

Biochemical Characterisation of 18 Accessions of Sweet Potato (*Ipomoea batatas* L. Lam.) Using Total Leaf and Tuberos Root Protein by SDS-PAGE

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Abstract

DNA-based markers, undeniably, provide potent tools for studying genetic relationships in plants; however, potential of protein-based markers using SDS-PAGE is quite promising, particularly in developing countries. Plant storage proteins serve as molecular reserves imperative for plant growth and maintenance and thus have proven to be essential for the survival of plant species. Storage proteins are also indispensable in plant defense mechanisms as insecticidal and anti-microbial proteins. They have also been widely employed as biochemical markers for genetic diversity studies. Eighteen sweet potato (*Ipomoea batatas* L. Lam) accessions with local or exotic origin were evaluated for genetic diversity using leaf and tuberos root protein via sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Marked variations observed in the electrophoregrams of total leaf and tuberos root proteins gave an indication of intense variability among the accessions. Molecular size of proteins ranged from 20.23 to 28.89 kDa and 22.44 to 97.19 kDa, in the total leaf and tuberos root proteins, respectively. Two pairs of accessions SA/BNARI and UE 007 as well as HMA 1 and US 029 were identified as duplicates based on their banding patterns.

Keywords: Biochemical, characterisation, SDS-PAGE, sweet potato, leaf & tuberos root protein

Introduction

Sweet potato (*Ipomoea batatas* L. Lam) belongs to the family Convolvulaceae and is grown primarily for its storage roots. Its germplasm exhibits great genetic variability in many characters. The successful conservation and genetic improvement of sweet potato, is dependent on the level of knowledge on germplasm diversity (Yada and Tukamuhabwa, 2010).

Genetic diversity studies are important to plant breeders, in the selection of parents for hybridisation as sound crop improvement depends upon the magnitude of genetic variability in base populations (Afuape et al., 2011). However, before selections are made, the germplasm collection should be characterised to identify unique traits useful to the plant breeder. The characterisation of sweet potato germplasm has traditionally relied on morphological characters and has been used for various purposes including identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance (Elameen et al., 2011; Karuri et al., 2010).

Morphological characterisation is the first step in the assessment of sweet potato diversity (Karuri et al., 2010). However, due to the asexual propagation of the crop, numerous duplicate accessions are present in many collections (Huamán et al., 1999). In Ghana as in many parts of the world, different accessions are given similar names and leading difficulties in precise identification of genotypes (Ahiakpa et al., 2013; Harlan, 1992). Furthermore, limited success has been achieved with morphological diversity analyses alone in the selection of parental genotypes for hybridisation schemes due to phenotypic plasticity and environmental impact on morphological traits (Lin et al. 2007; Price et al., 2003; Prakash et al., 1996).

Therefore, morphological characterisation is often complemented with other methods of characterisation to ensure precise identification of true genetic identities of accessions in any collection. Molecular markers are the most efficient markers for genetic diversity studies in many species (Rakoczy-Trojanowska and Bolibok, 2004) including sweet potato (Zhang et al., 2000). However, the use of biochemical markers based on total seed and tuberos root protein and enzyme by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique has proven to be a reliable, yet inexpensive method of developing genetic markers for identification and genetic analyses of several plant species, as they reveal differences between storage proteins or enzymes encoded by different alleles at a single (allozymes) or more gene loci (isozymes) (Oppong-Konadu et al., 2005; Rao, 2004).

Electrophoretic profiles of total proteins and isozymes have been used in genetic diversity studies in sweet potato and to identify duplicates in germplasm collections (Saha et al., 2000; Kennedy and Thompson, 1991). The main objective of this study was to characterise 18 accessions of sweet potato and to identify duplicates in the collection using total leaf and tuberous root proteins via SDS-PAGE to enhance future breeding work.

MATERIALS AND METHODS

Germplasm collection

Fresh young leaves and tubers harvested at maturity from 18 sweet potato accessions were placed on ice to prevent dehydration and maintain freshness and integrity of the samples. The table below shows names and origins of the sweet potato accessions used in the study.

Table 1: Sweet potato Accessions used for Biochemical Study

Code	Name	Origin	Type	Flesh Colour	Skin Colour
CR001	BLUE BLUE	Central Region	Local	Pale yellow	Cream
ER 001	Farmer's variety	Eastern Region	Local	Cream	Cream
FREEMA	Farmer's variety	Greater Accra Region	Local	Pale yellow	Cream
HMA1	Farmer's variety	Greater Accra Region	Local	Pale yellow	Cream
HMA2	Farmer's variety	Volta Region	Local	Cream	Cream
HMA3	Farmer's variety	Greater Accra Region	Local	Cream	Purple red
LOCAL 1	Farmer's variety	Greater Accra Region	Local	Pale yellow	Cream
LOCAL 2	Farmer's variety	Greater Accra Region	Local	Cream	Purple red
UE 007	BONAGAYELE	Upper East Region	Local	White	Purple red
CRI001	DAAK 008	Ashanti Region	Local	Pale orange	Purple red
CRI 054	Farmer's variety	Ashanti Region	Local	Pale yellow	Pink
CEMSA 74-228	CEMSA 74-228	Cuba	Exotic	Pale yellow	Pink
CRI027	TIS 86/0350	Nigeria	Exotic	Cream	Cream
SA/BNARI	Beauregard	South Africa	Exotic	Intermediate orange	Brownish orange
UK/BNARI	Betablast	United Kingdom	Exotic	Dark orange	Pink
US 004	Yellow Sunflower	China	Exotic	Pale yellow	Cream
US 020	L329	Papua New Guinea	Exotic	White	Pink
US 029	W-209	USDA/Repository	Exotic	Dark orange	Purple red

Sample preparation

Extraction of total leaf protein

Each leaf sample weighing 0.4 g was homogenised in a mortar using liquid nitrogen and 0.8 g of insoluble polyclar AT (N-vinyl-2-pyrrolidinone). The powdered mixture obtained was added to 9.5 ml extraction buffer and stirred for 15 minutes to ensure uniform dissolution in the buffer. It was centrifuged at 4°C for 45 minutes at 16000 rpm. The supernatant was then collected and 90% cold acetone was added to make up the volume to 20 ml. The protein was allowed to precipitate at -20°C for 2 hours, and centrifuged at 9000 rpm for 20 minutes at 4°C. The supernatant was discarded and the precipitate was washed once with 90% cold acetone and centrifuged at 9000 rpm for 10 minutes. The pellet formed was air-dried and re-suspended in 500 µl of the extraction buffer and stored at -20 °C until needed for electrophoresis. Fifteen micro-litres of each sample were used for gel electrophoresis.

Extraction of total tuberous root protein

One gram (1.0 g) of each tuber was pulverised in 1 ml extraction buffer (containing 0.5 M Tris pH 6.8, and 10% SDS). The supernatant was separated by centrifugation at 4000 rpm for 10 minutes at 4 °C. The supernatant was centrifuged again at 4000 rpm for 10 minutes at 4°C and the resulting supernatant kept for 3 hours at room temperature and used directly for electrophoresis.

Preparation of Gel slab

Four gelslabs were prepared according to Laemmli (1970). Two for analysis of sweet potato leaf proteins and two for tuberous root proteins. For preparation of each gel, the gel slab unit (BioRad SDS/PAGE Mini Protean II Gel) was assembled with the glass sandwich set in casting mode with 0.75 mm spacers in place. 12% resolving gel was prepared by adding 1.6 ml of dH₂O, 1.3 ml of Tris (pH 8.8), 2 ml of 30% acrylamide solution, 50 ml of 10% SDS, 50 µl 10% of APS and 2 ml of Tetramethylethylenediamine (TEMED) (all the reagents were added except TEMED); once the TEMED and ammonium per sulphate (APS) have been added to the polyacrylamide solution, it was polymerised in few minutes. The resolving gel was transferred into the slab unit using 1 ml micropipette.

Immediately after pouring the gel, 1-2 ml layer of butanol/dH₂O was gently added on top to avoid exposure to

air and to eliminate any bubbles from it. The gel was left to polymerise for 10 minutes. The layer of butanol/dH₂O was discarded and the gel washed with dH₂O. The stacking gel was poured directly on top of the resolving gel. The comb was gently and quickly inserted and the gel left to polymerise.

The gel slab unit was assembled into the electrophoresis chamber and the inner chamber filled with 1X running buffer. Additionally, 1X running buffer was poured into the outer chamber until the level was above the bottom of the glass plate. 5 µl of the marker and 15 µl of each sample were loaded onto the gel using long gel-loading tips.

Electrophoresis

The top of the electrophoresis apparatus (BioRad SDS/PAGE Mini Protean II Gel, Germany) was assembled and connected to a power source (SIGMA PS 500-2, USA). The proteins were allowed to run through the stacking gel at 100 V, the power was then turned up to increase the voltage until the display read ~200 V and the gel allowed to run for 45 minutes. The power was turned off and gel apparatus disassembled after the tracking dye reached the bottom of the resolving gel.

Staining and De-staining of Gel

The gels were stained with Coomassie Blue staining solution (1% Coomassie Brilliant Blue R-250, 45% methanol, 10% glacial acetic acid and 45% dH₂O) for 30 minutes and de-stained for 1 - 4 hours in a de-staining solution (7% glacial acetic acid, 20% methanol and 73% dH₂O).

Data analysis

Electrophoregrams for each accession were scored and the presence (1) or absence (0) of each band recorded. Presence and absence of bands were entered into a binary data matrix. The Simple-matching similarity matrix was used to construct a dendrogram by the complete-link method using a General statistical package (Genstat, vers. 9.2, UK). The relative mobility value (Rf) of each protein band was calculated using Image J software (vers. 1.44). A standard molecular weight marker (low range) consisting of phosphorylase B (106.0 kDa), bovine serum albumin (80.0 kDa), ovalbumin (49.5 kDa), carbonic anhydrase (32.5 kDa), soya bean trypsin inhibitor (27.5 kDa), and lysozyme (18.5 kDa) (Biorad, Prestained High and Low range SDS-PAGE Standards) were used for computing the molecular weights of the different protein bands.

RESULTS

Electrophoretic Patterns of Total Leaf and Tuberos Root proteins of 18 Accessions of Sweet potato

Figures 1 - 4 show the total leaf and tuberos root proteins for 18 accessions of sweet potato. One to four bands were detected for the leaf proteins and one to five bands for the tuberos root proteins. The most abundant (dark and thickest) bands for total leaf and tuberos root were 28.9 kDa and 38.68 kDa respectively as shown in Table 2. The other bands were smaller, close to 21 and 25 kDa for total leaf protein and 22 to 33 kDa for the tuberos root proteins. Most of the bands were faint; thus not abundant and difficult to visualise.

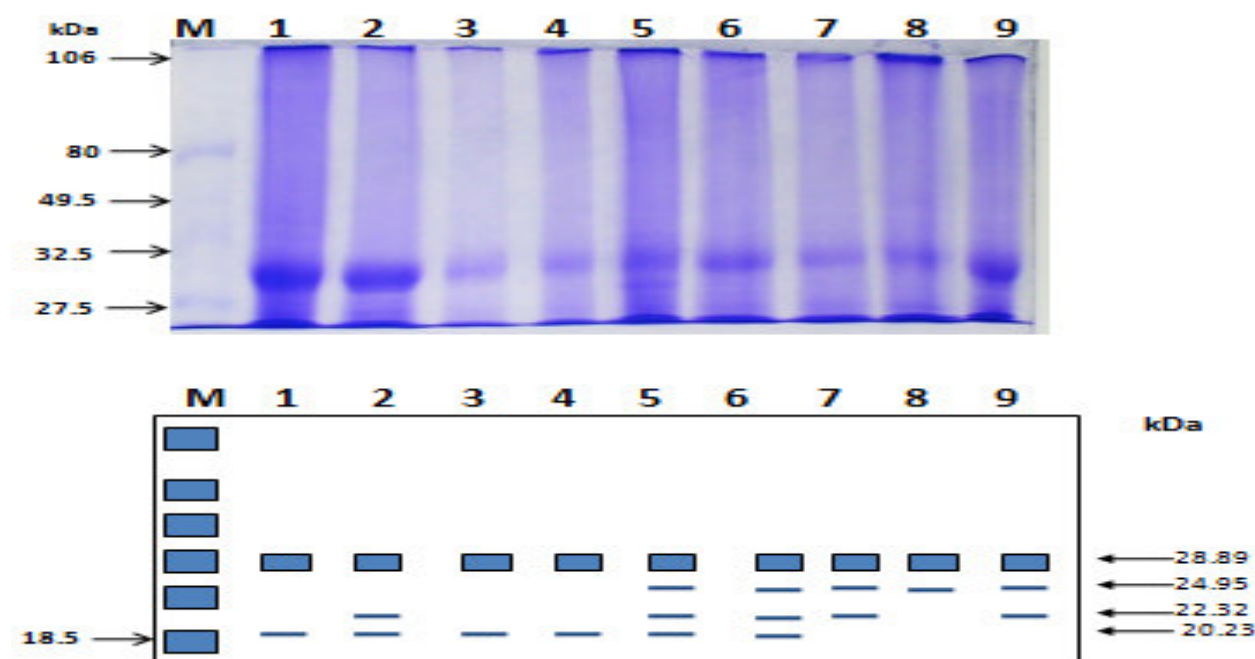


Fig. 1: Electrophoregrams showing banding patterns of sweet potato leaf proteins. M. Markers, 1. CRI 027; 2. UK/BNARI; 3. SA/BNARI; 4. HMA 3; 5. HMA 1; 6. HMA 2; 7. FREEMA; 8. CRI 054 and 9. US 020

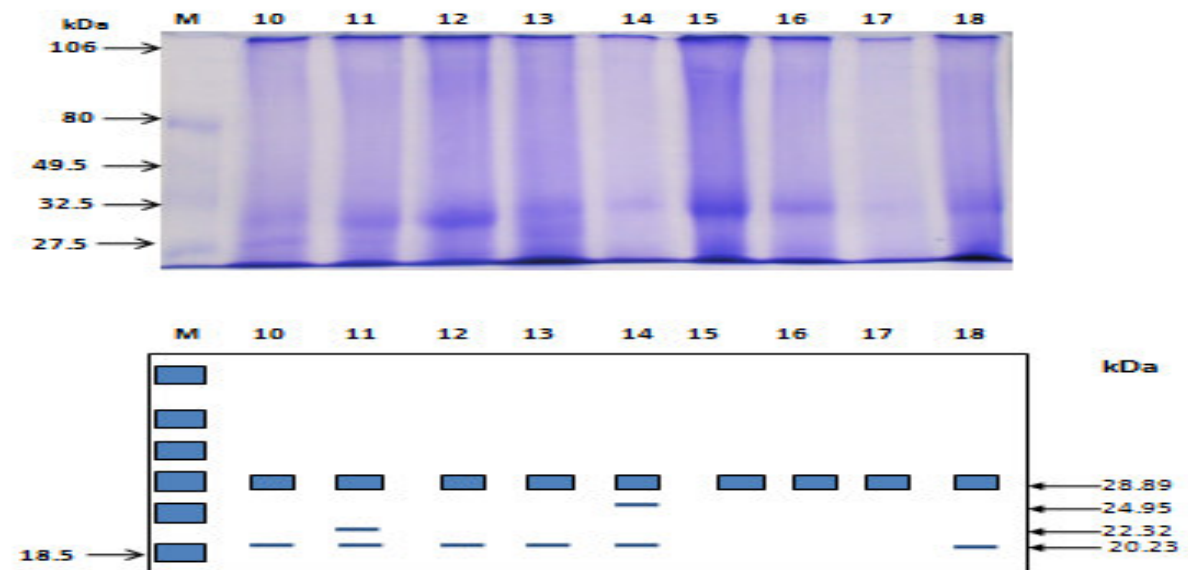


Fig. 2: Electrophoregrams showing banding patterns of sweet potato leaf proteins. M. Markers, 10. US 029; 11. CR 001; 12. US 004; 13. LOCAL 1; 14. CRI 001; 15. UE 007; 16. LOCAL 2; 17. CEMSA 74-228 and 18. ER 001.

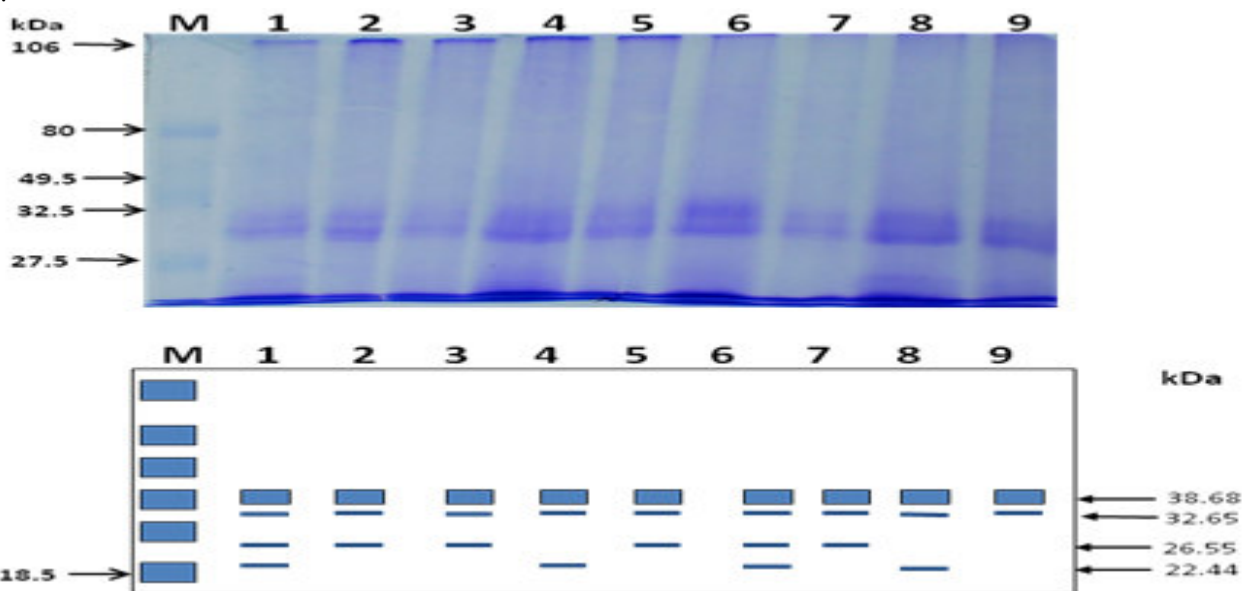


Fig. 3: Electrophoregrams showing banding patterns of sweet potato tuberous root proteins. M. Markers, 1. HMA 1; 2. UE 007; 3. FREEMA; 4. US 004; 5. SA/BNARI; 6. ER 001; 7. HMA 2; 8. UK/BNARI and 9. HMA 3

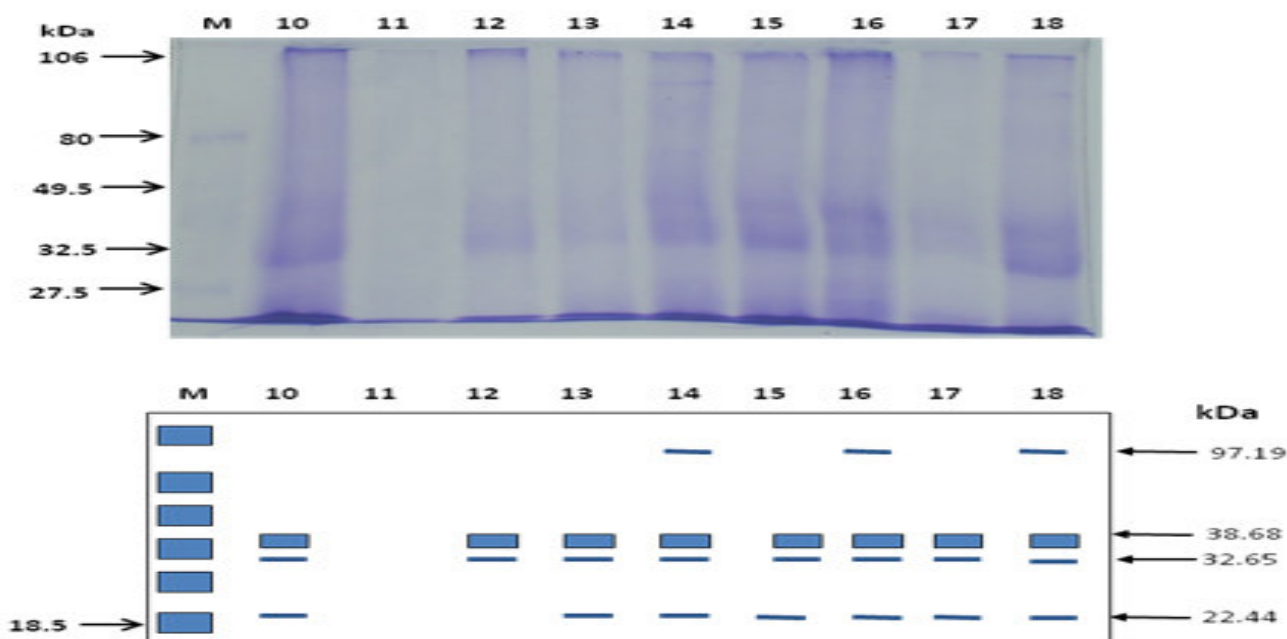


Fig. 4: Electrophoregrams showing banding patterns of sweet potato tuberous root proteins. M. Markers; 10. LOCAL 1; 11. CRI 027; 12. US 020; 13. CR 001; 14. US 029; 15. LOCAL 2; 16. CRI 001; 17. CRI 054 and 18. CEMSA 74-228

Table 2: Total leaf and tuberous root proteins of 18 Accessions of *I. batatas* as resolved on 12% SDS-PAGE

Protein Origin	Total number of protein bands	Band	Molecular weight (kDa)	Corresponding Rf values
Leaves	4	1	28.89	0.759
		2	24.95	0.833
		3	22.32	0.886
		4	20.23	0.933
Tubers	5	1	97.19	0.17
		2	38.68	0.62
		3	32.65	0.70
		4	26.55	0.80
		5	22.44	0.91

Cluster Analysis of 18 Accessions of Sweet potato based on electrophoretic banding patterns of total leaf and tuberous root proteins

Figure 5 shows the genetic relationship among the accessions revealed by electrophoretic banding patterns of total leaf and tuberous root proteins. The dendrogram is based on Simple Matching Coefficient using Complete-Linked Similarity Matrix. The accessions were separated into two clusters at a genetic distance of 33.7%. The first major cluster contains four accessions at 67% similarity index and further regrouped into two sub-clusters at a genetic distance of 78.2%. However, the other 14 accessions started to separate at 44.9% similarity index and further form clusters and sub-clusters. CRI 027 and US 020 were the most widely separated among the accessions.

Two pairs of accessions SA/BNARI: UE007 and HMA 2: FREEMA failed to separate at a similarity index of 100%, suggesting that they are duplicates. Similarly, three accessions UK BNARI: US004: LOCAL 1 exhibited 100% genetic similarity, forming a triplicate.

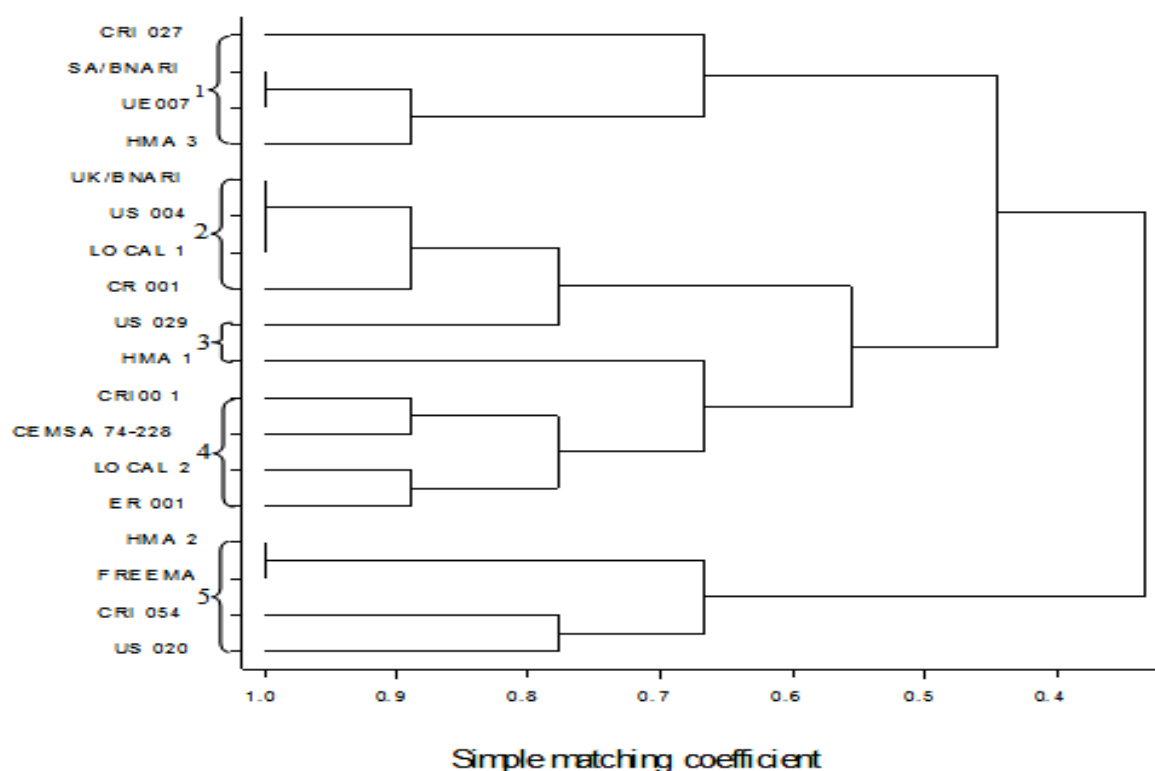


Fig. 5: A dendrogram showing the genetic relationship among the accessions based on Simple Matching Coefficient using Complete-Linked Matrix method

Discussion

Electrophoretic Pattern of Total Leaf and Tuberos Root Proteins of 18 Accessions of Ipomoea batatasL.

Each accession within the collection showed distinct electrophoretic banding pattern. The highest number of bands for the total leaf and tuberos root proteins were 4 and 5 respectively. The four banding patterns of the total leaf proteins is consistent with those observed by Saha et al. (2000) and Kennedy and Thompson (1991). The results obtained for the total tuberos root proteins however contrast with results of Ali and Javad (2007), (30 bands) and Saha et al. (2000) in sweet potatoes (18 bands).

The molecular weights of the total leaf protein bands ranged from 20.23 to 28.89 kDa and those of the total tuberos root proteins spanned from 22.44 to 97.19 kDa. These results are consistent with reports of Ali and Javad (2007) in potato; Hou et al. (2004), Hou and Lin (1998) as well as Wang and Yeh (1996) in sweet potato. Toyama et al. (2006); Hou et al. (2004) and Shewry (2003), reported that storage roots of sweet potato contain large quantities of two proteins, sporamin A and B, which account for over 80% of total soluble protein content. Additionally, Toyama et al. (2006); Hou et al. (2004; 1999) determined the molecular weight of sporamins in sweet potato tubers as being in the range of 22.4 to 38.9 kDa. Ohto et al. (1992); Hattori et al. (1991) and Nakamura et al. (1991) have established that sporamins are inducible in the leaves and petioles of sweet potato.

Cluster Analysis of 18 Accessions of Sweet potato based on electrophoretic banding patterns of total leaf and tuberos root proteins

The results of the cluster analysis revealed low genetic diversity among accessions with two major clusters at a genetic distance of 33.4%. The accessions were distributed in five cluster entries; however, local accessions were grouped in a relatively distinct manner. Accession US 020 and CRI 027 were widely separated confirming patterns of clustering observed in an earlier morphological study. Accessions are considered duplicates if their genetic similarity index is equal or greater than 95% (Anderson et al., 2007). FREEMA and HMA 2, as well as UE 007 and SA/BNARI may be considered as duplicates with similarity index of 100%. Similarly, UK/BNARI, US 004 and LOCAL 1 had similar total leaf and tuberos root banding pattern and were identified as single entry. This contrasts results of morphological study on the same accessions which identified only HMA 2 and ER 001 as duplicates (Sossah, 2012).

SA/BNARI, UK/BNARI and US 029 are orange-fleshed varieties but were classified as duplicates with white-, and pale yellow-fleshed varieties. This divergence may be attributable to the limited genetic base, evident in the total proteins of the leaves or environmental fluctuations impacting on expression of morphological traits (Sammour, 2005). Indeed, Prakash et al. (1996) noted that protein-based markers as isozymes and total proteins

have been used with limited success in sweet potato in genetic diversity studies as a result of low levels of polymorphism. Though, accessions may have limited genetic diversity, they can still be useful in genetic improvement programmes (Oppong-Konadu et al., 2005). CRI 027 and US 020 are the most widely separated accessions and may be considered for use in any future hybridisation programme, if their agronomic attributes are desirable.

Conclusion

Although, DNA-based markers provide powerful tools for detecting genetic relationships in plants, the capacity of protein markers using SDS-PAGE is quite high and effective, especially in developing countries. The variations observed in the electrophoregrams of the total leaf and tuberous root proteins in this study, though small, give an indication of the level of polymorphism among the accessions. The sizes of the bands observed, spanned from 20.23 to 28.89 kDa and 22.44 to 97.19 kDa, in the total leaf and tuberous root proteins respectively. Two pairs of accessions SA/BNARI and UE 007 as well as HMA 2 and FREEMA were identified as duplicates based on their banding pattern. Similarly, three entries, LOCAL 1, US 004 and UK/BNARI were also identified as a single entity based on their banding patterns for leaf and tuber proteins. CRI 027 and US 020 are the most widely divergent accessions. Integrating protein and DNA-based markers with agro-morphological markers for comprehensive genetic diversity studies in sweet potato may offer the greatest of prospect in the future.

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