration of potassium the following accessions resulted in having the highest concentrations BF137, WT026 , WT018 and MT076 with mean concentrations of 29.7±0.04, 29.6±0.00, 29.4±0.07and 28.9±0.10. The lowest significant concentrations were in accession GT032 with potassium concentration of 13.2±0.01. BT114 mutant accession resulted with the highest significant mean of calcium concentration at 19±0.00 while the least were accessions MF015 and MT076 each with a mean of 7±0.00.

The mutant accessions that registered significant positive improvement based on the study results were WT026 (ash, total starch, Phosphorous and potassium) ,WT018 (Potassium and calcium), MT110 (fat and crude protein), BT188 (crude protein and phosphorous), BT32 (Crude protein),BT114 calcium), MT076 and BT137 (Potassium) ,GT095 (%moisture). Therefore, any of the above 9 accessions can be adopted by farmers for different purposes. For further research, a study comparing the quantity of biomass produced by the different accessions would give evidence and recommendation on which accessions are most suited for biomass.

Correlation Analysis of proximate values and mineral composition in dolichos accessions: The estimated correlation of proximate and mineral values in dolichos lablab accessions is represented in Table 3. There was a significant positive correlation between percent ash levels on phosphorous and potassium concentration and between phosphorous to potassium concentration.

3.2 Discussion

In the present study, the dolichos mutant accessions were selected based on earliness and seed yield potential. The analysis of the accessions indicated higher performance in terms of proximate characteristics and/or mineral concentrations than commercial varieties. Similarly some accessions showed superiority over other mutant accessions and control (Commercial varieties) within and/or between the proximate values and mineral concentrations. Varied results on proximate analysis of Lablab beans have been reported by [7,3]. The mean

Table 2. Mean squares for percent proximate values and mineral contents of 24 *Lablab* Accessions

Accession	Mst %	Ash %	Fat %	CF %	CP %	TS %	P (100mg)	K (100mg)	Ca (100mg)
MT076	8.73±0.28	2.35±0.46	4.24±0.27	9.17±0.02	20.03±0.01	29.50±2.12	44±0.00	28.9±0.10	7±0.00
MF015	9.69±0.23	1.09±0.01	4.10±0.50	10.83±1.68	27.49±0.69	34.00±2.83	33±0.07	21.2±0.09	7±0.00
BF137	8.77±0.40	2.61±0.51	4.78±1.10	10.01±2.04	26.45±0.17	25.50±3.54	53±0.00	29.7±0.04	11±0.01
MF048	8.05±0.21	2.07±0.19	7.13±2.16	9.34±0.49	21.86±2.12	28.50±0.71	32±0.00	24.8±0.63	12±0.00
GT076	11.71±0.22	1.16±0.18	3.40±1.65	11.77±0.08	24.13±2.19	28.50±4.95	38±0.00	21.0±0.12	13±0.00
MT049	8.35±0.09	1.48±0.17	4.46±0.14	12.70±0.01	25.67±0.01	31.50±2.12	39±0.07	21.8±0.14	15±0.00
BT183	9.85±0.88	1.56±0.58	4.39±0.54	10.51±0.40	22.54±0.25	28.00±0.00	36±0.00	20.2±0.01	14±0.00
BT039	8.79±1.10	1.69±0.04	4.12±0.16	8.86±0.71	24.43±0.10	29.00±1.41	27±0.00	21.5±0.08	16±0.00
MT110	11.84±0.20	1.38±0.11	8.05±0.71	10.31±0.07	28.78±0.30	33.00±4.24	38±0.11	20.5±0.04	17±0.00
G	7.54±0.00	2.41±0.40	5.48±0.17	10.16±0.75	27.27±0.66	33.50±0.71	54±0.02	24.5±0.28	15±0.00
BT046	7.41±0.17	1.16±0.04	7.20±0.00	10.23±0.00	22.56±0.13	30.00±1.41	28±0.07	25.5±0.00	10±0.00
BT188	10.11±0.08	2.47±0.26	5.38±0.02	8.99±0.53	28.66±0.14	29.00±0.00	56±0.01	22.9±0.01	17±0.00
B	10.93±2.08	2.21±0.14	3.75±0.69	11.05±0.26	23.85±0.21	33.50±7.78	47±0.06	25.2±0.33	18±0.01
BF032	9.31±0.44	2.47±0.28	4.67±0.15	10.81±0.07	28.87±0.18	27.00±1.41	53±0.00	26.5±0.13	19±0.00
M	8.89±0.07	2.13±0.03	4.56±0.12	11.47±0.52	20.18±1.68	36.50±3.54	28±0.07	22.8±0.01	14±0.00
BF105	7.39±0.30	2.50±0.28	3.90±0.79	10.30±0.76	21.49±0.21	32.50±2.12	44±.01	25.7±0.00	15±0.01
GT095	7.35±0.03[b]	2.54±0.54	5.58±0.03	10.41±0.25	21.67±0.11	29.50±0.71	54±0.00	25.5±0.00	17±0.00
BT114	9.36±0.34	1.94±0.51	5.03±0.48	10.96±2.26	23.48±0.42	34.50±2.12	34±0.07	28.1±0.00	19±0.00
BT154	8.22±0.25	1.19±0.11	3.98±0.54	9.69±0.46	20.23±0.21	33.00±2.83	28±0.02	15.7±0.34	18±0.02
BT166	9.13±0.25	1.16±0.04	4.46±0.14	11.47±1.25	25.21±1.07	29.50±0.71	27±0.03	19.1±0.00	16±0.00
GT032	10.68±0.15	1.13±0.06	4.18±0.25	11.29±0.08	25.23±4.56	31.50±0.71	30±0.00	13.2±0.01	17±0.00
W	11.12±0.88	1.88±1.15	3.35±1.41	12.56±0.57	22.15±0.19	27.50±0.71	48±0.00	20.0±1.06	17±0.00
WT018	7.77±0.06	2.86±0.07	3.40±1.46	12.62±0.37	20.71±0.21	28.00±2.83	55±0.07	29.4±0.07	15±0.00
WT026	9.66±0.18	2.90±0.09	4.73±0.06	10.73±0.71	24.57±1.12	39.00±0.00	57±0.00	29.6±0.00	16±0.00
MEAN	9.19±0.99	1.928±0.66	4.76±1.33	10.674±1.23	24.06±2.93	30.91±3.76	40.8±0.11	23.45±0.47	14.7±0.43
CV%	5.99	19.28	16.77	8.18	5.25	9.00	10.54	12.16	4.03
P Value	***	***	***	***	***	***	***	***	***

*** = Significant at P≤0.001, (CV%). Coefficient of variation at the 5% level of significance according to Least Significant Difference (LSD)*

Where: Mst% Percent moisture; Ash%:percent ash, CF%:Percent fibre; CP%:Percent crude protein; TS%:Percent total starch; P:Phosphorous;K:Potassium and Ca: Calcium.

Table 3. Summary of correlations analysis of nutritional traits and mineral contents among mutant accessions

MST	ASH	FAT	CF	CP	TS	P	K	Ca	
MST	1.00								
ASH	-0.33	1.00							
FAT	-0.13	-0.07	1.00						
CF	0.23	-0.13	-0.41	1.00					
CP	0.39	-0.09	0.27	-0.08	1.00				
TS	0.04	0.00	0.06	0.06	-0.04	1.00			
P	-0.03	0.81*	-0.11	0.06	0.23	-0.14	1.00		
K	-0.38	0.78*	0.11	-0.11	-0.10	-0.01	0.59*	1.00	
Ca	0.22	0.13	-0.08	0.21	0.17	0.14	0.16	-0.21	1.00

**Significant at P < 0.05; Where: MST moisture Content; CF: Crude fibre; C: Crude protein; TS:Total starch P:Phosphorous; K:Potassium and Ca: Calcium*

values of the traits in the accessions increased significantly in the mutant lines compared to commercial varieties. The significant difference in the amount of percent ash, fat crude fibre, crude protein and total starch content among the dolichos accession indicate genetic variability that resulted due to mutation. The variations agrees with [11] and [12] that induced mutation can increase yield as well as other as quality traits such as protein content, fiber or baking quality. It is reported that mutation induction is fit for traits that have either not been favored by natural selection in the evolutionary process or have not been improved during previous plant breeding efforts. Ash content referred to inorganic residue with mineral as its primary content [13].The higher the level of ash content, the higher the level of mineral content would be. This finding are inferred by the correlation analysis during the study. Defining ash content is considered crucial for food source due to several reasons; one of which is that mineral content would define the physicochemical characteristic of the food source. The reduction in ash among the accessions to 1.92 % compared to 3.50% by [14] could have been contributed by high doses of irradiation 300gy and 400gy used to breed the accessions. The mean percent crude protein of 24.06% indicates an improvement of 1% from the results of [9] from dolichos lablab bred through classical hybridization. This result supported by the findings that radiation has direct impact on the protein biochemistry in legumes [15,16]. The appearance of low significant proximate and mineral contents among some of the mutant accessions compared commercial varieties is attributed to deleterious effect of mutations .According to [14,17] most induced mutations are deleterious, but when appropriate selection technique is applied, useful mutants can be recovered.

The study identified 9 mutant accessions with desirable mutations in either one or multiple different specialties .This concurs with reports that mutation breeding has recently been used for enhancing bioavailability of important nutrients in certain crops. Two barley mutant varieties with low phytic acid have been released for commercial production [18-19]. Through mutation technology rice mutant varieties with low glutelin content, such as LGC-1 and its derivatives, have been developed for people who must restrict protein intake, as it is the case of patients with kidney disease. Improvement of sorghum through mutation have identified high nutrition values in terms of protein and starch contents that it could be used an alternative food source [3]Therefore, any of the above 9 accessions can be adopted by plant breeders for different purposes and improvement of other dolichos germplasms.

4. CONCLUSION

Emphasis in all the developing countries is development of nutrient rich food crops. Mutation induction is an important tool in achievement of this milestone. The dolichos lablab accessions bred for earliness and yield potential can also result in fortification important nutritional values in orphaned food crops and other cash crops. In the present study, the high yielding mutant accessions were assessed for proximate and mineral contents and possibly be advanced to variety development and release.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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