Original Article

Fingerprinting of Rice (*Oryza sativa*) Landraces from Three Geopolitical Zones in Ebonyi State Nigeria using SDS-page

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ABSTRACT

The genetic variability among ten (10) accessions of Rice (*Oryza sativa*) from three (3) Geopolitical zones (Ikwo, Afikpo and Abakaliki) in Ebonyi State Nigeria were assessed using sodium dodecyl polyacrylamide gel electrophoresis (SDS - PAGE). There were variations in the banding patterns of the accessions and polymorphism was also observed. The banding patterns of the 10 accessions were examined and photographed. The number of reproducible bands was recorded by observation of gels and photographs. The molecular weights of the protein bands were deduced using molecular weights of the pro-mega standard and dendrogram was also constructed in order to place different clusters and groups of the 10 accessions in the right places for easy identification. Twelve polypeptide bands were obtained ranging from 10KDa to 245KDa. The Unweight Pair Group Method with Mean Algorithm (UPGMA) dendrogram grouped the ten accessions into two clusters and five groups. Some accessions appeared in the same group, while others were observed to be in different groups. Accessions (3, 5, 6 and 7) neither show any atom of similarities with each other nor with the remaining accessions in terms of banding patterns and number of bands (they are significantly different) so they were placed on Group 5. This significant difference for most of the characters is an indication of wide genetic variability among the collections, which could be exploited in selection and breeding for improved varieties.

Key words: Fingerprinting, Molecular markers, Rice land races, SDS-page, Genetic variation.

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INTRODUCTION

Rice is one of the most valuable cereal crops cultivated and consumed all over the world. It is one of the staple foods in several African counties, including Nigeria and constitutes a large portion of the diet on a regular basis.[1] Rice is cultivated in mostly all agro-ecological zones in Nigeria but on a relatively small scale. As asserted by,[2] Nigeria is the continent's leading consumer of rice, one of the largest producers of rice in Africa and simultaneously one of the largest rice importers in the world. Rice is an important food security crop, it is an essential cash crop for it is mainly small-scale producers who commonly sell 80% of total production and consume only 20%. Farm productivity of staple crops, in developing countries such as Nigeria is low due to traditional methods of farming, poor irrigation facilities, land fragmentation, the impact of climate change, misuse of modern agricultural technology, and less availability of credit.[3] Among the staple crops consumed in Nigeria, rice has risen to a position of eminence. Rice is the most important staple food for about half of the human race.[4] The annual consumption of rice in Nigeria was about 5 million MT while quantity supplied was 2.7 million MT, with a demand-supply gap of about 2.3 million MT, which is today filled in by importation.[5] Nigeria still ranks third with Iraq (after the Philippines and China) in the group of major

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rice importing countries in the world. Rice (*Oryza spp. L*.), a grain cereal, is an important staple food for the world's human population, providing more than 20% of the calories consumed worldwide.[6] It has the second highest production worldwide, after maize.[7] Rice is an important crop that has generated several studies in Nigeria.[5] Some studies had focused on adoption of improved rice variety, consumption and marketing of rice whilst others focused on resource use and technical efficiency.[8] A review of studies related to agricultural producers' efficiency shows there is a large body of literature dealing with farm-level technical efficiency.

The rice plant has been relegated to an unimportant position as it is grown predominantly by the older generation of farmers.[9] The bulk of the genetic resources of this crop are in the hands of these farmers, which threatens its survival. There is therefore, a need for germplasm collection, characterization and conservation of rice to prevent it from being lost. Information provided by characterization can be useful in identifying promising rice genotypes that could be recommended directly to farmers and other end users and also for incorporation into breeding activities for further improvement. Very little information is available on the nature and extent of genetic diversity of Nigerian accessions of rice particularly using molecular markers.

MATERIALS AND METHODS

Data collection

The seeds of the rice were collected from three (3) Geopolitical areas in Ebonyi State Nigeria (Afikpo, Abakaliki and Ikwo) on April 2020 and the leaves were collected and used for SDS-PAGE procedures at the University of Lagos (UNILAG) laboratory between 16th of June to 18th of June, 2020. Data collected were subjected to certain analysis in order to determine if there is any difference or similarities among the accessions.

Protein extraction

Protein extraction of the accessions was carried out in the Department of Microbiology, University of Lagos, (UNILAG). For the extraction of proteins for electrophoresis, the respective leaves were placed in an Eppendolf tube, extraction buffer 2x SDS Electrophoresis Buffer containing the following concentration: 15.1g Tris base, 72.0g glycine, 5.0g SDS and H_2O to 1000ml total volume was added and a plastic pestle was used to moisturize (ground) the various samples. The samples were heated on a Heat block machine at 100°C for 5 min. The crude proteins were recovered as clear supernatant on top of the tube. The supernatant was used for electrophoresis. Leaves proteins were separated by carrying out electrophoresis in the discontinuous buffer system using 10% gel slab (Acrylamide-bis-acrylamide gel) as the separating gel and 5% acrylamide-bis-acrylamide gel as the stacking gel.

Preparation of gel

Five percent (5%) stacking gel was prepared and it consisted of 3.0mL of H_2O, 1.3mL of 4x Tris.Cl/SDS pH 6.8, 0.9mL of 30% acrylamide/0.8% bisacrylamide, 80µl of 10% ammonium peroxodisulfate and 5µl of TEMED. (10%) Resolving gel was also prepared and it consisted of 5.0ml of 30% acryl/0.8% bisacrylamide, 6.3ml of H_2O, 3.7ml of 4x, Tris.Cl/SDS pH 8.8, 200µl of 10% ammonium peroxodisulfate and 10µl of TEMED.

Loading the electrophoresis tank

Ominipac mini-vertical gel apparatus was loaded with the resolving gel solution and topped with distilled water to eliminate oxygen. The stacking gel was loaded carefully to avoid bubbles and was allowed to polymerize completely. The comb was inserted for the stacking gel. After polymerization the water was poured off completely. The glass and gel sandwich were removed from the casting base, transferred into the electrophoretic tank and terminals were noted.

Loading the samples

The combs were gently removed, each sample was loaded carefully and the tank was filled with electrophoresis buffer (Tris-Glycine pH 8.3) made up of 9.0g of Tris base, 43.2g of glycine and 3.0g of SDS. The gels were run at 90 volts for 2 hr in an Ominipac mini-vertical Gel Apparatus using promega protein as a standard marker. Bromophenol blue (BPB) consisting of 0.01g of bromophenol blue, 8.00g of sucrose, 0.1g of SDS and 8.0ml of 0.25 M EDTA stock were added to the sample buffer as tracking dye to monitor the movement of protein molecules in the gel. The gel was run until the dye front reached the bottom of the gel.

Staining the gel

The gels were gently removed and washed with 500ml of the gel fixing solution which was made up of 500ml of 95% (v/v) ethanol, 300ml of distilled water and 100ml of reagent grade of acetic acid mixed and adjusted to total volume of 1000ml with water. This was to wash off SDS – containing gel buffer out of the gel. The solutions were later removed by aspiration. The gels

were washed in 500ml of gel-washing solution. The gel-washing solution was composed of 500ml of analytical grade methanol, 300ml of distilled water and 100ml of acetic acid. The solution was made up to a total volume of 1000ml with water. The gels were covered with 400ml of Coomassie blue stain composed of 0.4 g of Coomassie blue R350, 200 ml of 40% (v/v) methanol and 20% (v/v) acetic acid in distilled water at room temperature for 3 to 4 hr and were gently agitated. The Coomassie stains were removed after staining.

Destaining

Gels were covered with 250ml of the destaining solution. The destaining solution composed of 500 ml of methanol, 300ml of distilled water, 100ml of Acetic acid. These were mixed and adjusted to 1000ml with water. The destaining solution was changed severally until the protein bands were seen clearly without background staining of the gel.

Gel documentation and analysis

Banding patterns of the 10 accessions were examined and photographed. Each band was considered as a character and absence or presence was coded for analysis. The number of reproducible bands was recorded by observation of gels and photographs. The molecular weights of the protein bands of the accessions were deduced using molecular weights of the Pro-mega standard. A dendrogram was constructed using Numerical Taxonomic and Multivariate Analysis System Software (NTSYS - pc).

RESULTS

Evaluation of genetic variability

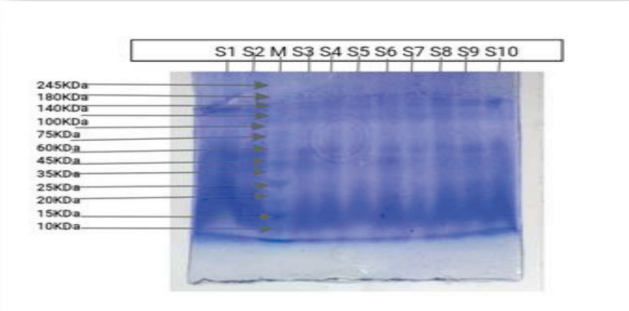

Plate 1: The electrophoretic banding pattern of Rice (*Oryza sativa*) accessions generated by SDS-PAGE. The Protein profiles of the accessions also showed variability on the basis of intensity, presence or absence of protein bands.

DISCUSSION AND CONCLUSION

This study assessed the genetic variabilities among ten accessions of *Oryza sativa* obtained from three (3) Geopolitical zones (Ikwo, Afikpo and Abakaliki) in Ebonyi State, Nigeria. Genetic differences among the accessions were detected by the presence or absence of bands and by their intensities as shown in Tables 1 and 2. This band differences could be used as basis for identification and variation among accessions. Variability in intensity observed in some bands indicates the quantity of protein peptide at a particular molecular weight. The variability in SDS-PAGE profile also indicates genetic diversity in

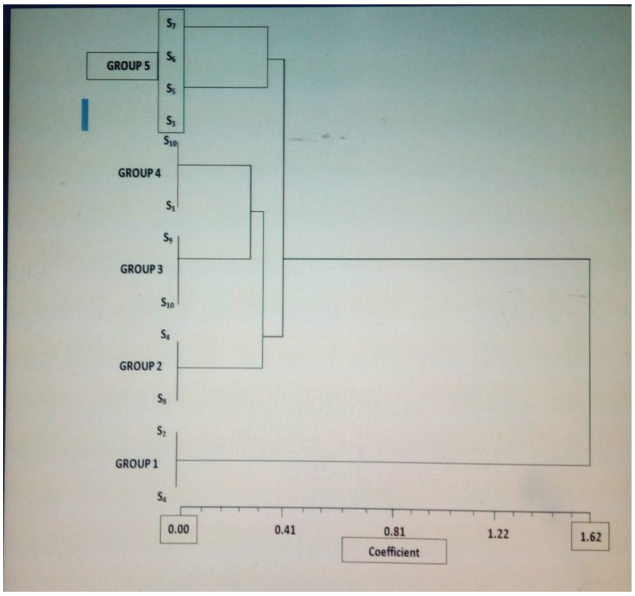

Figure 1: Dendrogram showing the relationship between the ten (10) accessions of rice (*Oryza sativa*).

Table 1: Number of bands present/absent in the accessions of rice.

S/N	Accessions	Number of Bands Present	Number of bands Absent
1	Meruwa	7	5
2	Iron	8	4
3	Cp(ferro 55)	6	6
4	Iron	8	4
5	Mirimiri	6	6
6	Masy	6	6
7	Nwangbanya	6	6
8	Meruwa	7	5
9	Egodi	7	5
10	R8(igbo)	7	5

Table 2: Intensity of protein banding pattern of 10 accessions of rice (*Oryza sativa*).

S/N	Molecular Weight	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
1.	245KDa	++	+	+	+	+	+	+	++	++	++
2.	180kDa	+	+	+	+	+	+	+	++	++	++
3.	140KDa	+	+	+	+	+	+	+	+	_	_
4.	100KDa	_	_	_	_	_	_	_	_	_	_
5.	75KDa	_	_	_	_	_	_	_	_	_	_
6.	60KDa	+++	++	++	++	+++	+	++	+	+++	++
7.	45KDa	+++	++	+++	+++	+++	+	++	+	+++	++
8.	35KDa	_	_	_	_	_	_	_	_	_	_
9.	25KDa	+++	++++	_	_	+++++	_	_	_	_	_
10.	20KDa	_	_	_	_	_	_	_	_	+++++	+++++
11.	15KDa	+++++	+++++	_	+++++	_	+++++	_	++++	++++	+++++
12.	10KDa	_	+++++	+++++	++++	_	_	+++++	+++	+++++	+++++
TB.	12	7	8	7	6	6	6	7	7	6	7

(KEY: TB =Total Bands, + light, ++ Dark, +++ Very Dark, ++++ intense, +++++ Very Intense and – Absence).

Table 3: Polymorphic distributions of protein peptides in SDS-Page analysis of rice accessions *(Oryza sativa).*

Protein Band	Molecular Weight	Present	Absent	Polymorphic / Monomorphic
1.	245KDa	10	0	monomorphic
2.	180KDa	10	0	monomorphic
3.	140KDa	8	2	Polymorphic
4.	100KDa	0	10	monomorphic
5.	75KDa	0	10	monomorphic
6.	60KDa	10	0	Monomorphic
7.	45KDa	10	0	monomorphic
8.	35KDa	0	10	monomorphic
9.	25KDa	3	7	Polymorphic
10.	20KDa	2	8	Polymorphic
11.	15KDa	8	2	Polymorphic
12.	10KDa	7	3	Polymorphic

rice genotypes collected from diverse environmental conditions.

The present study showed polymorphism indicating genetic variation as shown in Table 3. Polymorphism, according to Torkpo *et al.*,[10] could be examined for possible association with such important traits like drought resistance, disease resistance, nematode and parasitic weeds.

The data obtained from the electrophoregram of the ten accessions was subjected to cluster analysis using the Numerical Taxonomic and Multivariate Analysis System Software (NTSYS-pc) version 2.2. To determine the clustering and similarity coefficient of the generated matrix, the Unweighed Paired Group Method with Arithmetic Averaging (UPGMA) clustering method based on Jaccard's similarity coefficient was used. The dendrogram based on SDS-PAGE markers grouped the ten accessions into 2 main clusters and 4 groups with a similarity coefficient range of 0.41-1.62 as shown in Figure 1. S_2 and S_4 are more closely related to each other than they are to any other accessions. This shows that these accessions (S_2 and S_4) in Group 1 are identical and have less diversity. At 0.41 Jaccard's similarity coefficient S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, and S_{10} share common ancestor. S_4 and S_8 shared same banding patterns and they make up Group 2 but, they were gotten from different Geopolitical zones in Ebonyi State. S_9, and S_{10} which made up Group 3 also share the same banding pattern but, they were also gotten from different Geopolitical zones in Ebonyi state and they are more closely related to S_9, and S_{10} than they are to S_1, S_2, S_3, S_5, S_6, and S_7.

Cluster 1 comprised of two accessions which were collected from different Geopolitical zones in Ebonyi state; one (S_2) from Ikwo in Ebonyi state and the other one (S_4) from Afikpo in Ebonyi state.

Cluster II comprised of 4 accessions (S_1, S_8, S_9 and S_{10}). S_1 and S_{10} were gotten from the same Geopolitical zone (Abakaliki) in Ebonyi state, While S_8 and S_9 were gotten

from different Geopolitical zones (Afikpo and Ikwo respectively) in Ebonyi State.

In total, 12 protein bands were recorded ranging from the molecular weight of 10KDa to 245KDa. In the protein profile, some genotypes expressed a single band, while others showed multiple bands. Relationship of the ten (10) accessions of Rice was based on the estimated number of total bands present in the protein profiles. The accessions 2 and 4 (S_2 and S_4) yielded the highest number (8) of bands, while minimum bands were found in other accessions of the crop (S_3, S_5, S_6, and S_7).

The dendrogram obtained from the 10 accessions of Rice plant using the Numerical Taxonomy and Multivariate Analysis revealed 2 clusters and 5 groups. The clustered accessions indicate close genetic relatedness or proximity. Accessions 2 and 4 which are in the same group may be as a result of duplication of plant materials, while accession 3, 5, 6, and7 in group 5 could be genetically different from all the accessions even though some (accession 3 and 7) originated from the same Geopolitical zone (Ikwo) in Ebonyi state. Relative closeness can be explained by the fact that there is no cross boundary check among states and seed exchange between farmers may disseminate plants from one region to the other.[11] This may be the case of the accessions understudy, indicating that these accessions may have been moved from one geopolitical zone to the other, implying that the plants may have the same gene source.

Abakaliki and Ikwo accessions (1 and 9) were found in different groups (4 and 3) which is also an indication of genetic variability. Other accessions that were found in different groups indicate genetic variability and could create wider variation when crossed. According to Ahmed *et al.,*[12] genetic variation may occur due to natural selection, genetic diversification and environmental impacts. Bertozo *et al.,*[13] reported significant variation among different species of groundnut for seed storage proteins and suggested that different species of groundnut would be used to increase genetic diversity of the germplasm. Accession 1 was independent and distantly related to some accessions. Independent and distantly related accessions had the highest genetic diversity reflecting their genetic uniqueness.[14] Variation of the germplasm could be of enormous value to breeders for developing new cultivars and to design their hybridization program with greater success.[15] Maity *et al.,*[16] reported that contrasting parents may be identified and used in the crossing programme for generating wider variability for selection and crop improvement, based on distance between species of different clusters.

In conclusion, protein electrophoresis was able to show the differences and relationship among the 10 accessions of *Oryza sativa*. Genetic variations are useful for plant improvement. This will help in characterization and selection of planting materials for breeders. It will also help in preventing the duplication of plant materials while designing breeding programmes. The protein bands could be associated with different traits or characteristics of importance in breeding. For instance, Accessions S_8, S_9, S_{10} and S_3 may not be used together in a breeding programme. Using S_8 and S_9 or $S_{10}$and S_3would amount to duplication of the same material. Thus, accessions from the same group should not be used in a breeding programme, while accessions from different groups despite their origin could be used together in a breeding programme.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SDS: PAGE; Sodium dodecyl sulfate polyacrylamide gel electrophoresis; **Bp:** Base pair; **EDTA:** Ethylene Diamine Tetra Acetic acid; **NTSYS:** Numerical Taxonomic and Multivariate Analysis System Software; **KDa:** Kilodalton; **UPGMA:** Unweighted Pair Group Method with Mean Algorithm.

SUMMARY

- The greatest challenge of farmers in Nigeria is that there is little knowledge of the genetic diversity and molecular characterization of rice varieties in the country. In diseases outbreak difficulty is encountered on breeding approach that would be most suitable in managing the problem. This has led to a lot losses as a result of poor yield.
- This study has breached the gap by analyzing ten(10) accessions of *Oryza sativa* obtained from three (3) Geopolitical zones (Ikwo, Afikpo and Abakaliki) in Ebonyi State, Nigeria.
- Genetic variations were established among the accessions of rice cultivated in the state. Accessions

S_3,S_8, S_9, and S_{10} may not be used together in a breeding programme. This will help in preventing the duplication of plant materials while designing breeding programmes. Knowledge of the variations would lead to a better breading programme for farmers.

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