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EFFECT OF SODIUM AZIDE ON BAMBARA GROUNDNUT (*Vigna subterranea (*L.) Verdc.) AS REVEALED BY SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

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ABSTRACT

This study investigated the mutagenic effects of Sodium Azide (NaN_3) on the agro-morphological and protein content of eight Bambara groundnut genotypes. The seeds of six genotypes; TVSu-86, TVSu-91, TVSu-186, TVSu-235, TVSu-242, TVSu-350 were collected from the International Institute of Tropical Agriculture (IITA) and two landraces from Abia State and Enugu State North East, Nigeria local markets. The seeds were treated with five concentrations: 0.00%(control), 0.01%, 0.03%, 0.05% and 0.07% of NaN_3 after pre-soaking for 6hrs in distilled water and sown in pots arranged in a Complete Randomized Design with three replicates. There was reduction in germination percentage and growth characters as concentrations of NaN_3 increases. Early flowering was recorded at 37 days mutated with 0.07% of NaN_3 compared to control which flowered late at 42 days. NaN_3 (0.07%) caused lethal effect on Abia and Enugu landraces. There was no significant ($P>0.05$) difference in yield traits among mutants and control. Mutant seeds significantly ($P<0.05$) increased protein content (19.12%) at 0.05% of NaN_3 compared to control (18.5%). The number of seeds (0.99), seed yield (0.89) and pod yield (0.96) strongly correlated with seeds per pod (0.85). The SDS-PAGE revealed the presence of polypeptide bands in mutants compared to control. TVSu-235 and TVSu-350 genotypes had higher tolerance and yield traits to 0.01% concentration of NaN_3, thus could be further improved in subsequent breeding.

Keywords: Bambara groundnut, Sodium azide, SDS-PAGE, polypeptide bands.

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.)Verdc,), member of the family Fabaceae is an indigenous, underutilized African legume mainly grown by subsistence farmers for food (Bamshaiye et al., 2011). Bambara groundnut is the third most important leguminous crop after groundnut and cowpea in terms of production and consumption with which it shares its origin of genetic diversity (Olawuyi et al., 2015). Genetic variation is fundamental to successful breeding programs in vegetative and sexually propagated plants (Agbolade et al., 2016; Wongpiyasatid et al., 2000). This variation which occurs naturally or artificially by inducing with physical, biological or chemical mutagens has attracted the interest of plant breeders for many decades. Mutation breeding is an aspect of conventional plant breeding which

involves physical and chemical mutation in order to assess genetic variation resulting to improved varieties with better characteristics [Mensah and Obadoni (2007); Arulbalachandran (2009); Olawuyi et al., (2016); Mennella 2011].

Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, seed storage protein analysis represents a valid alternative and improved approach to varietal identification (Iqbal, 2005). Ghafoor (2002) reported that grain storage protein profiling based on SDS-PAGE can be employed for various purposes such as; characterization of germplasm, varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species and generation of pertinent information to complement evaluation (Ilbas, 2005).

Sodium azide (NaN_3) is one of the constituents of bactericide, pesticide and industrial nitrogen gas generator that may be lethal or beneficial in several plants such as barley, maize and some other crop species [Mostafa (2011); Asad et al., (2014)].

Therefore, this study investigated the effects of Sodium azide on the morphological, agronomical and protein contents of Bambara groundnut using SDS-PAGE.

EXPERIMENTAL PROCEDURES

Collection of seed samples, experimental location and experimental design

The seeds of six (6) Bambara genotypes namely: TVSu-86, TVSu-91, TVSu-186, TVSu-235, TVSu-242, TVSu-350 were collected from the germplasm unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and two (2) landraces (L_1 and L_2) were collected from Abia State ($5^o25'N$ $7^o30'E$) and Enugu State ($6^o30'N$ $7^o30'E$) respectively.

The *in vitro* and screen house experiments were carried out at the Genetics and Molecular Laboratory and Screen house of the Department of Botany, University of Ibadan, Nigeria respectively. The design was laid out in Complete Randomized Design (CRD) comprising of 8 genotypes, 5 concentrations and 3 replicates.

Preparation of sodium azide (NaN_3), pre-treatment of seeds and in vitro screening

The seeds of Bambara were pre-soaked in 100mls distilled water for 6 hours prior to treatment with Sodium azide. The seeds were then treated with concentrations of Sodium azide (0.01%, 0.03%, 0.05% and 0.07%) for 6 hours while untreated seeds (0.00%) served as control according to the method described by Asgharipour and Rafiei (2011). The five pre-soaked and treated seeds of bambara were blotted with Whatman filter paper to remove excess moisture for 20minutes and planted on Petri dishes lined with moist filter paper. 2ml of distilled water was added daily to the petri dish according to the method described by IPGRI (2020).

Research design and Screen house experiment

10kg of sterilized top soil was measured using a weighing scale and put into each of 120 perforated polythene bags. Three treated and untreated seeds of each Bambara genotype were sown 3 inches deep inside polythene bag filled with sterilized soil and spaced at a distance of 30cm in a completely randomized design (CRD) with three

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replicates. Watering and other agronomic practices were also carried out during growth and development of the plant until maturity.

Determination of Quantitative characters and Protein contents

Observations on morphological traits of NaN_3 induced mutants and controls were carried out and measured using measuring tape and visual counting for a period of 14 weeks after planting following the IPGRI, IITA and BAMNET (AOAC 1996) Descriptors for Bambara groundnut. The crude protein content was determined using micro Kjeldahl method as described in AOAC (Weber 1975) and Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) was analysed using 12% vertical slab polyacrylamide gel for the separation of polypeptide bands according to the procedure described by Cokkizgin and Cokkizgin (2010).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using SAS generalized linear model (GLM) software and means with significant differences were separated by Duncan multiple range test (DMRT) ($P \leq 0.05$).The final germination percentage (FGP) was obtained using Olawuyi and Okoli (2017) method as stated below;

$$\text{FGP} = \frac{\text{number of germinated seeds after 8 days}}{\text{total number of seeds planted}}$$

RESULTS AND DISCUSSION

The result in Table 1 shows the interactive effect of genotype, concentrations and growth stage on germination and growth characters of Bambara groundnut. The concentration of Sodium azide produced highly significant ($p<0.001$) effects on plant height, leaf length, leaf width, number of leaves and number of branches while it showed no significant effect on the germination percentage. The genotypic effect was highly significant ($p<0.001$) on germination percentage, plant height, leaf length and width, number of leaves and branches. The growth stage was highly significant in germination percentage and all growth characters with the exception of leaf length. Similar result were obtained for the interactive effect of Genotype × Concentration in all growth characters except in the germination percentage while the interactive effect of Concentration × Week (growth stage) was highly significant in germination percentage and all growth character.

Table 1. Mean square interaction of genotype, concentrations of Sodium azide and growth stage on germination and growth characters of Bambara groundnut

Source of variation	Df	Germination percentage	Plant height	Leaf length	Number of leaves	Number of branches	Leaf width
Concentration	4	9.69^{ns}	2121.02***	134.37***	42476.98***	5564.41***	12.95***
Genotype	7	385.48***	3321.67***	274.36***	143861.73***	17441.72***	25.47***
Replicate	2	2.32^{ns}	60.89^{ns}	11.92^{ns}	5453.61^{ns}	562.16^{ns}	0.39^{ns}
Week	13	17146.10***	34.80***	2.07^{ns}	91419.258***	10820.11***	2.28***
Genotype × concentration	24	87.58^{ns}	892.50***	52.29***	26745.69***	2853.22***	5.81***
Concentration × replicate	8	74.82^{ns}	120.31***	5.18***	25610.86***	3196.51***	0.71***
Concentration × week	52	99.59***	30.14***	2.18***	1828.05***	222.47***	0.59***
Genotype × replicate	14	73.01^{ns}	66.85***	3.29***	3478.95***	412.41***	0.90***
Genotype × week	91	385.60***	47.53***	3.61***	3786.25***	424.07***	0.45***
Week × replicate	26	10.52^{ns}	11.59^{ns}	0.69^{ns}	539.00^{ns}	64.23^{ns}	0.13^{ns}

Genotype × concentration × replicate	47	45.91^{ns}	117.29***	10.33***	13250.09***	1560.97***	0.89***
Genotype × concentration × week	312	87.58***	32.89***	3.05***	2166.30**	223.45***	0.53***
Concentration × week × replicate	104	74.82***	15.29^{ns}	1.52*	1629.64***	214.31***	0.23^{ns}
Genotype × week × replicate	182	73.01***	18.88*	1.39^{ns}	1094.67^{ns}	127.02^{ns}	0.23^{ns}
Error	611	611	562	562	560	560	533
Corrected total	1497	1497	1446	1446	1444	1444	1414

*Note: * = significant at p<0.05, ** = highly significant at p<0.01, *** = highly significant at p<0.001*.

The interactive effects of genotype, concentration of Sodium azide and growth stage (week) on agronomic and yield characters of bambara groundnut as seen in Table 2 revealed that the concentration of Sodium azide was highly significant (p<0.001) on number of days to flowering but not significant to yield characters while the genotypic effect was highly significant in both agronomic and yield characters. Similar results were obtained in the growth stage (week) which was highly significant in all agronomic and yield characters of Bambara groundnut. The interaction of Genotype × Concentration produced highly significant effect on number of days to flowering only. The interaction of Concentration × Week had significant expression on all agronomic and yield characters with the exception of seed yield per plant. The interaction of Genotype × Week was highly significant in all agronomic and yield characters also the interactive effect of Genotype × Concentration × Week was highly significant in number of days to flowering but not significant in yield characters.

Table 2. Mean square interactive effect of genotype, concentration of Sodium azide and growth stages on agronomic and yield characters of Bambara groundnut

Source of variation	df	Number of days to flowering	Seed yield per plant (g)	Seeds per pod	Pod yield per plant (g)	Number of seeds per plant	Number of pods per plant
Concentration	4	34.51***	0.35^{ns}	0.01^{ns}	1.21^{ns}	2.22^{ns}	2.19^{ns}
Genotype	7	135.99***	1.63***	0.02***	3.64***	7.78***	7.64***
Replicate	2	2.29^{ns}	8.32^{ns}	0.32^{ns}	18.55^{ns}	46.02^{ns}	45.11^{ns}
Week	13	10809.03***	8.55***	0.33***	19.40***	48.70***	47.78***
Genotype × concentration	24	18.39***	0.43^{ns}	0.004^{ns}	0.52^{ns}	1.06^{ns}	1.04^{ns}
Concentration × replicate	8	18.12***	0.22^{ns}	0.01^{ns}	0.68^{ns}	1.23^{ns}	1.23^{ns}
Concentration × week	52	27.13***	0.33ns	0.01***	1.22***	2.24**	2.22***
Genotype × replicate	14	3.34^{ns}	1.73***	0.03***	3.99***	8.76***	8.61***
Genotype × week	91	113.7*	1.63***	0.03***	3.68***	7.87***	7.74***
Week × replicate	26	1.71^{ns}	5.50**	0.21***	11.89***	30.42***	29.83***
Genotype × concentration × replicate	47	3.44^{ns}	0.46^{ns}	0.01^{ns}	0.60^{ns}	1.22^{ns}	1.19^{ns}
Genotype × concentration × week	309	14.20***	0.43^{ns}	0.004^{ns}	0.52^{ns}	1.06^{ns}	1.04^{ns}
Concentration × week × replicate	104	14.29***	0.21^{ns}	0.05^{ns}	0.68^{ns}	1.24^{ns}	1.23^{ns}
Genotype × week × replicate	182	2.48^{ns}	1.71***	0.03***	3.99***	8.76***	8.61***
Error	594	3.54	0.46	0.005	0.61	1.22	1.19
Corrected total	1477						

*Note: * = significant at p<0.05, ** = highly significant at p<0.01, *** = highly significant at p<0.001*

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The result in table 3 reveals the germination and growth response of different genotypes of Bambara groundnut to Sodium azide mutagen. The highest (5.24%) and lowest (1.59%) were recorded in TVSu-86 and L_1, respectively. The germination percentage of L_1 genotype differ significantly from TVSu-86, TVSu-186, TVSu-350 and TVSu-235, but not significantly different from TVSu-242 and TVSu-91. The plant height of TVSu-86, TVSu-91, TVSu-186, TVSu-235, TVSu-242, L_1 and L_2 differ significantly from each other while plant height of TVSu-235 and TVSu-350 did not differ from each other significantly. The leaf length of L_1 genotype did not differ significantly from that of L_2 but differs significantly from other genotypes. The leaf length of TVSu-235 and TVSu-242 did not differ significantly from each other. TVSu-186 had the highest leaf length (7.11 cm) while TVSu-91 had the lowest leaf length (2.98 cm). TVSu-86 had the highest number of leaves (124.09) while L_2 genotype had the smallest (43.64) number of leaves. The number of leaves of TVSu-91 was significantly different from all other genotypes while the number of leaves of TVSu-186, TVSu-235 and TVSu-350 were not different from each other significantly. The number of branches of TVSu-86 was not significantly different from TVSu-186. The highest number of branches (42.49) was recorded in TVSu-235. Number of branches recorded in Tvsu-235 was significantly different from those in TVSu-91, TVSu-242, and the landraces. The leaf width of L_1 genotype was significantly different from other genotypes while those of TVSu-235 and TVSu-350 were not significantly different from each other.

Table 3. Genotypic effect on germination and growth response of Bambara groundnut to Sodium azide

Genotype	Germination percentage *(%)*	Plant length *(cm)*	Leaf length *(cm)*	Number of leaves	Number of branches	Leaf width *(cm)*
TVSu-86	5.24^{a}	21.51^{d}	5.45^{d}	124.09^{a}	42.49^{a}	1.93^{d}
TVSu-91	2.54^{bc}	12.80^{g}	2.98^{f}	57.74^{d}	19.63^{e}	1.16^{f}
TVSu-186	4.77^{a}	26.21^{a}	7.11^{a}	117.78^{ab}	41.03^{ab}	2.26^{b}
TVSu-235	4.92^{a}	25.08^{b}	6.07^{c}	118.28^{ab}	40.20^{ab}	2.44^{a}
TVSu-242	2.38^{bc}	23.00^{c}	5.95^{c}	73.46^{c}	26.02^{c}	2.13^{c}
TVSu-350	3.81^{ab}	25.53^{ab}	6.69^{b}	110.19^{b}	38.65^{b}	2.43^{a}
L_1	1.59^{c}	18.76^{f}	4.73^{e}	68.52^{c}	23.28^{d}	2.19^{bc}
L_2	4.76^{a}	20.00^{e}	4.92^{e}	43.64^{e}	15.05^{f}	1.69^{e}

Means with different letter within a column differ significantly at $p<0.05$

The effect of different concentrations of Sodium azide on growth of Bambara groundnut as seen in Table 4 reveals that the germination percentage of control (0.00%) was significantly different from those mutated with 0.03% and 0.07%, while those mutated with 0.01% of Sodium azide was not significantly different from those mutated with 0.03 %, 0.05% and 0.07% of Sodium azide. The highest (25.10 cm) and lowest (18.15 cm) plant height were recorded in control and 0.07% of Sodium

azide respectively. The plant height of Bambara groundnut mutated with Sodium azide differ from each other significantly. The leaf length and width, number of leaves and branches of both mutated and unmutated Bambara groundnut differ from each other significantly. The highest and smallest leaf length and width were recorded in control and those mutated with 0.07% of Sodium azide, respectively. The highest (108.46) and lowest (93.12) number of leaves were recorded in untreated and those mutated with 0.01% of Sodium azide, respectively. The highest (37.51) and lowest (28.30) number of branches were recorded in unmutated (control) and those mutated with 0.03% of Sodium azide.

Table 4. Effect of Sodium azide concentrations on growth of Bambara groundnut

Sodium azide concentrations *(%)*	Germination *(%)*	Plant length *(cm)*	Leaf length *(cm)*	Number of leaves	Number of branches	Leaf width *(cm)*
0.00 (Control)	4.37^{a}	25.10^{a}	6.33^{a}	108.46^{a}	37.51^{a}	2.28^{a}
0.01	3.74^{ab}	22.41^{b}	5.84^{b}	93.12^{b}	32.77^{b}	2.29^{a}
0.03	3.18^{b}	20.12^{c}	5.18^{c}	83.35^{c}	28.30^{c}	1.94^{c}
0.05	3.86^{ab}	23.03^{b}	5.83^{b}	95.48^{b}	32.59^{b}	2.09^{b}
0.07	2.98^{b}	18.15^{d}	4.58^{d}	93.38^{b}	32.36^{b}	1.69^{d}

Means with different letter within a column differ significantly at p<0.05

The result in Table 5 shows the response of the effect of Sodium azide concentrations on protein content, agronomic and yield characters of Bambara groundnut. There is a significant difference in the number of days to flowering among the genotypes except for TVSu-86, TVSu-235 and TVSu-350 which are not significantly different while the seed yield per plant had no significant difference among the genotypes except TVSu-235 which had the highest seed yield of 0.25g. There was no significant increase in the protein content of 0.00% and 0.01% of Sodium azide but there was significant difference in the protein content of 0.03% and 0.05% of Sodium azide.

Table 5. Effect of Sodium azide concentrations on protein content, agronomic and yield characters of Bambara groundnut

Sodium azide concentrations	Number of days to flowering	Seed yield per plant *(g)*	Seeds per pod	Pod yield per plant *(g)*	Number of seeds per plant	Number of pods per plant	Protein content *(%)*
0.00% (Control)	3.49^{a}	0.12^{a}	0.02^{a}	0.22^{a}	0.31^{a}	0.30^{a}	18.75^{c}
0.01%	3.04^{b}	0.07^{a}	0.02^{a}	0.11^{a}	0.28^{a}	0.20^{a}	18.76^{c}
0.03%	2.60^{c}	0.05^{a}	0.01^{a}	0.20^{a}	0.20^{a}	0.14^{a}	18.95^{b}
0.05%	3.15^{b}	0.10^{a}	0.02^{a}	0.16^{a}	0.27^{a}	0.27^{a}	19.12^{a}
0.07%	2.08^{d}	0.10^{a}	0.00^{a}	0.11^{a}	0.18^{a}	0.18^{a}	19.06^{a}

Means with different letter within a column differ significantly at p<0.05

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The highest protein content (19.12%) was recorded in Bambara groundnut treated with 0.05% of Sodium azide while the lowest protein content (18.75%) was recorded in untreaated (control) Bambara groundnut. The genotypic effect of Sodium azide on the protein content of Bambara groundnut differed from each other significantly $p<0.05$ The highest (19.38%) and lowest (18.50%) protein content were recorded in TVSu-235 and TVSu-86, respectively as seen in Table 6.

Table 6. Genotypic effects on protein contents, agronomic and yield of Bambara groundnut induced with Sodium azide

Genotype	Number of days to flowering	Seed yield per plant *(g)*	Seeds per pod	Pod yield per plant *(g)*	Number of seeds per plant	Number of pods per plant	Protein content *(%)*
TVSu-86	2.86^{cd}	0.17^{ab}	0.03^{a}	0.22^{abc}	0.32^{ab}	0.31^{ab}	18.50^{e}
TVSu-91	2.04^{e}	0.02^{b}	0.01^{bc}	0.03^{dc}	0.06^{bc}	0.05^{bc}	19.15^{b}
TVSu-186	3.44^{b}	0.05^{b}	0.02^{ab}	0.08^{bcd}	0.14^{bc}	0.14^{bc}	18.85^{d}
TVSu-235	2.49^{d}	0.25^{a}	0.03^{a}	0.37^{a}	0.50^{a}	0.49^{a}	19.38^{a}
TVSu-242	3.59^{b}	0.02^{b}	0.01^{bc}	0.04^{dc}	0.08^{bc}	0.08^{bc}	19.00^{c}
TVSu-350	3.21^{bc}	0.14^{ab}	0.02^{ab}	0.25^{ab}	0.46^{a}	0.46^{a}	18.80^{d}
L_1	1.89^{e}	0.00^{b}	0.00^{c}	0.02^{dc}	0.00^{c}	0.02^{d}	-
L_2	6.76^{a}	0.00^{b}	0.00^{c}	0.00^{d}	0.00^{c}	0.00^{c}	-

Means with different letter within a column differ significantly at $p<0.05$

The Correlation coefficient of Bambara groundnut genotype to Sodium azide in Table 7 showed that the number of branches is positively correlated with plant height and number of leaves at r=0.510 and r=0.98; $p<0.05$ and $p<0.01$ respectively. The number of leaves, leaf length and leaf width at r=0.50; r=0.85; and r=0.74 are positively and strongly associated with plant height at $p<0.05$ and 0.01 respectively, while the leaf length shows positive and strong relationship with leaf width at r=0.71. The pod per plant is strongly and positively related with seed yield and seed per pod at r=0.89 and r=0.85 at $p<0.01$ respectively, while the number of seed per plant is strongly and positively related with seed yield and seed per pod at r=0.90 and r=0.85 at $p<0.01$ respectively. Also, the seed yield (g) and number of seed per pod at r=0.94 and r=0.86 are strongly and positively correlated with pod yield while the seed yield is positively and strongly correlated with seeds per pod at r=0.84 at $p<0.01$. The pod per plant is strongly and positively associated with number of seeds and pod yield at r=0.99 and r=0.96 at $p<0.01$ respectively while the number of seeds is positively and strongly correlated with pod yield at r=0.96; $p<0.01$

Table 7: Correlation coefficient of growth, agronomic and yield characters of Bambara groundnut induced with Sodium azide.

	G %	NB	PH	NL	LL	LW	DF	PP	NS	PY	SY	S/P	G	C	W	R
Germination %																
Number of branches	-0.24															
Plant height	0.03	0.51*														
Number of leaves	-0.23	0.98**	0.50*													
Leaf length	0.04	0.48	0.85**	0.47												
Leaf width	-0.05	0.45	0.74**	0.45	0.71**											
Days to flowering	-0.05	0.10	0.04	0.07	0.03	0.05										
Pods per plant	-0.024	0.09	0.03	0.07	0.03	0.04	0.35									
Number of seeds	-0.02	0.09	0.03	0.07	0.03	0.04	0.35	0.99**								
Pod yield (g)	-0.02	0.08	0.02	0.07	0.03	0.04	0.33	0.96**	0.96**							
Seed yield (g)	-0.02	0.07	0.02	0.06	0.02	0.03	0.29	0.89**	0.90**	0.94**						
Seeds per pod	-0.03	0.08	0.03	0.06	0.03	0.05	0.38	0.85**	0.85**	0.86**	0.84**					
Genotype	-0.04	-0.12	0.18	-0.12	0.18	-0.12	0.21	0.25	0.01	-0.01	-0.02	-0.04				
Concentration	-0.02	-0.06	-0.23	-0.06	-0.21	-0.22	0.02	-0.02	-0.02	-0.02	-0.01	-0.02	-0.09			
Week	-0.37	0.51*	0.11	0.49	0.05	0.16	0.40	0.18	0.18	0.17	0.16	0.20	-0.02	-0.03		
Replicates	-0.00	0.01	0.04	0.02	0.01	0.01	0.00	-0.12	-0.12	-0.12	-0.11	-0.14	0.01	0.03	-0.00	

*Note: * = related (correlated) at p<0.05, ** = strongly related at p<0.01. G% = Germination percentage, NB = Number of branches, PH = Plant height, NL = Number of leaves, LL = Leaf length, LW = Leaf width, DF = Days to flowering, PP = Pods per plant, NS = Number of seeds, PY = Pod yield, SY = Seed yield, S/P = Seeds per pod, G = Genotype, C = Concentration, W = Week and R = Replicate*

There were variations in the expression of the mutagenic effect of Sodium azide at different concentrations on the bambara genotypes based on the morphological and agronomical traits. The mutant traits were expressed by the plants TVSu-186(0.07%) with purple seedling, stunted growth and death of plant within 5days-10days after planting (Plate 1a). TVSu-186(0.00%) had normal plant growth with green seedling at 5 days after planting (Plate 1b). Abia (L_1) landraces induced with 0.05% Sodium azide showed creeping stem and yellow leaf colour at 4 weeks after planting (Plate 2a). TVSu-91 induced with 0.05% Sodium azide showed two different leaf shape in a single plant stand at 4 weeks after planting (Plate 2b). Abia (L_1) genotype induced with 0.01% Sodium azide showed crinkled (rough and twisting) leaves at 4 weeks after planting (Plate 3). TVSu-86 (0.07%) induced with Sodium azide possessed two seeds per pod after harvest (Plate 4). The observed effect of Sodium azide mutagen on the number of polypeptide band using SDS- PAGE showed that there was increase in the number of polypeptide band as the concentration of Sodium azide increased (Plate 5).

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Plate 1a. TVSu-186 (0.07%) induced with Sodium azide showing purple seedling

Plate1b. TVSu-186 (0.00%) control showing normal colour

Plate 2a. Abia landraces induced with 0.05% Sodium azide showed creeping Stem and yellow leaf colour.

Plate 2b .TVSu-91 induced with 0.05% Sodium azide having two different leaf shape in a single plant stand.

Plate 3. Abia landrace induced with 0.01% Sodium azide showed crinkled (rough and twisting) leaves.

Plate 4. TVSu-86 (0.07%) produced mutants with two seeds per pod which was not observed in other concentrations.

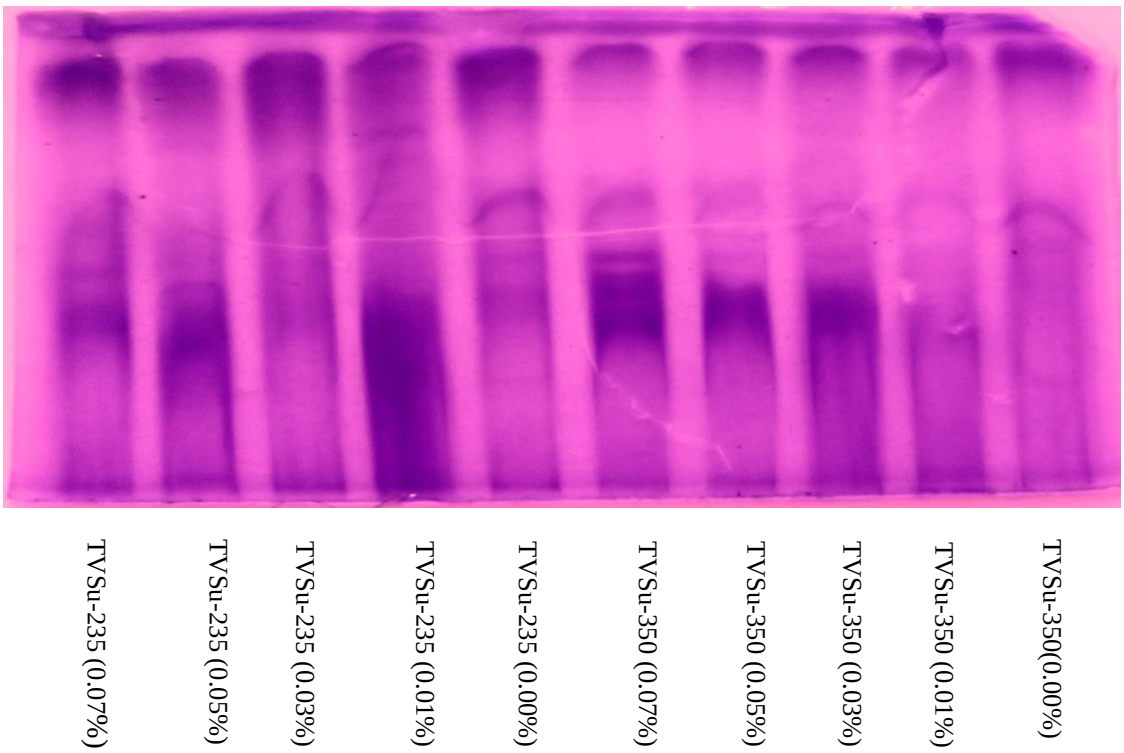

Plate 5. SDS-polyacrylamide gel electrophoregram of seed protein profile.

CONCLUSION

There was reduction in the germination percentage and growth characters as the concentration of Sodium azide increases as shown in (Table 1). This could be as a result of the lethal effect caused by the mutagen. Similar reductions were also observed in maize in the reports made by Odeigah and Osanyinpeju (1998).

Early flowering was recorded in Bambara groundnut (Table 2) treated with 0.07% of Sodium azide compared to the control which flowered late. There was no significant difference in yield traits among mutants and control, but the mutants and control of landraces could not survive the environment, therefore did not produce yield (Table 3).

The SDS-PAGE analysis revealed the presence of polypeptide bands in mutants which could be due to the damaging effect of the mutagen on different genes responsible for the polypeptide subunits. Similar occurrence had also been reported in Cowpea by [Mahamune, et al., 2017; Odeigah and Osanyinpeju (1998)].

The consumption of any legumes may result in flatulence and constipation which could be attributed to higher concentration of Sodium azide in pesticide used in controlling pest damaging field crops (Mostafa 2011). Mutant seeds had significant increase in protein content. The concentration of 0.05% Sodium azide had the highest protein content (19.12%), while control had (18.5%). The increase in protein content at higher concentration of Sodium azide could be due to increase in auxin

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level. Similar results were observed in French bean by Mahamune, et al., 2017.

The concentration of 0.05% Sodium azide mutagen reduced germination, growth and yield but increased the protein content of Bambara groundnut compared with control. TVSu-235 and TVSu-350 genotypes had higher tolerance and yield response to Sodium azide concentrations therefore; they could be further utilised in crop improvement. The growth and yield traits should also be encouraged in mutagenic breeding of other leguminous crops.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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