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Genetic Purity Estimates of Maize Seed Using Protein Profiles from Sodium Dodecyl-Sulphate – Poly-Acrylamide Gel Electrophoresis (SDS-PAGE)

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ABSTRACT

Estimates of genetic purity of seed based on morphological characters commonly used in most part of the Sub-saharan Africa requires complete growing cycle of the plant species, thus time and resources intensive. This study examined the use of SDS-PAGE as a faster and concise means of estimating genetic purity of seed devoid of environmental influence. Sodium Dodecyl-Sulphate – Poly-Acrylamide Gel Electrophoresis analysis was conducted on single seed of three replicates each of samples of four inbred parents and five maize hybrids sourced from Research Institute and Agro-dealer respectively. The frequencies of occurrence of the bands and similarity indices were calculated from the electrophoretic profiles scores which were later subjected to cluster analysis for dendogram grouping. Results revealed 14 types and a total of 212 protein bands. Minimum genetic similarity index (0.67) was recorded among seeds of KU 1414-STR while the highest genetic similarity distance (2.10) was recorded among seeds of Oba Super 2 collected from Agro-dealer. SDS-PAGE detected genetic variability among maize seed samples as clusters of maize samples that are genetically similar were clear. SDS-PAGE procedure could be adopted as fast and reliable tool devoid of environmental influence for genetic purity analysis in crops.

Keywords: Genetic purity, Maize seed, SDS-PAGE, Protein Profiles, Nigeria.

INTRODUCTION

Seed quality can be grouped into genetic, physical, physiological and pathological. The genetic quality of seed lot has been identified as very important because it determines to a large extent the response of the variety to other agronomic and ecological characteristics of the site of production (Cromwell *et al*, 1992). High precision evaluation of genetic purity of parental lines and early generation seeds (EGS) (breeder and foundation seed) in the seed value chain is therefore crucial for the successful delivery of high quality seeds to the end users (farmers). Genetic purity of seed is of great significance in seed science thus making seed identification and varietal purity testing very essential in effective agricultural production system. Genetic purity test is conducted to verify deviation from genuineness of the variety during its multiplication (Chetan-Kumar *et al*, 2012). Estimates of genetic purity based on morphological characters (Grow out Test) is the most common method used to determine genetic purity of seed lot in most part of the Sub-saharan Africa (Awaludin *et al*, 2013) This method, requires complete growing cycle of the plant species, thus time and resources intensive (Komori and Nitta, 2004). It is also of little precision because the crop characters can be influenced by the environment. Several other approaches have been used to determine genetic purity of seed. These include high-performance liquid chromatography (RP-HPLC) of seed storage proteins (Scanlon *et al*., 1989), DNA-based molecular markers (Perry, 2004), Ultrathin-layer isoelectric focusing (UTLIEF) of seed protein (ISTA, 1999) and so many others, but most of these method becomes too costly in most developing countries due to high tech equipment that is required to run the test. Analysis of protein composition of seeds has also been identified as a good indicator of genetic constituent of seeds. SDS-PAGE of proteins is mostly used method to discriminate varieties because the protein banding pattern is unique for any particular genotype and is independent of seed vigor and physiological seed activity (Kamel *et al*., 2003). It is relatively cheaper than most molecular analysis and has been successfully used to resolve taxonomic and evolutionary problems of several plants (Rabbani *et al*, 2001) and is not influenced by environmental factors. However, most of the research report using this tool has focused mainly on cultivar identification or diversity among cultivars. This experiment was designed to use a biochemical technique (SDS-PAGE) to estimate and quantify the genetic purity of maize with the aim of having a cheaper, faster and concise means of estimating genetic purity of seed devoid of environmental influence.

MATERIALS AND METHODS

The experiment was conducted in the Biotechnology Centre of Federal University of Agriculture, Abeokuta, Nigeria.

Seed materials

Seeds of inbred parents of commonly grown hybrid maize in Nigeria (9071 and 1368 for Oba Super 1, 4001 and KU-1414-SR for Oba Super 2 respectively) as well as the research hybrids of Oba-Super-1 and Oba-Super 2 kept solely as reference sample for research purpose were sourced from International Institute of Tropical Agriculture gene bank, while commercial hybrids of same varieties (Oba Super 1, Oba Super 2) and Oba-98 that were produced from parental materials handled by seed companies were purchased in an Agro-dealer shop in Ibadan, Nigeria.

Sample preparation and soluble protein extraction

Single seed in three replicates of the maize hybrids and their inbred parents were ground separately and 0.3 g of finely crushed maize powder of each sample was measured into 1.5 ml eppendorff tube and 800 µl of 0.1M Tris-HCl pH 7.6 extraction buffer was added before it was vortexed for 2 minutes for thorough mixing for the extraction of the soluble proteins. The solution was centrifuged at 10,000 rpm for 2 minutes, and then the supernatant was collected into a new eppendorf tube and stored at 4^{o}C.

Gel preparation

Two types of gels (12% separating and 4% stacking gels) were prepared for the electrophoresis. The separation gel (10 ml) was composed of deionized water (3350µl), 1.5M Tris-HCl (2500µl), 10% sodium dodecyl sulphate (100µl), acrylamide/bisacrylamide solution (4000µl), 10% ammonium per sulphate (75µl) and TEMED (7.5µl) while the stacking gel (10ml) contained deionized water (6100µl), 0.5M Tris HCl at pH of 6.8 (2500µl), 10% sodium dodecyl sulphate (100µl), acrylamide/bi-sacrylamide solution (1330µl), 10% ammonium per sulphate (50 µl) and TEMED (10 µl).

Electrophoresis procedure

The seed protein analysis was done with aid of 10 ml capacity of Bio-Rad Mini Protean II system. β-mercaptoethanol (7.5%) in sample buffer was used for the preparation of the protein samples. The supernatant and the sample buffer were added at ratio 4:1 and heated at 95^{o}C for 5 minutes in a water bath. The mixture was then loaded into the Bio-Rad Mini Protean II Cell starting with separating gel and after polymerization, loading comb was inserted and the stacking gel was carefully loaded. The mixture was run at 150V for 50 minutes according to Ramiro *et al*., (1995) and Maselli *et al*., (1996). At the completion of the electrophoresis, the gels were carefully removed under water and stained in the solution of 0.1% Coomassie Blue in 1:4 acetic acid and methanol for 45 minutes. The gels were de-stained for 24 hours in acetic acid and methanol solutions at the ratio of 1:4 in 40% distilled water. The de-stained gels were then scanned and the bands visually scored 1 for presence of band and 0 for absence of band (Lazaro and Aguinagalde, 1998).

Analysis of electrophoregrams

The electrophoretic profiles scores were subjected to cluster analysis using unweighted pair group method with arithmetic means (UPGMA) for phenogram or dendogram grouping with the aid PAST computer software version 3.12 (Hammer *et al* 2001) Frequencies of occurrence of the bands were calculated using the following formula (1):

$$\text{Frequency of occurrence} = \frac{Number\ of\ times\ the\ band\ occured}{Total\ number\ of\ samples} \quad \ldots\ldots. (1)$$

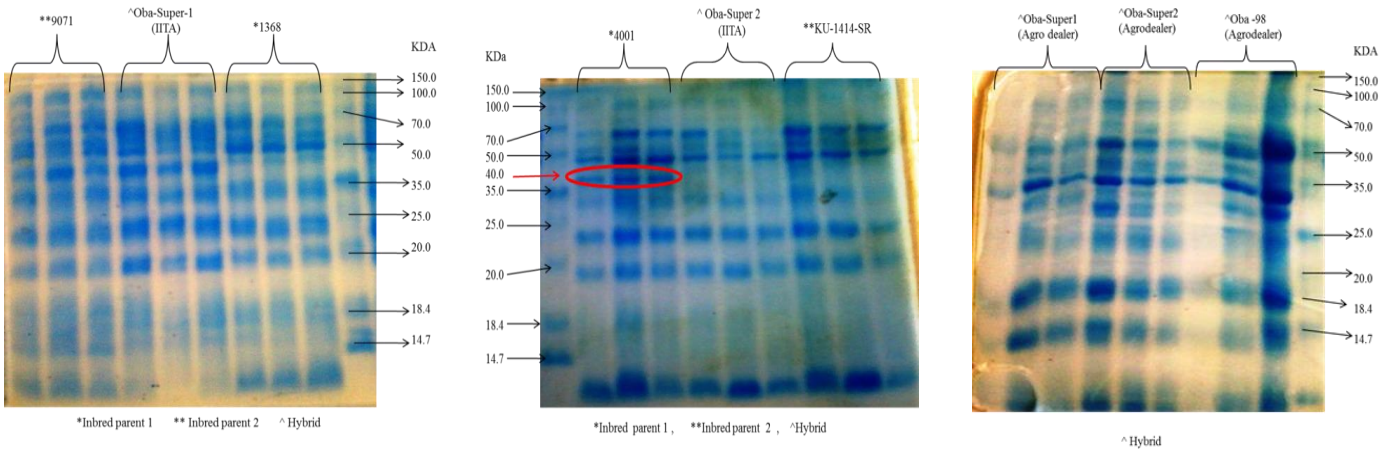


Figure 1: Electrophoregrams of total seed protein profiles of the tested samples. Each lane is the profile of each sample of the inbred line and the hybrids

RESULTS

Distribution and frequencies of occurrence of the seed protein bands from SDS-PAGE.

The distribution of identified protein bands across the maize lines and their frequencies revealed fourteen (14) types of protein bands of molecular weight ranging from 14.7 to 100 KDa. A total of 212 protein bands were visible (Table 1). The frequencies of occurrence of each of the fourteen protein bands were between the ranges of 0.11 and 0.96. The heaviest (100KDa) molecular weight protein band occurred in nineteen maize samples while the lightest (14.7KDa) molecular weight occurred only in 9 samples. The calculated frequency of protein band with the molecular weight of 50KDa was 0.96 and it was almost monomorphic because it occurred in 26 out of 27 maize samples. The protein band with the molecular weight of 40KDa has the least calculated frequency of 0.11. The bands occurred only on three samples of an inbred line (4001) which was one of the inbred parents of Oba Super 2 (Figure 1).

Similarity indices within the seeds of some maize inbred lines and maize hybrids based on protein profiles of SDS-PAGE

The similarity distance coefficients within seed lots of the inbred lines and hybrids calculated based on presence and absence of bands of SDS-PAGE revealed six levels (0.00, 1.00, 1.41, 2.00, 2.45 and 2.65) of similarity distances while comparison of similarity distance of same variety (Oba Super-1 and Oba Super-2) collected from different sources (IITA and Agro-dealer) revealed four levels (1.73, 2.24, 2.45 and 2.83) of similarity distances (Table 2). All the hybrid samples and the inbred lines except 4001 and Oba Super 2 collected from Agro-dealer recorded minimum similarity distance (0.00) between either seed 1 and 2 or between seed 2 and 3. The highest similarity distance coefficients (2.65) was recorded among seeds of an inbred line (1368) and Oba Super 1 collected from Agrodealer shop. Similarly, Oba Super-2 and Oba-98 collected from Agrodealer shop recorded 2.45 similarity distance coefficient (Table 2).

Average genetic distance indices among seeds of inbred lines and the hybrid seeds based on protein profiles of SDS-PAGE

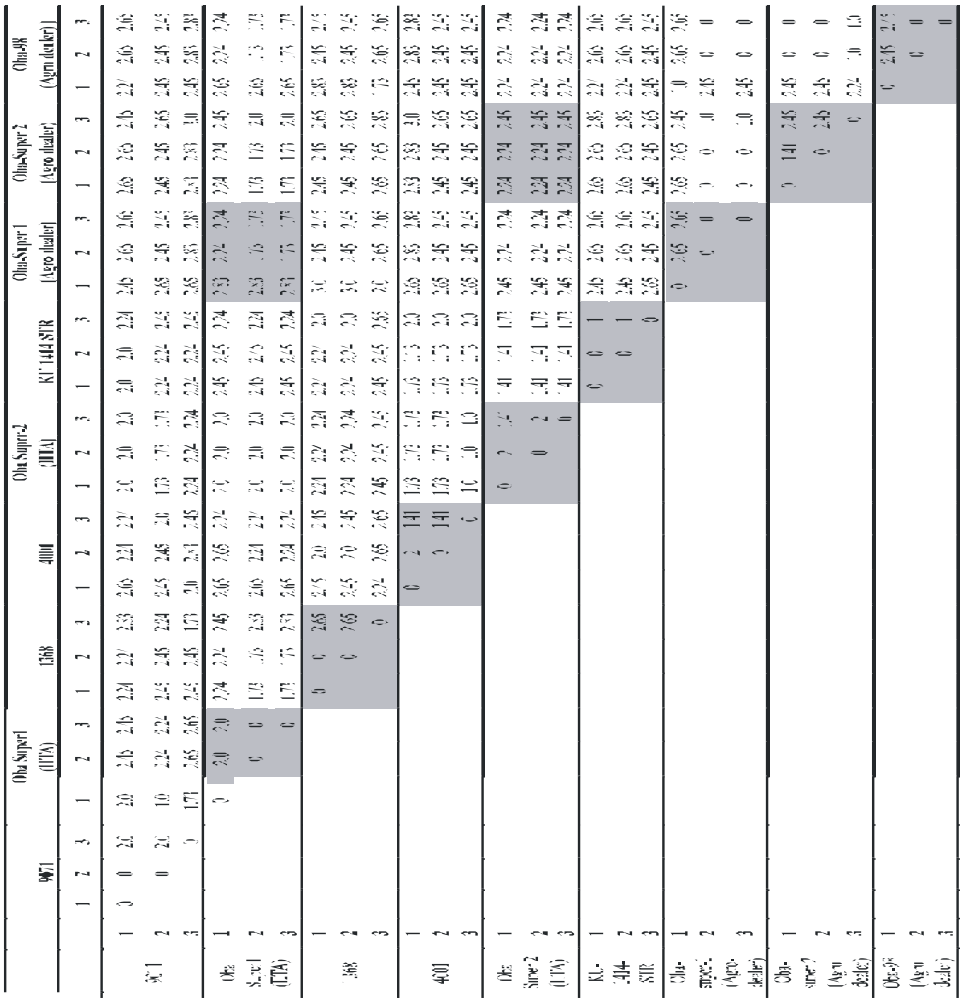
The average genetic distance indices used in estimating the genetic purity among the seeds of inbred lines and the hybrids based on SDS-PAGE analysis are presented in Table 3. Average genetic similarity indices ranges between 0.67 and 2.10. Minimum genetic similarity index (0.67) was recorded among seeds of KU 1414-STR while the highest genetic similarity distance (2.10) was recorded among seeds of Oba Super 2 collected from Agro-dealer. Genetic similarity distance among inbred lines ranges between 0.67 (recorded in KU 1414-STR) and 1.77 (recorded in 1368). The least average genetic similarity index among seeds of hybrid maize (1.33) was recorded from Oba Super 1 collected from IITA while the highest average similarity index (2.10) was recorded from Oba Super 2 collected from Agro dealer.

Table 1: Frequency of occurrence of identified seed protein bands from SDS-PAGE

Alleles/seed protein bands and their Molecular weight (KDa)	Number of occurrence in 27 maize samples	Calculated frequency of occurrence
1(100)	19	0.70
2 (80)	7	0.22
3 (70)	23	0.85
4 (50)	26	0.96
5 (45)	23	0.85
6 (40)	3	0.11
7 (35)	13	0.48
8 (32)	22	0.81
9 (30)	7	0.26
10 (22)	20	0.74
11 (20)	8	0.30
12 (19)	13	0.48
13 (18.4)	15	0.56
14 (14.7)	9	0.33
Total	212	

The figures in the first column represent different bands in descending order of their molecular weight while values in parenthesis represent molecular weight of the protein bands.

Table 2: Similarity indices within the seeds of hybrids and inbred lines based on protein profiles of SDS-PAGE



**Cells of similarity distances in comparison are yellow filled; Source of seed is written in parenthesis*

Table 3: Average genetic similarity distance indices between seeds of inbred lines and the hybrids based on protein profiles of SDSPAGE

Maize seed samples	Genetic similarity distance between			Average genetic distance between the seed lots
	Seed 1and 2	Seed 1 and 3	Seed 2 and 3	
9071	0	2	2	1.33
Oba Super 1(IITA)	2	2	0	1.33
1368	0	2.65	2.65	1.77
4001	2	1.41	1.41	1.61
Oba Super2 (IITA)	2	1.41	2	1.80
KU-1414-STR	0	1	1	0.67
Oba Super-1 (Agro-dealer)	2.65	2.65	0	1.77
Oba Super-2 (Agro-dealer)	1.41	2.45	2.45	2.10
Oba- 98 (Agro-dealer)	2.45	2.45	0	1.63

Source of seed in parenthesis

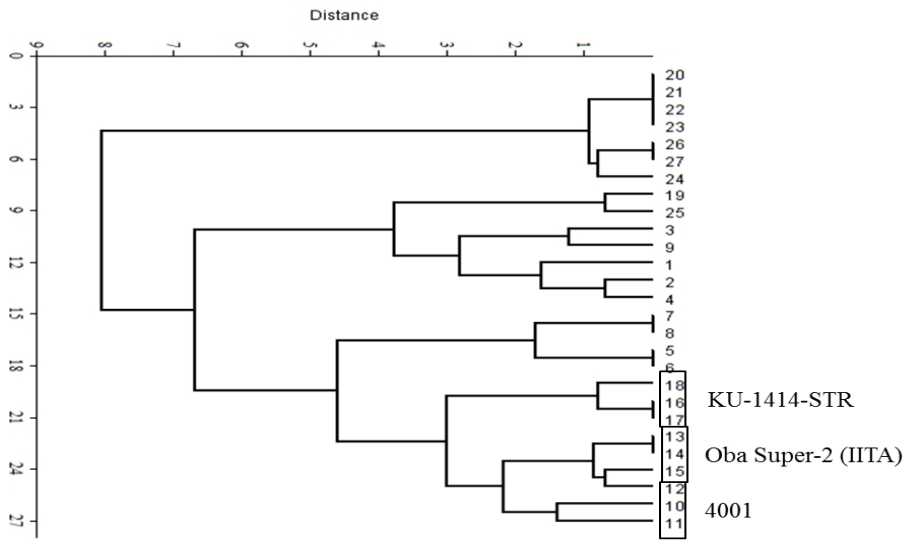


Figure 2: Relationship between seeds of the inbred lines and the hybrid maize varieties as revealed by cluster analysis based on genetic distance estimates of protein profiles of SDS-PAGE. Where **1** = 9071 (1); **2** = 9071 (2); **3** = 9071 (3); **4** = Oba Super 1 (IITA) (1); **5** = Oba Super 1 (IITA) (2); **6** = Oba Super 1 (IITA) (3); **7** = 1368 (1); **8** = 1368 (2); **9** = 1368 (3); **10** = 4001(1); **11** = 4001(2); **12** = 4001(3); **13** = Oba Super 2 (IITA) (1); **14** = Oba Super 2(IITA) (2); **15** = Oba Super 2 (IITA) (3); **16** = KU1414STR (1); **17** = KU1414STR (2); **18** = KU1414STR (3); **19** = Oba Super 1 (Agro Dealer) (1); **20** = Oba Super 1 (Agro Dealer) (2)
21 = Oba Super 1 (Agro Dealer) (3); **22** = Oba Super 2 (Agro Dealer) (1); **23** = Oba Super 2 (Agro Dealer) (2); **24** = Oba Super 2 (Agro Dealer) (3); **25** = Oba 98 (Agro Dealer) (1); **26** = Oba 98 (Agro Dealer) (2); **27** = Oba 98 (Agro Dealer) (3)

Average genetic distance indices among seeds of inbred lines and the hybrid seeds based on protein profiles of SDS-PAGE

The average genetic distance indices used in estimating the genetic purity among the seeds of inbred lines and the hybrids based on SDS-PAGE analysis are presented in Table 3. Average genetic similarity indices ranges between 0.67 and 2.10. Minimum genetic similarity index (0.67) was recorded among seeds of KU 1414-STR while the highest genetic similarity distance (2.10) was recorded among seeds of Oba Super 2 collected from Agro-dealer. Genetic similarity distance among inbred lines ranges between 0.67 (recorded in KU 1414-STR) and 1.77 (recorded in 1368). The least average genetic similarity index among seeds of hybrid maize (1.33) was recorded from Oba Super 1 collected from IITA while the highest average similarity index (2.10) was recorded from Oba Super 2 collected from Agro dealer.

Relationship between seeds of inbred lines and hybrid maize varieties based on protein profiles of SDS-PAGE

The dendogram constructed from similarity coefficients calculated based on SDSPAGE analysis for the inbred lines and hybrid seeds of maize revealed that the three seeds of Oba Super 2, 4001 and KU-1414-STR collected from IITA were grouped close to each other with one of the seed slightly different from the other two (Figure 2). Most of the other inbred lines and hybrids have only two seeds each grouped together while the other one is quite far from the other two. Oba super 2 collected from IITA clustered closely to the parental inbred lines (4001 and KU-1414-STR).

DISCUSSION

Two principal classes of protein (storage proteins and the lectins) dominate the protein content of many plant seeds (Langston-Unkefer and Gade, 1984). In this study, the frequency of protein bands with heavier molecular weights (45 to 100 KDa) except 80KDa detected in the maize seed sample was almost monomorphic. According to Machuka, (2001), the molecular weights of these proteins correspond to globulins (64-45 KDa) and albumins (122-65KDa). Young-Min *et al*, (2001) has reported that, though maize kernels contain albumins, globulins, prolamins and glutelins only two types of storage protein predominate maize seed. These are globulin found in the embryo (Kriz, 1999) and prolamins (zein fractions) with a group of alcohol-soluble polypeptides found in the endosperm, the major site of storage protein accumulation (Wilson, 1983). The result obtained in this study therefore imply that meaningful discrimination among maize samples through the use of SDS-PAGE can best be achieved by focusing on proteins with lighter molecular weight (zein proteins).

The calculated genetic similarity and distance indices which showed that varied similarity level existed among the maize samples are an indication that the seeds were not completely true to type. High similarity distance indices recorded between each hybrid seeds sourced from IITA and Agro-dealer is an indication that SDS-PAGE was able to detect genetic differences between the seeds of the varieties. This result is in agreement with Wang *et al*. (1994) that used seed proteins for characterization of maize hybrids and inbred lines from different geographical regions. The difference detected between the varieties can be as a result of handling during production since they were sourced from different organisations. The dendogram drawn from the similarity and distance indices of SDS-PAGE analysis revealed diverse level of relationship. All seeds that are closely related at 0.00 level of similarity, clustered into one group explain that such seeds have genetic similarities.

CONCLUSION

The storage proteins of cereals are of immense importance in determining the quality. Electrophoretic profiles observed in SDS-PADE analysis revealed diverse clusters of the maize samples based on their genetic variability. Therefore, a more precise variability among the seed samples can be revealed at molecular level using SDS-PAGE. Since seed protein analysis are not sensitive to environmental fluctuations and the banding patterns are stable. SDS-PAGE can be advocated for cultivar identification and purity analysis in crops so that breeders, seed scientists, seed regulatory agencies and seed companies can quickly ascertain the purity of seed lots without necessarily waiting for a complete growing cycle of the crop.

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