STUDIES ON BAMBARA GROUNDNUT (Vigna subterranea (L.)Verdc.) INDUCED WITH SODIUM AZIDE USING SODIUM DODECYL SULPHATE POLYACRLYAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

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Abstract

This study investigated the mutagenic tolerance of eight Bambara groundnut genotypes to Sodium azide (NaN3) using SDS-PAGE. The seeds of six genotypes; TVSu-86, TVSu-91, TVSu-186, TVSu-235, TVSu-242, TVSu-350 were collected from IITA and two landraces from Abia and Enugu local markets. The seeds were treated with five concentrations: 0.00%(control), 0.01%, 0.03%, 0.05% and 0.07% of NaN3 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 NaN3 increases. Early flowering was recorded at 37 days mutated with 0.07% of NaN3 compared to control which flowered late at 42 days. NaN3(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 NaN3 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 NaN3, thus could be further improved in subsequent breeding.

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.)Verdc,), member of the family Fabaceae is an indigenous, underutilized African legume crop mainly grown by subsistence farmers for food (Bamishaiye *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 (Aremu *et al.,* 2006). Genetic variation is fundamental to successful breeding programs in vegetatively and sexually propagated plants (Olawuyi*et al* ., 2015; Agbolade *et al* ., 2016). 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 (Wongpiyasatid *et al* ., 2000; Mensah and Obadoni, 2007; Arulbalachandran *et al* ., 2009; Olawuyi *et al* ., 2016).

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 (Mennella *et al* ., 1999). Iqbal *et al* . (2005) 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 (Ghafoor*et al* ., 2002).

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 (Ilbas *et al* ., 2005; Mostafa, 2011).

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

MATERIALS AND METHODS

Collection of seed samples, experimental location and experimental design

Six (6) Seeds of Bambara Genotypes: 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 were collected from Abia State and Enugu State, Nigeria.

The *in vitro* and screen house experiments were carried out at the Genetics and Molecular Laboratory and Nursery farm of the Department of Botany, University of Ibadan, Nigeria respectively. The design was laid out in Complete Randomized Design (CRD) comprising of 6 genotypes, 2 landraces, 5 concentrations and 3 replicates.

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

The seeds of Bambara were treated with concentrations of sodium azide (0.01%, 0.03%, 0.05% and 0.07%) after pre-soaked in 100ml of distilled for six(6) hours at room temperature (25°C), while untreated seeds (0.00%) served as control according to the method described by Asad *et al.* (2014). Five seeds of both treated and pre-soaked seeds 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 Asgharipour and Rafiei (2011).

Research design and Screen house experiment

10kg of sterilized top soil was measured using a weighing scale and put into 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). 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 (2000) Descriptors for Bambara groundnut. The crude protein content was determined using micro Kjeldahl method as described in AOAC (1996) 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 Weber and Osborn (1975).

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[?]0.05).The final germination percentage (FGP) was obtained using Cokkizgin and Cokkizgin (2010) method as stated below;

$$\text{FGP} = \frac{number\ of\ germinated\ seeds\ after\ 8\ days}{\text{total number of seeds planted}} \quad \times \quad 100$$

RESULTS AND DISCUSSION

There was reduction in the germination percentage and growth characters as the concentration of sodium azide increases. 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 Olawuyi and Okoli (2017).

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
Concentration	4	9.69^{ns}	2121.02***	134.37***	42476.98***
Genotype	7	385.48***	3321.67***	274.36***	143861.73***
Replicate	2	2.32^{ns}	60.89 ^{ns}	11.92 ^{ns}	5453.61 ^{ns}
Week	13	17146.10***	34.80***	2.07^{ns}	91419.258***
Genotype × concentration	24	87.58^{ns}	892.50***	52.29***	26745.69***
Concentration × replicate	8	74.82^{ns}	120.31***	5.18***	25610.86***
Concentration × week	52	99.59***	30.14***	2.18***	1828.05***
Genotype × replicate	14	73.01^{ns}	66.85***	3.29***	3478.95***
Genotype × week	91	385.60***	47.53***	3.61***	3786.25***
Week × replicate	26	10.52^{ns}	11.59^{ns}	0.69^{ns}	539.00^{ns}
Genotype × concentration × replicate	47	45.91^{ns}	117.29***	10.33***	13250.09***
Genotype × concentration × week	312	87.58***	32.89***	3.05***	2166.30**
Concentration × week × replicate	104	74.82***	15.29^{ns}	1.52*	1629.64***
Genotype × week × replicate	182	73.01***	18.88*	1.39^{ns}	1094.67^{ns}
Error	611	611	562	562	560
Corrected total	1497	1497	1446	1446	1444

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

Early flowering was recorded in Bambara groundnut mutated 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 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	P
Concentration	4	34.51***	0.35^{ns}	0.01^{ns}	1
Genotype	7	135.99***	1.63***	0.02***	3
Replicate	2	2.29^{ns}	8.32 ^{ns}	0.32 ^{ns}	1
Week	13	10809.03***	8.55***	0.33***	1
Genotype × concentration	24	18.39***	0.43^{ns}	0.004^{ns}	0
Concentration × replicate	8	18.12***	0.22^{ns}	0.01^{ns}	0
Concentration × week	52	27.13***	0.33ns	0.01***	1
Genotype × replicate	14	3.34^{ns}	1.73***	0.03***	3
Genotype × week	91	113.7*	1.63***	0.03***	3
Week × replicate	26	1.71^{ns}	5.50**	0.21***	1
Genotype × concentration × replicate	47	3.44^{ns}	0.46^{ns}	0.01^{ns}	0
Genotype × concentration × week	309	14.20***	0.43^{ns}	0.004^{ns}	0
Concentration × week × replicate	104	14.29***	0.21^{ns}	0.05^{ns}	0
Genotype × week × replicate	182	2.48^{ns}	1.71***	0.03***	3
Error	594	3.54	0.46	0.005	0

Source of variation	df	Number of days to flowering	Seed yield per plant (g)	Seeds per pod	P
Corrected total	1477				

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

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
TVSu 86	5.24^{a}	21.51^{d}	5.45^{d}	124.09^{a}	42.49^{a}
TVSu 91	2.54^{bc}	12.80^{g}	2.98^{f}	57.74^{d}	19.63^{e}
TVSu 186	4.77^{a}	26.21^{a}	7.11^{a}	117.78^{ab}	41.03^{ab}
TVSu 235	4.92^{a}	25.08^{b}	6.07^{c}	118.28^{ab}	40.20^{ab}
TVSu 242	2.38^{bc}	23.00^{c}	5.95^{c}	73.46^{c}	26.02^{c}
TVSu 350	3.81^{ab}	25.53^{ab}	6.69^{b}	110.19^{b}	38.65^{b}
Abia	1.59^{c}	18.76^{f}	4.73^{e}	68.52^{c}	23.28^{d}
Enugu	4.76^{a}	20.00^{e}	4.92^{e}	43.64^{e}	15.05^{f}

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

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
0.00 (Control)	4.37^{a}	25.10^{a}	6.33^{a}	108.46^{a}	37.51^{a}
0.01	3.74^{ab}	22.41^{b}	5.84^{b}	93.12^{b}	32.77^{b}
0.03	3.18^{b}	20.12^{c}	5.18^{c}	83.35^{c}	28.30^{c}
0.05	3.86^{ab}	23.03^{b}	5.83^{b}	95.48^{b}	32.59^{b}
0.07	2.98^{b}	18.15^{d}	4.58^{d}	93.38^{b}	32.36^{b}

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

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 level. Similar results were observed in French bean by Mahamune*et al* . (2017).

Table 5. 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
TVSu 86	2.86^{cd}	0.17^{ab}	0.03^{a}	0.22^{abc}	0.32^{ab}
TVSu 91	2.04^{e}	0.02^{b}	0.01^{bc}	0.03^{dc}	0.06^{bc}
TVSu 186	3.44^{b}	0.05^{b}	0.02^{ab}	0.08^{bcd}	0.14^{bc}
TVSu 235	2.49^{d}	0.25^{a}	0.03^{a}	0.37^{a}	0.50^{a}
TVSu 242	3.59^{b}	0.02^{b}	0.01^{bc}	0.04^{dc}	0.08^{bc}
TVSu 350	3.21^{bc}	0.14^{ab}	0.02^{ab}	0.25^{ab}	0.46^{a}
Abia	1.89^{e}	0.00^{b}	0.00^{c}	0.02^{dc}	0.00^{c}
Enugu	6.76^{a}	0.00^{b}	0.00^{c}	0.00^{d}	0.00^{c}

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

Table 6. Effect of sodium azide concentrations on protein contents, 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 plan
0.00% (Control)	3.49[a]	0.12[a]	0.02[a]	0.22[a]
0.01%	3.04[b]	0.07[a]	0.02[a]	0.11[a]
0.03%	2.60[c]	0.05[a]	0.01[a]	0.20[a]
0.05%	3.15[b]	0.10[a]	0.02[a]	0.16[a]
0.07%	2.08[d]	0.10[a]	0.00[a]	0.11[a]

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

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
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
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
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

*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*

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 Odeigah *et al.*(1998) and Mahamune *et al* . (2017).

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 (Ilbas *et al.*, 2005).

CONCLUSION

The concentration of 0.07% 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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Week	13	10809.03***	8.55***	0.33***	1
Genotype × concentration	24	18.39***	0.43^{ns}	0.004^{ns}	0
Concentration × replicate	8	18.12***	0.22^{ns}	0.01^{ns}	0
Concentration × week	52	27.13***	0.33ns	0.01***	1
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TVSu 91	2.54^{bc}	12.80^{g}	2.98^{f}	57.74^{d}	19.63^{e}
TVSu 186	4.77^{a}	26.21^{a}	7.11^{a}	117.78^{ab}	41.03^{ab}
TVSu 235	4.92^{a}	25.08^{b}	6.07^{c}	118.28^{ab}	40.20^{ab}
TVSu 242	2.38^{bc}	23.00^{c}	5.95^{c}	73.46^{c}	26.02^{c}
TVSu 350	3.81^{ab}	25.53^{ab}	6.69^{b}	110.19^{b}	38.65^{b}
Abia	1.59^{c}	18.76^{f}	4.73^{e}	68.52^{c}	23.28^{d}
Enugu	4.76^{a}	20.00^{e}	4.92^{e}	43.64^{e}	15.05^{f}

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

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
0.00 (Control)	4.37^{a}	25.10^{a}	6.33^{a}	108.46^{a}	37.51^{a}
0.01	3.74^{ab}	22.41^{b}	5.84^{b}	93.12^{b}	32.77^{b}
0.03	3.18^{b}	20.12^{c}	5.18^{c}	83.35^{c}	28.30^{c}
0.05	3.86^{ab}	23.03^{b}	5.83^{b}	95.48^{b}	32.59^{b}
0.07	2.98^{b}	18.15^{d}	4.58^{d}	93.38^{b}	32.36^{b}

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

Table 5. 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
TVSu 86	2.86^{cd}	0.17^{ab}	0.03^{a}	0.22^{abc}	0.32^{ab}
TVSu 91	2.04^{e}	0.02^{b}	0.01^{bc}	0.03^{dc}	0.06^{bc}
TVSu 186	3.44^{b}	0.05^{b}	0.02^{ab}	0.08^{bcd}	0.14^{bc}
TVSu 235	2.49^{d}	0.25^{a}	0.03^{a}	0.37^{a}	0.50^{a}
TVSu 242	3.59^{b}	0.02^{b}	0.01^{bc}	0.04^{dc}	0.08^{bc}
TVSu 350	3.21^{bc}	0.14^{ab}	0.02^{ab}	0.25^{ab}	0.46^{a}
Abia	1.89^{e}	0.00^{b}	0.00^{c}	0.02^{dc}	0.00^{c}
Enugu	6.76^{a}	0.00^{b}	0.00^{c}	0.00^{d}	0.00^{c}

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

Table 6. Effect of sodium azide concentrations on protein contents, 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 plan
0.00% (Control)	3.49^{a}	0.12^{a}	0.02^{a}	0.22^{a}
0.01%	3.04^{b}	0.07^{a}	0.02^{a}	0.11^{a}
0.03%	2.60^{c}	0.05^{a}	0.01^{a}	0.20^{a}
0.05%	3.15^{b}	0.10^{a}	0.02^{a}	0.16^{a}
0.07%	2.08^{d}	0.10^{a}	0.00^{a}	0.11^{a}

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

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
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
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
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

*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*

Plate 1a. TVSu-186 (0.07%) induced with **Plate 1b** . TVSu-186 (0.00%) control Sodium azide showing purple seedling showing normal colour

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

Plate 2b .TVSu-91 induced with 0.05% Sodium azide had two different leaves 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.

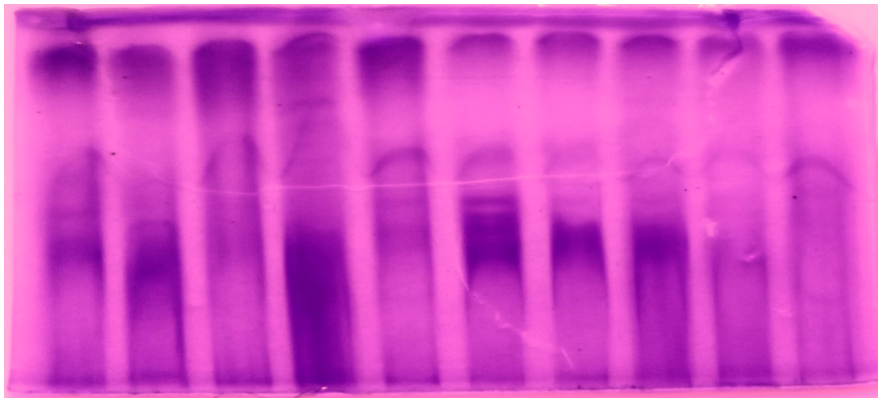

Plate 5 . SDS-polyacrylamide gel electrophoregram of seed protein profile.

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`ArtiCle Figures.pdf` available at https://authorea.com/users/361769/articles/483110-studies-on-bambara-groundnut-vigna-subterranea-l-verdc-induced-with-sodium-azide-using-sodium-dodecyl-sulphate-polyacrlyamide-gel-electrophoresis-sds-page