INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0224/2023/29–5–339–344
DOI: 10.17957/IJAB/15.2038
http://www.fspublishers.org

Full Length Article

Genetic Variability of Ten Accessions of *Afzelia africana* from Southern Nigeria using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Chioma Chinyere Isuosuo, Sheily Nneka Egonu, Obiora Emmanuel Udoh, Dilibe Clifford Urama, Emmanuel Gabriel Njoku*, Favour Urunwah Orah and Jessica Chinwendu Ezeani
Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria
*For correspondence: gabriel.njoku@unn.edu.ng
Received 25 May 2022; Accepted 16 December 2022; Published 13 April 2023

Abstract

The genetic variability of ten accessions of *Afzelia africana* Sm. seeds were obtained from ten States in Southern Nigeria was determined using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The electropherogram revealed three distinct polymorphic bands ranging from 33–104 kDa. The dendrogram showed the relationship among the accessions based on the similarity index using the Unweighted Pair Group Method with Arithmetic Means (UPGMA), revealing two clusters. Cluster 1 comprised accession from Rivers State. Accessions from other states were grouped in the second cluster, consisting of two groups. Majority of the accessions clustering in one group indicated low genetic variability. The accession from Rivers State is the root of dendrogram, which indicates that it may be the oldest in the evolutionary trend. Accessions in the same group should not be combined in a breeding programme. SDS-PAGE as a protein marker has proven to be helpful in the identification of genetic variation in *A. africana*.

Keywords: Underutilized crops; Mahogany bean; Biochemical marker; Genetic diversity; Dendrogram

Introduction

Population growth, inadequate supply of protein and overconsumption of a cereal-based diet are some of the leading causes of protein and energy malnutrition in developing countries. The high cost of proteins has encouraged the use of protein sources from underutilized legumes, especially for the rural communities (Bolanle 2010; Singh *et al.* 2022). These include wild legumes, which serve as reliable and cheap sources of nutrition and medicine (Igbe and Okhuarobo 2018). It has been observed that monotonous food intake has affected the health and well-being of man. Therefore, there is an awareness to inculcate underutilized and indigenous crops in improving food security and human well-being (Agulanna 2020).

The underutilized legumes are vital and economic sources of proteins, carbohydrates, and calories which are essential in human nutrition (Nwosu 2012). They are classified as underutilized due to little or no documentation or unstated information on their nutritional or dietetic usefulness. Also, a decrease in the attention given to their production, usefulness and consumption has not encouraged the realization of their potential of contributing to national income (Agulanna 2020). Other descriptions such as orphan, minor, new and neglected crops have been used to characterize these crop species with untapped potentials (Padulosi and Hoeschle-Zeledon 2004). Nwosu (2012) emphasized the need to explore the use of these local foods to meet the demands of the growing population of Nigeria.

One of such local foods is *Afzelia africana*, commonly known as mahogany bean. It is called Akparata, Yiase, Apa, Ukpo, and Kawo by Igbos, Tivs, Yoruba, Idoma, and Hausa, respectively. It is an underutilized deciduous tree, also called Counter wood tree or African Oak belonging to the family Fabaceae (Odimegwu *et al.* 2015). *A. africana* is rich in proteins, crude fiber, ash content, and lipids (Ayanwale *et al.* 2007; Egwujeh and Yusuf 2015). The protein and mineral content are comparable to meat, egg, and fish. It has a long shelf life and requires little purification due to the presence of palmitic and oleic acids, making it suitable for the production of alkyl resins and shoe polish (Omokpariola *et al*. 2021). The processed seed flour is used as a soup thickener (Adebayo and Ojo 2013), leaves as vegetables in food preparation, and the flowers as condiments in sauces (Gérard and Louppe 2011).

The roots, leaves, bark, fruit ash have medicinal properties used in the treatment of different ailments (Gérard and Louppe 2011; Partey *et al*. 2018). The wood is resistant to insects and can withstand variations in humidity without shrinkage; this makes it suitable for woodworks,

To cite this paper: Isuosuo CC, SN Egonu, OE Udoh, DC Urama, EG Njoku, FU Orah, JC Ezeani (2023). Genetic variability of ten accessions of *Afzelia africana* from Southern Nigeria using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). *Intl J Agric Biol* 29:339–344

furniture making, and constructions and is highly priced internationally (Gérard and Louppe 2011; Mensah *et al.* 2016). Roasted seeds are used to rear chicken (Olorunmaiye *et al*. 2019), while the trees are pruned for livestock feed (Amahowe *et al.* 2018). The leaves have been described to be the most palatable livestock feed in West Africa (Nacoulma *et al*. 2017).

Although *A. africana* is valuable due to its multiple uses. It has been over-exploited, leading to a decline in its natural population (Houehanou *et al.* 2019). It has been identified as a threatened species in the "Red List of Threatened Species" (Hills 2020). Efforts promoting the conservation and management of the species are being carried out, but little is known about the population genetics. Information on the genetic variation within the species is lacking, which is vital for the efficient utilization of plants (Nadeem *et al.* 2018).

The advancement in the use of markers for the exploitation and identification of plant genetic diversity is one of the most critical developments in the field of molecular genetic studies (Idrees and Irshad 2014). It has enabled plant breeders to overcome the challenge of the limited gene pool of domesticated species by identifying valuable genes that are key in the improvement of traits (Eldakak *et al*. 2013). Kaga *et al*. (1996) described the assessment of genetic diversity as a fundamental step in crop improvement, which provides tools in gene bank management, germplasm tagging, identifying or eliminating duplicates in the gene stock, creating core collections, and cataloguing populations for genome mapping experiments.

Rafalski and Tingey (1993) reported SDS-PAGE to be a powerful tool and commonly used technique in population genetic studies. Furthermore, Riggs *et al.* (2003) and Khan and Ali (2017) described it as a dependable method of detecting variation without environmental influence. The variations observed in protein profiles and seed storage proteins have been reported to be important in specie classification, interspecific diversity, and phylogenetic or evolutionary relationships among species and plant domestication, in relation to genetic resource conservation and breeding (Javaid *et al.* 2004; Hameed *et al.* 2009; Shaye *et al*. 2018). Seed protein patterns have been classified as a good tool for identification of accessions that cannot be differentiated using morphological criteria alone (Potokina *et al*. 2000).

Genetic diversity studies on *A. africana* using SDS-PAGE are limited. Efforts have been made to characterize this plant using nuclear satellite markers (Houehanou *et al*. 2019). However, in some protein-rich plants, SDS-PAGE has been a helpful tool in the assessment of genetic diversity in the Fabaceae family (Omanhinmin and Ogunbodede 2013; Alege and Abu 2014; Oladejo *et al*. 2019; Pandey *et al*. 2020). We predict that SDS-PAGE electrophoresis will be able to show the genetic relationship among the 10 accessions of *A. africana*. Thus, the specific objective of this study was to detect genetic variability and determine the phylogenetic relationship of ten accessions of *A. africana*.

Materials and Methods

Collection of samples

Experiments were performed in the Classic Biomedical Laboratory, Nsukka, Enugu State, Nigeria, in August of the year 2021. Seeds of 10 accessions of *A. africana* (Akparata) were collected from 10 different localities in Nigeria. These localities include Edo (ED), Rivers (RI), Abia (AB), Lagos (LA), Ebonyi (EB), Abakaliki (AI), Awka (AW), Umuahia (UA), Owerri (OW), and Oji River (OI). The pods of *Afzelia* seeds were exposed to heat using a gas cylinder and a frying pan. Thereafter, the pods were cracked, and the seeds were extracted and kept in a cool, dry place before running the experiment in the Classic Biomedical Laboratory, Nsukka.

Protein extraction

The seeds were grinded separately into a fine powder using an electric blender. The respective powder (0.2 g each sample) was placed in tube and homogenized thoroughly by vortexing with an extraction buffer containing 2.5% SDS in 0.2 M Tris (pH 6.8), 1.5 M Tris-HCL (pH 8.8), 10% glycerol and 5% 2-mercarptoethanol. The mixture was centrifuged in the Eppendorf tube at 10,000 rpm for 5 min at 4^{o}C to obtain clear supernatants.

SDS-PAGE assay

The seed proteins were subjected to SDS-PAGE using the Laemmli (1970) modified method. Gel electrophoresis was carried out in an Ominipac mini gel electrophoresis apparatus using 4% stacking gel and 10% resolving gel performed in 1X electrophoresis buffer (pH 8.3). Bromophenol blue consisting of 0.01 g of bromophenol blue, 8.00 g of sucrose, 0.1 g of SDS and 8.0 mL of 0.25 M EDTA stock were added to the sample buffer as tracking dye to monitor the movement of the proteins. The gel was gradually run from 60 V through 80 V to 100 V for 2 h using the unstained protein standard molecular marker. At the end of electrophoresis, the gel was removed, covered with 500 mL of the gel-fixing solution and then covered with 400 mL of Coomassie stain. The staining solution contained 0.4 g of Coomassie blue R350, 200 mL of 40% (v/v) methanol and 20% (v/v) acetic acid. The gel was stained at room temperature for 3–4 h with gentle agitation. The staining solution was removed by aspiration after staining. The excess of stain was removed by washing gels in 50% (v/v) methanol in water with 10% (v/v) acetic acid. The solution was changed several times until the protein bands were seen without background staining of the gel. The process was repeated twice and the electropherogram with the clearest protein bands were used in the gel documentation and analysis.

Gel documentation and analysis

The electropherogram was photographed and examined. The molecular weight of the accessions was obtained using the unstained protein standard. The bands were coded based on presence and absence. The of level of intensities of the bands were used to determine the genetic diversity and relationship of the accessions using the Numerical Taxonomic and Multivariate Analysis System Software (NTSYSpc) version 2.2 by constructing a dendrogram.

Results

As regards genetic variability, *A. Africana* seed electropherogram revealed three distinct bands ranging from 33.42 to 104 kDa (Table 1). Observed polymorphism was based on the presence of bands (Table 1; Fig. 1). Protein band 1 (104.47 kDa) was observed to have seven polymorphic bands, whereas bands 1 and 2 with molecular weights 42.04 and 33.42 kDa, respectively were observed to have ten polymorphic bands (Table 2).

The data obtained from the protein profile of the accessions were subjected to cluster analysis using the NTSYSpc version 2.2. The UPGMA dendrogram grouped 10 accessions into 2 clusters consisting of four groups (Fig. 2). At similarity coefficient of 0.11, cluster 1 was observed from the accession from Rivers State, while accessions from other States were in the second cluster at a coefficient of 0.18. Four groups emerged at a coefficient similarity of 0.02, and groups 2, 3 and 4 emerged at the coefficient similarity of (0.01). Group 1 consists of accessions from Edo and Abia State. Group 2 consists of accessions from Lagos, Akwa Ibom, Awka, and Umuahia. Group 3 consists of accessions from Owerri and Orji River, and group 4 consists of accession from Ebonyi. Although accessions in groups 2 and 3 clustered differently, they were observed at the same coefficient of similarity. The accessions from Rivers State were observed to be the root of the dendrogram (Fig. 2).

Discussion

Seed protein electrophoresis showed the differences and relationships among 10 accessions of *A. africana*. It supports the use of seed storage proteins as a tool for identifying genetic variability. In this regard, Sharma *et al.* (2010) and Omanhinmin and Ogunbodede (2013) reported that electrophoretic analysis of seed storage protein is now widely recognized technique for cultivar identification in breeding species. In assessing genetic variability in Saudi tomatoes, Shaye *et al.* (2018) provided sufficient proof of genetic variability based on the seed storage proteins.

Similarities in some of the banding patterns suggest low genetic variation among the accessions. The variation observed could be the result of differences in the degree of intensity, the presence and the absence of the bands. Polymorphism was detected among the accessions which indicates that the proteins separation was in different forms due to the environmental factors. Shaye *et al.* (2018) opined that similarity in banding patterns could result from common ancestry that is very close in time. Low genetic variation is suggested to be a result of genetic drift and inbreeding within a population implying low polymorphism (Robert 1997; Peddakasim *et al.* 2015). The similarity in banding patterns was also observed by Ikram (2021) in some varieties of pumpkins and by Odeigah and Osanyinpeju (1998) in Bambara nuts. This was reportedly due to common characters in the landraces, which indicated that the genes encoding the proteins may be abundant (Akinwusi and Illoh 1995). Ullah *et al.* (2009) reported that uniform or similar banding patterns observed in some accessions could be due to these proteins being conserved. Accessions with thick or differences in the degree of band intensity may indicate differences in the quantity of proteins. Alwhibi (2017) attributed this to changes in protein groups of plant cell as a result of the adjustment to changes in the environment enabled by several factors controlled by the genes.

The dendrogram revealed that the accessions have a common origin (Fig. 2). However, the accession from Rivers State is an independent group and also the root of the dendrogram, which suggests that it may be older in the evolutionary trend. Osawuru *et al*. (2015) described evolutionary divergence as one of the causes of genetic diversity. Cluster 1 (RI) and cluster 2 (ED, AB, LA, AW, AI, UA, OR, OW, EB) are distantly related, which suggests that they could be genetically different (Fig. 2), and hence could be combined in a breeding programme. Oladejo *et al.* (2019) suggested that these genes could be isolated using molecular tools to complement and fasten breeding projects. Accessions in group 1 (ED, AB) could be combined with accessions in groups 2, 3 and 4. Turi *et al.* (2010) suggested that a cross between accessions could create a higher or larger genetic base even with low genetic diversity. Despite the fact that the accessions were collected from different and distant locations, some accessions were observed in the same cluster. Accessions in the same group or cluster, although collected from different states, could be genetically similar or closely related. Thus, as opined by Oladejo *et al*. (2019), this may be due to the accessions having common specific attributes or traits. In addition, accessions in group 2 (LA, AW, AI, UA) and accessions in group 3 (OR and OW) have the same coefficient similarities and hence, could be genetically the same but were planted in different environments.

Accession from Ebonyi State (group 1) occurred at a higher similarity coefficient than accessions in groups 2 and 3 and, therefore, may be genetically distinct from accessions in groups 2 and 3, although they occurred in the same cluster (Fig. 2). Group 2 and 3 might have evolved later in the evolutionary trend and also with same coefficient similarity with group 1 indicating they may be closely

Table 1: Presence and absence of protein bands showing degrees of intensity

Band No	Molecular Weight	Unstained Proteins Standard	ED	RI	AB	LA	EB	AW	AI	UA	OR	OW
1	142	200	—	—	—	+	+	+	+	+	+	+
2	47.0435	150	+++++	+++	++++++	++++++	++++	++++++	++++++	++++++	+++++	+++++
3	33.419	100	++	+++	++	++	++	++	++	++	++	++
4		85										
5		70										
6		60										
7		50										
8		40										
9		30										
10		25										
11		20										
12		15										
13		10										

+ signifies the presence of bands and the degree of intensity - signifies absence of bands

Table 2: Number of polymorphic bands present in accessions 1-10

Band No	Protein bands (kDa)	No. of polymorphic bands
1	104.472	**7**
2	42.0435	10
3	33.419	10

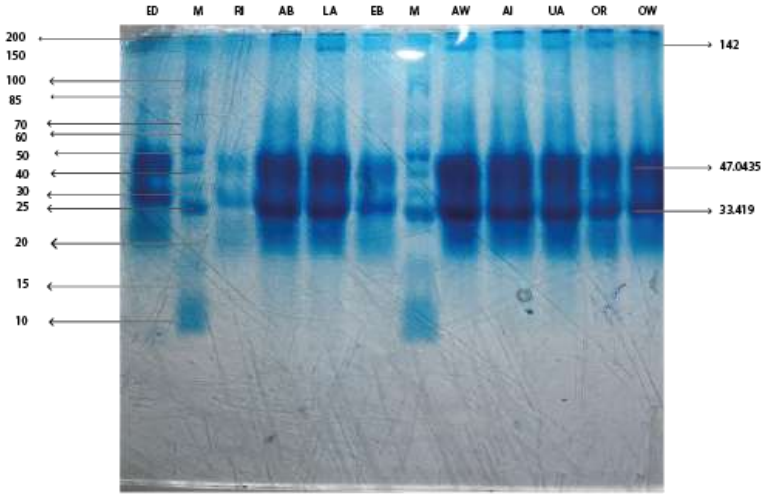

Fig. 1: SDS-PAGE electropherogram of seed protein of ten accessions of *A. africana*
M is the unstained protein standard molecular marker

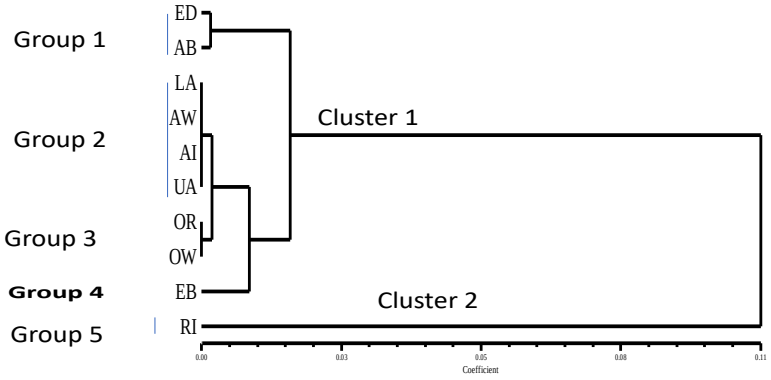

Fig. 2: Dendrogram showing the relationship between the ten accessions of *A. africana*

related and may have evolved at the same time. Isuosuo and Akaneme (2013) opined that clustering might indicate close proximity or relatedness. The accessions with the same similarity coefficients may be an indication of relative closeness. Yi *et al.* (2008) suggested that relative closeness may be as a result of no cross-boundary checks among states and seed exchange between farmers, which are distributed from one region to another. This indicates that some of these accessions used in the study might have migrated from one state to another, meaning that the seeds could be genetically the same.

Conclusion

Accessions in cluster 1 and cluster 2 can be combined in a breeding program, while accessions in the same group may not be used in the same breeding program. Therefore, the efforts should be made in germplasm collection and the use of higher molecular markers in determining genetic diversity and the development of improved varieties of *A. africana*.

Author Contributions

CCI, FUO and JCE planned the experiment, EGN and CCI interpreted the results and made the write up and OEU statistically analysed the data.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

References

Adebayo SF, OC Ojo (2013). Nutrient composition and functional properties of *Afzelia africana* seed. *J Environ Sci Toxicol Food Technol* 6:2319–2402

Agulanna FT (2020). The role of indigenous and underutilized crops in the enhancement of health and food security in Nigeria. *Afr J Biomed Res* 23:305–312

Akinwusi O, HC Illoh (1995). Crude protein electrophoresis seeds of some species of *Hibiscus*. *Nig J Bot* 8:71–76

Alege GO, NE Abu (2014). Seed protein electrophoresis of some members of the family Fabaceae. *Afr J Biotechnol* 13:3730–3735

Amahowe OI, SS Biaou, AK Natta, RO Balagueman (2018). Multiple disturbance patterns and population structure of a tropical tree species, *Afzelia africana* (Leguminosae– Caesalpinioideae), in two contrasting bioclimatic zones of the Republic of Benin. *Southern For* 80:95–103

Alwhibi SM (2017). Characterization of seed storage protein patterns of *Heliotropium digynum*. *Saudi J Biol Sci* 24:1404–1409

Ayanwale BA, CO Obun, AV Ayanwale (2007). Effect of raw and roasted wild *Afzelia africana* seed meal-based diets on broiler chickens. *Intl J Poult Sci* 6:27–30

Bolanle AO (2010). Nutritional composition of *Afzelia africana. Biosci, Biotechnol Res Asia* 7:101–106

Egwujeh SID, PA Yusuf (2015). Chemical compositions of aril cap of african oak (*Afzelia africana*) seed. *Eur J Food Sci Technol* 3:41–47

Eldakak M, SIM Milad, AI Nawar, JS Rohila (2013). Proteomics: A biotechnology tool for crop improvement. *Front Plant Sci* 4:35

Gérard J, D Louppe (2011). *Afzelia Africana* Sm. ex Pers. In: *PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale)*. Lemmens RHMJ, D Louppe, AA Oteng-Amoako (Eds). Wageningen, Netherlands

Hameed A, TM Shah, BM Atta, N Iqbal, MA Haq, H Ali (2009). Comparative seed storage protein profiling of Kabuli chickpea genotypes. *Pak J Bot* 41:703–710

Hills R (2020). *Afzelia africana*. The IUCN Red List of Threatened Species 2020. Website: https://www.iucnredlist.org/species/33032/67742420

Houehanou TD, K Prinz, F Hellwig (2019). Characterization of 15 nuclear microsatellite markers for *Afzelia africana* (Fabaceae) and related species. *Appl Plant Sci* 7:e1249

Idrees M, M Irshad (2014). Molecular markers in plants for analysis of genetic diversity: A review. *Eur Acad Res* 2:1513–1540

Igbe I, A Okhuarobo (2018). Expanding the insights into the usefulness of *Brachystegia eurycoma* Harm: A review of its medicinal and nutritional values. *J Intercult Ethnopharmacol* 7:82–94

Ikram M, N Ali, M Ishaq, M Romman, Y Islam, N Khan (2021). Genetic variation among seedling of pumpkins genotypes through SDS-PAGE. *Amer J Plant Sci* 12:711–719

Isuosuo CC, FI Akaneme (2014). Genetic variability assessment of accessions of *Corchorus olitorius* L. using sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Afr J Biotechnol* 13:2004–2009

Javaid A, A Ghafoor, R Anwar (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak J Bot* 36:25–29

Kaga A, N Tomooka, Y Egawa, K Hosaka, O Kamijima (1996). Species relationships in the subgenus Ceratotropis (genus *Vigna*) as revealed by RAPD analysis. *Euphytica* 88:17–24

Khan N, S Ali (2017). Advances in the detection of genetic diversity in bread wheat. *Amer J Agric Sci* 4:29–36

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685

Mensah S, TD Houéhanou, AE Assogbajo, KA Anyomi, A Ouedraogo, RG Kakaï (2016). Latitudinal variation in the woody species diversity of *Afzelia africana* Sm. habitats in West Africa. *Trop Ecol* 57:717–726

Nacoulma BM, AM Lykke, S Traoré, B Sinsin, A Thiombiano (2017). Impact of bark and foliage harvesting on fruit production of the multipurpose tree *Afzelia africana* in Burkina Faso (West Africa). *Agrofor Syst* 91:565–576

Nadeem MA, MA Nawaz, MQ Shahid, Y Doğan, G Comertpay, M Yıldız, R Hatipoğlu, F Ahmad, A Alsaleh, N Labhane, H Özkan, G Chung, FS Baloch (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnol Biotechnol Equip* 32:261–285

Nwosu JN (2012). The rheological and proximate properties of some food thickeners ("Ukpo", "Achi" and "Ofo") as affected by processing. *Intl J Basic Appl Sci* 1:313–321

Odeigah PGC, AOP Osanyinpeju (1998). Evaluating the genetic biodiversity of Bambara groundnut accessions from Nigeria using SDS-polyacrylamide gel electrophoresis. *Genet Res Crop Evol* 45:451–458

Odimegwu EN, JN Nwosu, MC Umelo, IA Olawuni, LO Akajiaku, BN Oga (2015). Effect of processing on the proximate and functional properties of "Akparata" (*Afzelia africana*) flour. *Eur J Food Sci Technol* 3:48–60

Oladejo AS, AO Bolaji, IO Obisesan, OG Omitogun (2019). SDS-Page characterization of some elite cowpea (*Vigna Unguiculata* L. WALP) varieties. *Nig J Biotechnol* 36:45–51

Olorunmaiye KS, LE Apeh, HA Madandola, MO Oguntoye (2019). Proximate and phytochemical composition of African mahogany (*Afzelia africana*) seed and African mesquite (*Prosopis africana*) pod. *J Appl Sci Environ Manage* 23:249–252

Omanhinmin CA, OO Ogunbodede (2013). Genetic diversity, taxonomy and legumins implications of seed storage protein profiling in Fabaceae. *Afr J Biotechnol* 12:2157–2163

Omokpariola DO, VU Okechukwu, PL Omokpariola (2021). Effects of processing on the nutritive and anti-nutritive properties of *Afzelia africana*. *Adv J Chem* 3:188–198

Osawuru ME, MC Ogwu, RO Aiwansoba (2015). Hierarchical approaches to the analysis of genetic diversity in plants: A systematic overview. *Univ Mauritius Res J* 21:1–36

Padulosi S, I Hoeschle-Zeledon (2004). Underutilized plant species: What are they? *LEISA Mag* 20:5–6

Pandey AK, AK Singh, P Singh, RP Vyas, RK Singh, H Bhandari (2020). Characterization and identification of pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes based on quality of seed protein. *Legume Res* 43:165–171

Partey ST, F Avornyo, M Ouédraogo, R Zougmoré (2018). *Candidate Fodder Species for Goat Production in Northern Ghana*. CCAFS Info Note. Bamako, Mali

Peddakasim D, P Suneetha, SU Lakshmi, R Srideepthi, MSR Krishna (2015). Seed storage protein profiling and phylogenetic relationships of *Capsicum annum* L. cultivars using SDS-PAGE. *Res J Biotechnol* 10:1–4

Potokina E, AV Duncan, EE Eggi, N Tomooka (2000). Population diversity of the *Vicia sativa* agg. (Fabaceae) in the flora of the former USSR deduced from RAPD and seed protein analyses. *Genet Res Crop Evol* 47:171–183

Rafalski JA, SV Tingey (1993). Genetic diagnosis in plant breeding: RAPDs microsatellite and machines. *Trends Genet* 9:275–280

Riggs DIM, RW Robinson, JC Howell, S Reiners, CA McClurg, R Rouse, HC Wien, RJ Glatz, RD Morse, TA Zitter, MT McGrath, KL Everts, MP Hoffmann, PD Cutis, S Hadcock (2003). *Pumpkin Production Guide, NRAES-123.* Northeast Regional Agric. Engineering Service, Ithaca, New York, USA

Robert C (1997). Importance of genetic variation to the viability of mammalian populations. *J Mammal* 78:320–355

Sharma GM, C Mundoma, M Seavy, KH Roux, SK Sathe (2010). Purification and biochemical characterization of Brazil nut (*Bertholletia excelsa* L.) seed storage proteins. *J Agric Food Chem* 58:5714–5723

Shaye NA, H Migdadi, A Charbaji, S Alsayegh, S Daoud, W AL-Anazia, S Alghamdi (2018). Genetic variation among Saudi tomato (*Solanum lycopersicum* L.) landraces studied using SDS-PAGE and SRAP markers. *Saudi J Biol Sci* 25:1007–1015

Singh N, P Jain, M Ujinwal, S Langyan (2022). Escalate protein plates from legumes for sustainable human nutrition. *Front Nutr* 9:977986

Turi NA, M Farhatullah, A Rabbani, NU Khan, M Akmal, ZH Pervaiz, MU Aslam (2010). Paper Study of total seed storage protein in indigenous *Brassica* species based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). *Afr J Biotechnol* 9:7595–7602

Ullah I, P Khaliq, P Abdullah, I Mallock (2009). Genetic diversity in pea (*Pisum sativum*) based on SDS-PAGE. *Pak J Agric Res* 22:120–126

Yi SS, SA Jatoi, T Fujunura, S Yamanaka, J Watanabe, KN Watanebe (2008). Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-centre of origin. *Plant Breed* 127:189–196