International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 2 Issue 6‖ June2013 ‖ PP.13-20

Polyacrylamide Gel Electrophoresis Determination of Genetic Variabilities Among 24 Underutilized Legume Accessions.

[1] Agbolade, J.O., [2].Okonji R., [3*]Olakunle, T.P., [4]Olayiwola, O.A., [5].Akinro E.B., And [6]Aasa-Sadique, A.D.

[1] Department Of Biological Sciences, Oduduwa University, Ipetumodu, Ile-Ife, Nigeria.
[2]Department Of Biochemistry,Obafemi Awolowo University, Ile-Ife, Nigeria.
[3] Department Of Applied Sciences, Osun State Polytechnic, Iree, Nigeria.*
[4] Department Of Chemical Sciences, Oduduwa University, Ipetumodu, Ile-Ife, Nigeria.
[5] Department Of Science Laboratory Technology, Osun State Polytechnic, Iree, Nigeria.
[6] Department Of Basic Sciences, Osun State Polytechnic, Iree, Nigeria.

ABSTRACT : *Twelve species of twenty four accessions miscellaneous legumes were obtained from germplasm unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. These were accessed for their genetic relationship and phylogenic relatedness through electrophoretic analysis of the seed proteins. Protein characterization with standard marker revealed that seeds of the 24 accessions contained proteins (albumin, globulin and vicilin) with molecular weights ranging from 65 - 122 kda, 45 – 64 kda and 15 – 44 kda respectively. The proportions of albumin to globulin, albumin to vicilin, globulin to vicilin and albumin to vicilin to globulin were ½, 5/24, 1/3, and 5/24 respectively. All the accessions had at least two proteins in common. The study revealed intra-specific similarities and inter-specific genetic diversity in protein contents among the 24 miscellaneous legumes accessions.*

KEYWORDS*: Germplasm, miscellaneous legumes, phylogenetic, electrophoretic, genetic, marker.*

I. INTRODUCTION

The term legume is applied broadly to all plants of the pea and bean family (Leguminosae) which comprises the Caesalpinaceae (Senna family), Mimosaceae (Locust bean family) and Pappillonacea (comprising about 10 tribes) [1],[2]. Legume is a simple dry fruit formed from a superior monocarpus pistil; the pericarp dehisces at maturity longitudinally along both sides to liberate the seeds therein. The seeds are usually arranged along one of the margins of the fruits [3],[2]. The family Leguminosae embraces a large group of dicotyledonous plants having their fruits as pods, which may be round or flat, sometimes winged, straight or curved, of variable length, fibrous or fleshy and which often split open at maturity. The flowers are mostly complete and irregular. The calyx has five, more or less unequal and partially united sepals, and these flowers are usually hermaphroditic. The leaves are usually alternate, pinnately compound or trifoliolate [4], [5].Grain legumes have become a major component of grain – based farming systems in many parts of the world. In terms of world economy and plant utility, grain legumes are grouped into two as major and minor species . The major species include the industrial legumes such as soybean and groundnut, which are extremely important in the world economy. Others are common beans (*Phaseolus vulgaris),* chickenpea *(Cicer arietinum),* and pea *(Pisum sativum).* Minor species exist in a wide range of diversity either as cultivated or wild species across various regions of the world and are usually cultivated by the traditional farmers. The wild species of the minor grain legumes include kersting groundnut *(Kerstingiella geocarpa),* marama bean *(Tylosema esculentum).* The minor grain legumes have also been referred to as miscellaneous, neglected, underutilized, under-cultivated or lesser – known legumes [6],[7], *,[8]*.The miscellaneous legumes are the minor grain legumes that have received very little research attention when compared with the major grain legumes such as cowpea and soybean. This neglect has lead to the loss or genetic erosion of the germplasm of many of the minor legumes. Most of the research efforts on miscellaneous legumes improvements have been on chemical composition and nutritional values. Majority of the representative miscellaneous legumes have not witnessed considerable research attention over the years [9],[10]. Consumption of legumes has been highly correlated with reduced mortality resulting from coronary heart disease [6]. Studies have also been carried out on the *invitro* multi-enzyme digestibility of flowers proteins of six varieties of African Yam bean[11].[12],[13],[14] have also reported on the nutritional values of African yam bean.

The current study aims at carrying out electrophoretic analysis of the seed proteins of twelve species of twenty four accessions underutilized legumes including Bambara groundnut (*Vigna subterranean (L.) Thouars)* (TVSu 1126 and TVSu 1415), Green gram (*Vigna radiata (L.) R.Wilczek) (TVr 145 and TVr 1001*), Jack bean

(*Canavalia ensiformis (L.*) DC) (TCe1 and TCe3), Mung bean (*Vigna mungo (L.*) Hepper) (TVm 12 and TVm 13), Pigeon pea (*Cajanus cajan (L.*) DC) (TCc 8127 and TCc8156), Rice bean (*Vigna angularis (L.*) Thouars) (TVa 1 and TVa 1173), African yam bean (*Sphenostylis stenocarpa (Hochst Ex.A. Rich*) Harms (TSs 137 and TSs 156), *Kersting* groundnut (*kerstingiella geocarpa (Harms*), (TKg 6 and TKg 12) lablab (*Lablab purpureus (var. lignosus*) (TLn 21 and TLn 29), Mexican yam bean (*Pachyrhizus tuberosus (Lam.*) (TPtu 1 and TPtu 5), Sword bean (*Canavalian gladiata (Jacq.*) DC) (TCg 1 and TCg 4) and Winged bean (*Psophocarpus tetragonolobus (L.*) DC) (TPt 12 and TPt 18) with a view to revealing their genetic variations.

II. MATERIALS AND METHOD

Polyacrylamide gel electrophoresis was carried out according to the procedure described by Pharmacia [15] on 7.5% rod gel. Dried seeds of each of the twenty four accessions of the miscellaneous legumes were blended with domestic blender. One (1) gram each of the seed powder was suspended in 10ml of 0.2M Phosphate Buffer Saline (PBS) (pH7.2) for 12 hours with occasional stirring to ensure complete dissolution of all the soluble proteins.The crude homogenate was thereafter centrifuged at 4000rpm for 20minutes to remove cellular debris. The protein sample was prepared by adding 50 µl of the resultant supernatant to 20µl of the tracking dye, bromophenol blue (0.05 % bromophenol blue in 0.01 M sodium phosphate buffer, pH 7.2) as described by Weber and Osborn [16]. One drop of glycerol was added to this mixture to make it dense. An aliquot of 20 µl of the mixture was then layered on 7.5% acrylamide gel. The gels were run at 8 mA per gel at room temperature. After electrophoresis the gels were stained without fixation for 2 hr with a staining solution (1.25 g Coomassie Brilliant Blue R250, 227 ml of methanol, 46 ml of glacial acetic acid made up to 500 ml with distilled water). Destaining was carried out in a solution containing 7.5% methanol and 5% glacial acetic acid. Photographs of the gels were taken and schematic representation was made.

III. MOLECULAR DATA ANALYSIS

electrophoretic gel. Data on the location of the bands were converted to their respective molecular weight in kilo Graduated meter rule was used to measure the location of each band on the Dalton. Protein types apportioned to each accession were characterized using standard marker based on their molecular weights. Dendrogram was generated to show the grouping of the 24 accessions of the miscellaneous legumes according to their respective molecular weights. Venn diagram was also used to represent the similarities in the protein composition of the species and accessions.

IV. RESULT AND DISCUSSION

The results of the electrophoresis of crude protein from the twenty four accessions of the twelve species of miscellaneous legume studied are shown in Plates 1 and 2. Table 1 shows the list of the accession used for the electrophoresis and the number of bands observed for each accession. Table 2 shows the relationship among the species and the accession of the miscellaneous legumes studied in terms of the number of bands the species and the accession had in common with one another, with respect to their molecular weights while Table3 shows characterization of the proteins based on their molecular weight. The bands were arbitrarily classified as slow, intermediate, fast and very fast on the basis of the mobility. Bands occurring in the region between 0 - 1.9cm of the gel length were termed slow bands, those in the region between 2cm and 4cm were termed intermediate, and those in the region between 4.1cm and 6.0cm were termed fast bands while very fast bands are those occurring between the region of 6.1cm and 8.1cm and those beyond. The result of electrophoresis shows that some of members of the population are quite dissimilar both in terms of number and intensity of the bands while some other ones show a certain degree of relatedness, especially more among the subspecies. They revealed discernible identities which were indications of intra- specific relationships among the accession of each species of the miscellaneous legumes studied. The result was in accordance with that of[2] .The number of bands observed varied from one (1) in TPtu1 and TPtu18 (*Pachyrizus tuberosus),* TCe1 and TCe3 (*Canavalia ensiformis)* (Plate 20) to six in TPt18 (*Psophocarpus tetragonolobus)* and TCc8156 (*Cajanus cajan)* (Plate 20). All the populations had at least one major band.

Plate 1: Crude Protein electrophoresis of the twenty four accessions of the miscellaneous legumes

A	- TCg1	– Sword bean	M -	TLn21	- Lablab bean Lablab
B	- TCg4	- Sword bean	N -	TLn29	- Lablab bean
C	- TPtu1	- Mexican bean	O –	TSs 137	– African yam bean
D	– TPtu5	– Mexican bean	P –	TSs156	– African yam bean
E	– TPt12	– Winged bean –	Q -	TVSul126	– Bambara groundnuts
F	– TPt18	– Winged bean -	R -	TVSu1415	– Bambara groundnuts
G	- TCe1	- Jack bean	S –	TKg6	– Kersting groundnut
H	– TCe3	– Jack bean	T -	TKg12	- Kersting groundnut
I	– TVal	– Rice bean	U –	TVr145	– Green gram
J	–TVal173	– Rice bean	V –	TVr1001	– Green gram
K	–TVm12	– Mung bean	W -	TCc8127	– Pigeon pea
L	– TVm13	– Mung bean	X –	TCc8156	– Pigeon pea

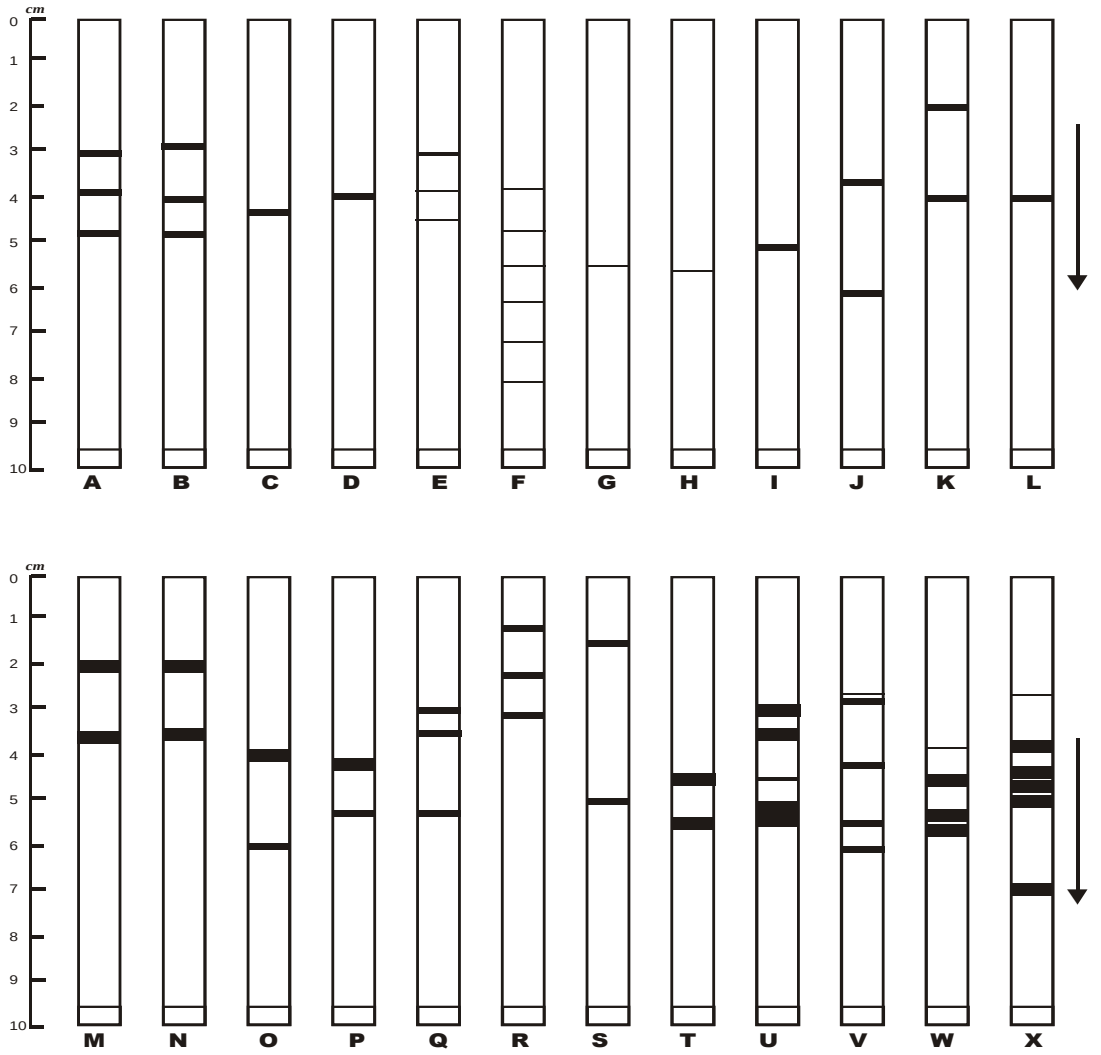


Plate 6: Schematic representation of the crude protein electrophoresis of twelve species of twenty four accessions of the miscellaneous legumes studied.

- A – TCg1 – Sword bean
- B – TCg4 - Sword bean
- C – TPtu1 – Mexican yam bean
- D – TPtu1 – Mexican yam bean
- E– TPt12 – Winged bean
- F – TPt18 – Winged bean
- G – TCel – Jack bean
- H – TCe3 – Jack bean
- I – TVal – Rice bean
- J – TVal173 – Rice bean
- K – TVm12 – Mung bean
- L – TVm13 – Mung bean
- M – TLn21 – Lablab bean
- N – TLn29 – Lablab bean
- O – TSs 137 – African yam bean
- P – TSs156 – African yam bean
- Q – TVSul126 – Bambara groundnuts
- R – TVSu1415 - Bambara groundnuts
- S – TKg6 – Kersting groundnut
- T – TKg6 – Kersting groundnut
- U – TVr145 – Green gram
- V – TVr1001 – Green gram
- W – TCc8127 – Pigeon pea
- X - TCc8156 – Pigeon pea

Table 1: Relationships Between Species And Accessions of Miscellaneous Legumes Studied on The Basis of the Relative Mobility of the Bands and their Closeness to One Another.

NAME OF SPECIES (ACC. NO)	SLOW BANDS (0-1.9) cm	INTERMEDIATE BANDS (2.0-4.0) cm	FAST BANDS (4.1-6.0) cm	VERY FAST BANDS (6.1-8.1) cm	TOTAL NUMBER OF BANDS (TNB)	UNIQUE BANDS
TCg1	-	2	1	-	3	-
TCg4	-	2	1	-	3	-
TPtu1	-	-	1	-	1	1
TPtu5	-	1	-	-	1	-
TPt12	-	2	1	-	3	-
TPt18	-	1	2	3	6	4
TCe1	-	-	1	-	1	-
TCe3	-	-	1	-	1	1
TVa1	-	1	1	-	2	2
TVa1173	-	1	-	1	2	1
TVm12	-	2	-	-	2	-
TVm13	-	1	-	-	1	-
TLn21	-	2	-	-	2	2
TLn29	-	2	-	-	2	1
TSs137	-	1	1	-	2	2
TSs156	-	-	2	-	2	3
TVSu1126	-	2	1	-	3	-
TVSu1415	1	2	-	-	3	3
TKg6	1	-	1	-	2	1
TKg12	-	1	2	-	3	-
TVr145	-	2	3	-	5	2
TVr1001	-	2	2	1	5	5
TCc8127	-	1	3	-	4	3
TCc8156	-	2	3	1	6	2

A – TCg1 – Sword bean
B – TCg4 – Sword bean
C – TPtu1 – Mexican yam bean
D – TPtu5 – Mexican yam bean
E – TPt12 – Winged bean
F – TPt18 – Winged bean
G – TCe1 – Jack bean
H – TCe3 – Jack bean
I – TVal – Rice bean
J – Tva1173 – Rice bean
K – TVm12 – Mung bean
L – TVm13 – Mung bean
M – TLn21 – Lablab bean
N – TLn29 – Lablab bean
O – TSs 137 – African yam bean
P – TSs156 – African yam bean
Q – TVSul126 – Bambara groundnuts
R – TVSu1415 – Bambara groundnuts
S – TKg6 – Kersting groundnut
T – TKg12 – Kersting groundnut
U – TVr145 – Green gram
V – TVr1001 – Green gram
W – TCc8127 – Pigeon pea
X – TCc8156 – Pigeon pea

Table 2: Pattern of Bands distribution among the Miscellaneous Legumes relative to their Molecular Weights

	Molecular weight (Kda)													
Values	**6.5**	**14**	**20**	**29**	**36**	**41**	**45**	**50**	**55**	**63**	**66**	**69**	**84**	**97**
TCg1	0	0	0	0	0	0	1	0	1	0	1	0	0	0
TCg4	0	0	0	0	0	0	1	0	1	0	1	0	0	0
TPtu1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
TPtu5	0	0	0	0	0	0	0	0	1	0	0	0	0	0
TPt12	0	0	0	0	0	0	1	0	1	0	1	0	0	0
TPt18	0	0	1	1	1	1	1	0	1	0	0	0	0	0
TCe1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
TCe3	0	0	0	0	0	1	0	0	0	0	0	0	0	0
TVa1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
TVa1173	0	0	0	0	1	0	0	0	1	0	0	0	0	0
TVm12	0	0	0	0	0	0	0	0	1	0	0	0	1	0
TVm13	0	0	0	0	0	0	0	0	1	0	0	0	0	0

TLn21	0	0	0	0	0	0	0	0	0	1	0	0	1	0
TLn29	0	0	0	0	0	0	0	0	0	1	0	0	1	0
TSs156	0	0	0	0	0	1	0	0	1	0	0	0	0	0
TSs156	0	0	0	0	0	1	0	0	1	0	0	0	0	0
TVSu1126	0	0	0	0	0	1	0	0	0	1	1	0	0	0
TVSu1415	0	0	0	0	0	0	0	0	0	0	1	0	1	1
TKg6	0	0	0	0	0	0	1	0	0	0	0	0	0	1
TKg12	0	0	0	0	0	1	0	1	0	0	0	0	0	0
TVr145	0	0	0	0	1	1	1	1	0	1	1	0	0	0
TVr1001	0	0	0	0	1	1	0	0	1	0	1	1	0	0
TCc8127	0	0	0	0	1	1	0	0	1	1	0	0	0	0
TCc8156	0	0	0	1	0	1	1	1	0	1	0	1	0	0

Legend: 1 represents presence of band, 0 represents absence of band.

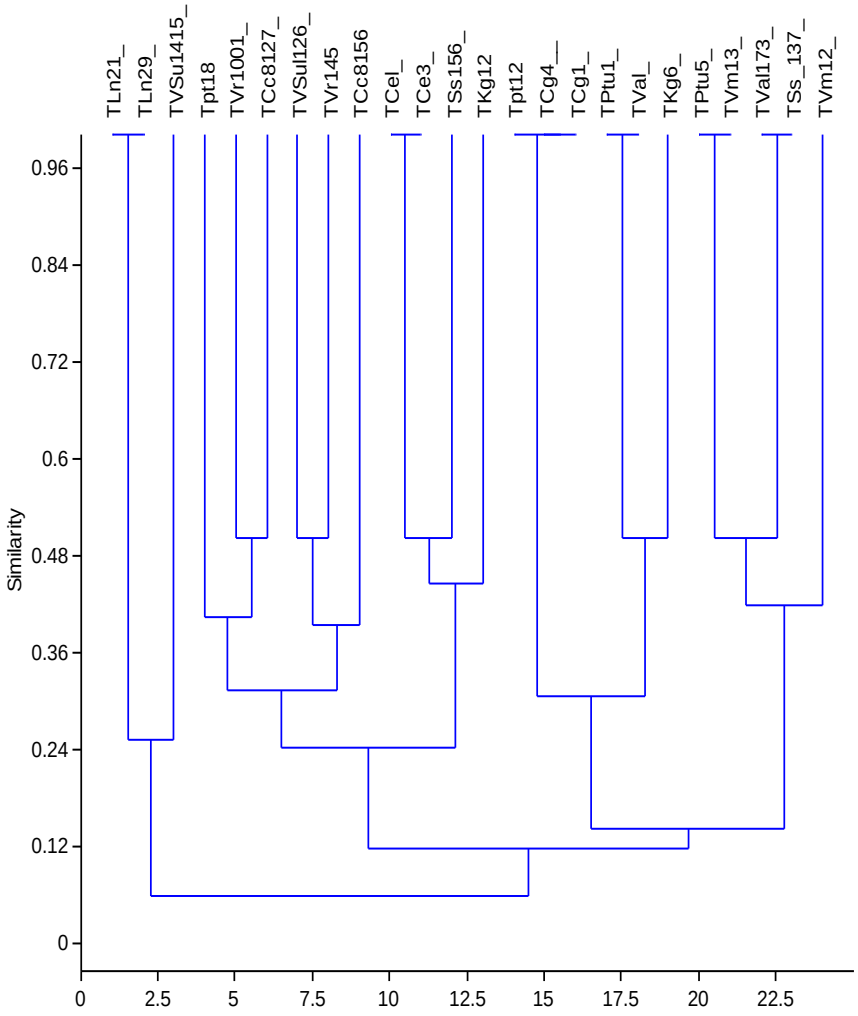
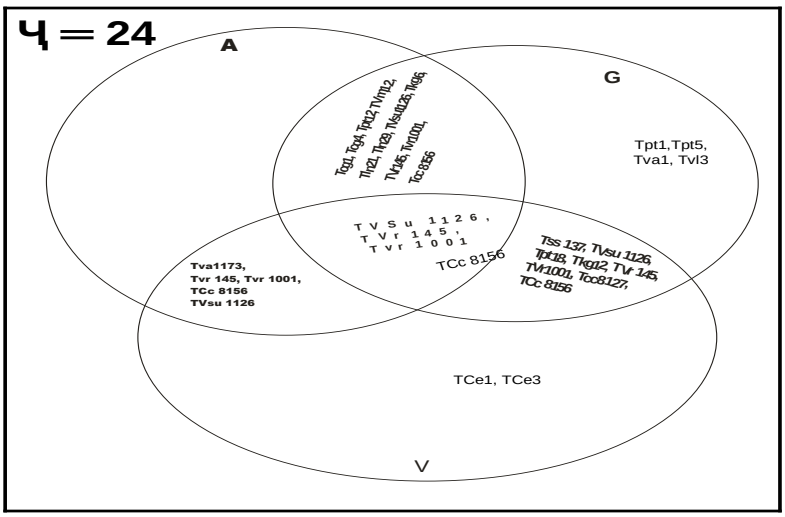


Fig.3: Molecular Dendrogram showing the grouping of the 24 accessions of miscellaneous legumes

Table 3: Characterization of the Proteins based on their Molecular Weights

Taxa	Molecular weights/possible protein (Kda)				
	Total bands	Albumins (65-122)	Globulins (45-64)	Vicilins (15-44)	Alpha-amylase inhibitors (10-14)
TCg1	3	1	2	0	0
TCg4	3	1	2	0	0
TPtu1	1	0	1	0	0
TPtu5	1	0	1	0	0
TPt12	3	1	2	0	0
TPt18	6	0	2	4	0
TCe1	1	0	0	1	0
TCe3	1	0	0	1	0
TVa1	1	0	1	0	0
TVa1173	2	1	0	1	0
TVm12	2	1	1	0	0
TVm13	1	0	1	0	0
TLn21	2	1	1	0	0
TLn29	2	1	1	0	0
TSs156	2	0	1	1	0
TSs156	2	0	2	0	0
TVSu1126	3	1	1	1	0
TVSu1415	3	3	0	0	0
TKg6	2	1	1	0	0
TKg12	2	0	1	1	0
TVr145	6	1	3	2	0
TVr1001	5	2	1	2	0
TCc8127	4	0	2	2	0
TCc8156	6	1	3	2	0

Similarities and differences in protein composition of the miscellaneous legumes based on grouping by Venn diagram are presented in Figure 1. Four separate groups could be distinguished. Accessions TSs137, TPt18, TKg12, TCc8127, TCc8156, TVsu1126, TVr145, TVr1001 contained proteins globulin and vicilin, TCg1, TCg4, TPt12, TVm12, TVr145, TVr1001, TVsu1126, TLn21, TLn29, TKg6, TCc8156 had proteins albumins and globulins in their seeds. Albumins and vicilin proteins were found in TVa1173, TVr145, TVr1001, TVsu1126 and TCc8156 while all the three proteins albumins, globulins and vicilin were found in accessions TVsu1126, TVr145, TVr1001, TCc8156. Accession TCc8156 (*Cajanus cajan)* was found to be more closely associated with the *Vigna Species* in protein composition. TPt1, TPt5, TVa1, TVm13 accessions had only protein globulin, TCe1 and TCe3 had only vicilin as being unique to them and there was no single specie or accession with only albumin.



Legend: A-Albumins; G-Globulins and V-Vicilins

Fig.4: Venn diagram indicating similarities and differences in protein composition of the Miscellaneous Legumes species and accessions studied

The study clearly presents characteristic protein types and differentiations among the 12 species of the 24 accessions miscellaneous legumes. Features observed and values recorded are representatives of the genetic

variabilities and similarities among these plants and should be used to establish relationship among the studied taxa which is in line with the result obtained by Popoola[5].

REFERENCES

[1] Apyanwu, A.C. (1981) 'A Textbook of Agriculture'. 1st Edition. African Educational Publisher (Nig). Pp 103-104.

[2] Agbolade,J.O.,Adegbite, A.E., Olawuyi, O.J., Aina D.A. and Popoola J.O. (2011). Comparative Study on the Epidermal Features of Under-Utilized Legume Accessions.Scholarly *Journal of Agricultural Science.Vol*.1(3)pp133-139.

[3] Brenan, J.P. (1963). Geographical Relationship of Leguminoseae'. Proc. Fifth Plenary Meeting of A.E.T.F.A.T., New York. Pp 102-105.

[4] King, H.G. (1963). Pasture for the South'. 4th Edition. The Inter Estate Printers and Publishers, Inc. Dancville, III Pp. 1-12.

[5] Popoola J.O.,Adegbite A.E.,Obembe O.O.,and Agbolade J.O.(2011). Reproductive Mechanisms and Polen Characterization in some Accessions of an Underutilized Legume (Shenostylis stenocarpa Hochst Ex.A.Rich) harms. *International Journal of Biodersity Conservation.Vol*.3(6).pp 185-192.

[6] Aremu, M. O., Olaofe, O. and T. E. Akintayo (2006). A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours'. *Pakistan Journal of Nutrition*. 5(1): 34-38.

[7] Omitogun, O.G., Okeola, O.G., Fasidi, I.O., Obisesan, I.O., and J. Machuka (2001). A galactose-specific lectin from Sphenostylis stenocarpa is insecticidal against cowpea genetic engineering. African Crop Science Proceedings, 5: 111-116.

[8] Adeboye, O. C. (2008). Reducing the oligosaccharide and anti-nutritional factor contents of two under-utilized grain legumes of Southwest Nigeria'. Acta Horticulturae, International Society for Horticultural Science.

[9] Bandoin and Mergai (2001). Yam bean Sphenostylis stenocarpa (Hochst.ex A. Rich) Harms. In: R. H. Raemaekers (ed.) Crop Production in Tropical Africa. *Directorate General for International Cooperation (DGIC)* Brussels., Belgium. Pp. 372-377.

[10] Bioversity International (2009). http://www.bioversityinternational.org/scientific information/themes/neglected and underutilized species/overview.html (accessed 01/05/201)

[11] Oshodi, A.A. and M.M. Ekperigin (1989). Functional properties of pigeon pea (Cajanus Cajan) flour'. Food Chem. 34: 1-5.

[12] Okigbo, B.N (1992). Plant Conservation in Africa'. IITA, Ibadan, Nigeria. 25p.

[13] Evans, I.M. and Boulter, D. (1974). Amino Acid Composition of Seed Meals of Yam Bean (Sphenomostylis stenocarpa) and Lima Bean (Phaseolus lunatus). *J.Science Food Agriculture* 25(8):919-912.

[14] Apata, D. F. and Ologhobo, A. D. (1997). Tropism Inhibitor and other anti-nutritional factors in tropical legume seeds. Tropical Science, 37:52-59

[15] Pharmacia's Manual (1983). Polyacrylamide Gel Electrophoresis Laboratory Techniques. February Revised Edition.

[16] Weber, K.M. and Osborn (1975). Protein and Sodium Dodecyle Sulphate Molecular Weight Determination, Polyacrylamide Gels and Related Procedures "In the Proteins".