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Investigation of the Genetic Diversity of Selected Wild and Cultivated Sorghum Germplasm Using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

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ABSTRACT

Thirty-five (35), wild (5) and cultivated (30) *Sorghum bicolor* germplasms were collected from 5 different agro-ecological zones in Nigeria. Proteins were extracted from six weeks old fresh leaves (1.0 g) with 10ml of 0.85% (w/v) sodium chloride (NaCl) solution, filtered and followed by centrifugation at room temperature at 3,000 rpm for 10 min. The supernatants were employed for protein analyses on polyacrylamide gel electrophoresis in the presence of SDS. The protein concentrations of the cultivars from North East, North West, South and wild were not statistically different ($P < 0.05$) but in the same range with those of cultivars from North Central agro-ecological zone. Analyses of proteins revealed a total of 141 protein bands produced by the cultivars, 21 % has relative molecular mass of 190kD, 24% (22kD), 21% (11kD) and 24% (10kD). However, proteins with 21kD and 26kD were specific to only cultivars from North Central and wild collections. The proportion of differentiated protein (Pp) ranged between 0.06 and 0.24 while the dendrogram constructs revealed little variation between the cultivated and wild *sorghum* accessions.

INTRODUCTION

Sorghum (*Sorghum bicolor,* L.), with related millets is a major source of energy and protein in the semi-arid tropic (SAT) areas. It is the fifth leading cereal in terms of world production and one of the major coarse grain cereals grown as a rain-fed crop in the SAT areas. In both Africa and India, nearly all *Sorghum* grain is used directly as human food, sometimes providing up to 50% of dietary consumption (Doggett 1988; Pushpamma 1993).

Sorghum bicolor (L.) belongs to a large genus of the tribe Andropogoneae in the family Gramineae and has been cultivated in Africa for over 3,000 years. Most cultivars are annuals while few are perennials. Cultivated and most weedy *sorghum* are non-rhizomatous, culms nodes are either glabrous or shortly tomentose (Dahlberg *et al*., 2000, 2007).

SDS-PAGE is a simple, convenient, rapid and widely used technique for the analyses of proteins and holds its popularity to its power to resolution of mixture of complex proteins (Smith 1994, Srivastava 2008). According to Gottlieb (1972), the advantage of electrophoretic technique is that variation in protein band patterns could be related to genes that code for various polypeptides. It is employed to detect differences in polymorphic proteins in different cultivars. Since protein composition is genetically determined, it is not subjected to environmental conditions as well as the location of the genes coding for particular proteins in several cases (Aiken and Gardiner 1991, Cai and Bulletin 1992). Electrophoretic analyses of proteins have advantages over traditional identification techniques in that it offers a rapid and reliable evaluation of genetic differences among most cultivars (Ferguson and Grabe 1986, Gardiner and Fered 1987, 1988), it is a promising tool to distinguish cultivars of a particular crop species (Illoh 1990, Moller and Spoor 1993, Jha and Ohri 1996, Essiet and Illoh 1997, Folorunso and Olorode, 2002). The choice of protein as an index of biomarker in this study is because it is easier to establish cultivar relationships and involves simple technology that can easily be adapted in countries like Nigeria. Using the solubility-based classification scheme, sorghum proteins are classified as albumins, globulins, kafirins, cross-linked kafirins and glutelins. The sorghum protein content ranges between 6% and 18% with an average of 11% (Jambunathan *et al*., 1975 Lasztity 1996, Hamaker-Bruce and Bugusu, 2007). This study was designed to investigate the genetic variations between the wild and cultivated sorghum species in the six agro-ecological zones in Nigeria with a view to establishing relatedness between the varieties. Also the study will allow for the discovery of useful traits (usually inherent in the wild relatives) that could be used for the improvement of the cultivated varieties. The protein bands, being an expression of the Sorghum DNA, were analysed to know the number and their possible migratory position in the electrophoretic field; thus enhancing their discovery and importance in breeding practices towards Sorghum improvement.

MATERIALS AND METHODS

Materials

Thirty five (35) Sorghum accessions were obtained from the National Centre for Genetic Resources and Biotechnology, Moor-Plantation, Ibadan, Nigeria and from the six agro-ecological zones in Nigeria. The sorghum accessions represented 30 cultivated and 5 wild from Kaduna, Kano, Niger, Nassarawa, Plateau, Gombe, Bauchi, Jigawa, Kogi and oyo States, Nigeria.

Methods

Planting of Sorghum Seeds

Thirty five Jiffy pots were filled with humus soil to a height of 10 cm and well watered. 3 seeds of each *Sorghum* accession (wild and cultivated) were planted in each pot in duplicates. The seeds were allowed to germinate and grow at the Nursery of the Reforestation Project in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria for 6 weeks. The seedlings were watered at regular intervals of 2 days.

Extraction of leaf total soluble proteins

Six weeks old fresh leaves (1.0 g) of the *Sorghum* seedlings were cut into bits, crushed to a fine paste using acid washed glass beads as abrasive. The powder was then suspended in extracting medium and homogenized with 10 ml of 0.85 % (w/v) NaCl. The slurries were transferred into clean vial bottles and left overnight at 4℃ and followed by filtration and centrifugation at 3,000 rpm for 10 min., on Bench Microcentrifuge (Model 800D). The supernatants were collected into clean sterile bottles and used for the estimation of soluble total protein and electrophoretic analyses.

Estimation of Protein Concentrations

The total protein concentrations in each protein extract was estimated using alkaline-copper reagent method of Schactek and Pollack (1973) as slightly modified by Oyedapo *et al*. (2004) using bovine albumin (2.5 mg/ml). The absorbance was read at 650 nm against protein reagent blank. The amount of protein in each sample was estimated using the expression:

$$\text{Concentration of protein (mg/ml)} = \frac{\text{Abs of test} - \text{Abs of blank}}{\text{Abs of std} - \text{Abs of blank}} \times \text{df} \times \text{conc of std.}$$

where abs of test (absorbance of protein extract); abs of std (absorbance of standard); df (dilution factor, 5). The protein concentration was expressed as mg/g fresh leaf equivalent.

SDS-Polyacrylamide Gel Electrophoresis

The SDS – PAGE analysis was carried out using the Bio-Rad Mini Protein III slab cell (10 ml capacity) using a discontinuous buffering system according to a modified method that was based on those earlier reported by Laemmli (1970) and Smith (1994). The protein samples and marker proteins were prepared by solubilization in equal volumes of sample buffer (62.5 mM Tris- HCl, pH 6.8; 10% (w/v) SDS; 10% (v/v) β-mercaptoethanol; and 0.01% (w/v) bromophenol) and followed by heating at 100 ° C for 5 min. The protein samples (50 µl) and marker proteins (50 µl) were carefully loaded into the gels and electrophoresis was carried out at room temperature at constant current of 3mA/column until the tracking dye was about 0.5 cm to the bottom of the gels. The gels were removed, washed, the lengths and dye fronts measured. The gels were stained overnight with 0.025% (w/v) Coomasie Brilliant Blue R250 in a mixture of MeOH : CH_3COOH : H_2O (65 : 25: 10; v/v/v) and followed by destaining in a mixture of CH_3COOH: MeOH: H_2O; 3:7:20 (v/v/v) until protein bands were visible. The distances migrated by the proteins were measured and relative mobility (rm) of each protein was estimated. Leaf protein bands were evaluated visually , protein bands photographed and scored as present (1) or absent (0) at each of the observable loci (Fig. 1). The molecular weight of each protein was estimated from the standard calibration curve of relative mobility and logarithm of molecular weights of maker proteins.

Differentiation of Protein Banding Patterns

This was evaluated by the proportion of differentiated protein bands (Pp) which was calculated as Pp = Dn / Tn, where Tn is the total number of protein bands, Dn is the number of differentiated protein bands between the cultivars, which was obtained by subtracting the number of protein bands non-identical for the cultivars from the total number of protein bands, Tn.

Statistical Analysis:

Data were expressed as mean ± SEM (n= 5 readings). Dendrograms were equally constructed using the Paleontological statistical software package version 1.7 for phylogenetic studies. Values of $p< 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1.0 : Summary of Protein Concentrations (mg/g) of 35 *Sorghum* Collections from Agro-ecological Zones

Agro-ecological zone	Protein concentration (mg/g fresh leaf)
1. North Central (14)	18.4 ± 0.8^a
2. North West (6)	22.6 ± 1.8^b
3. North East (9)	22.0 ± 0.8^b
4. South West (1)	23.6 ± 0.2^c
5. Wild Collections (5)	23.0 ± 1.0^c

Each value represented the mean ± SEM (n = 5 readings). Figures with the same superscripts are not statistically different, while those with different superscripts are statistically different. Value of $p< 0.05$ was taken as statistically significant.

Table 3.0: Proportion of Differentiated Protein (Pp) Among Sorghum Cultivars from Agro-Ecological Zones

Agro-ecological zone	North Central	North East	North West	South West	Wild Collection
Proportion of Differentiated Proteins	5/52 (0.10)	2/36 (0.06)	6/25 (0.24)	-	5/26 (0.19)

Sorghum bicolor **cultivars on SDS-PAGE.**

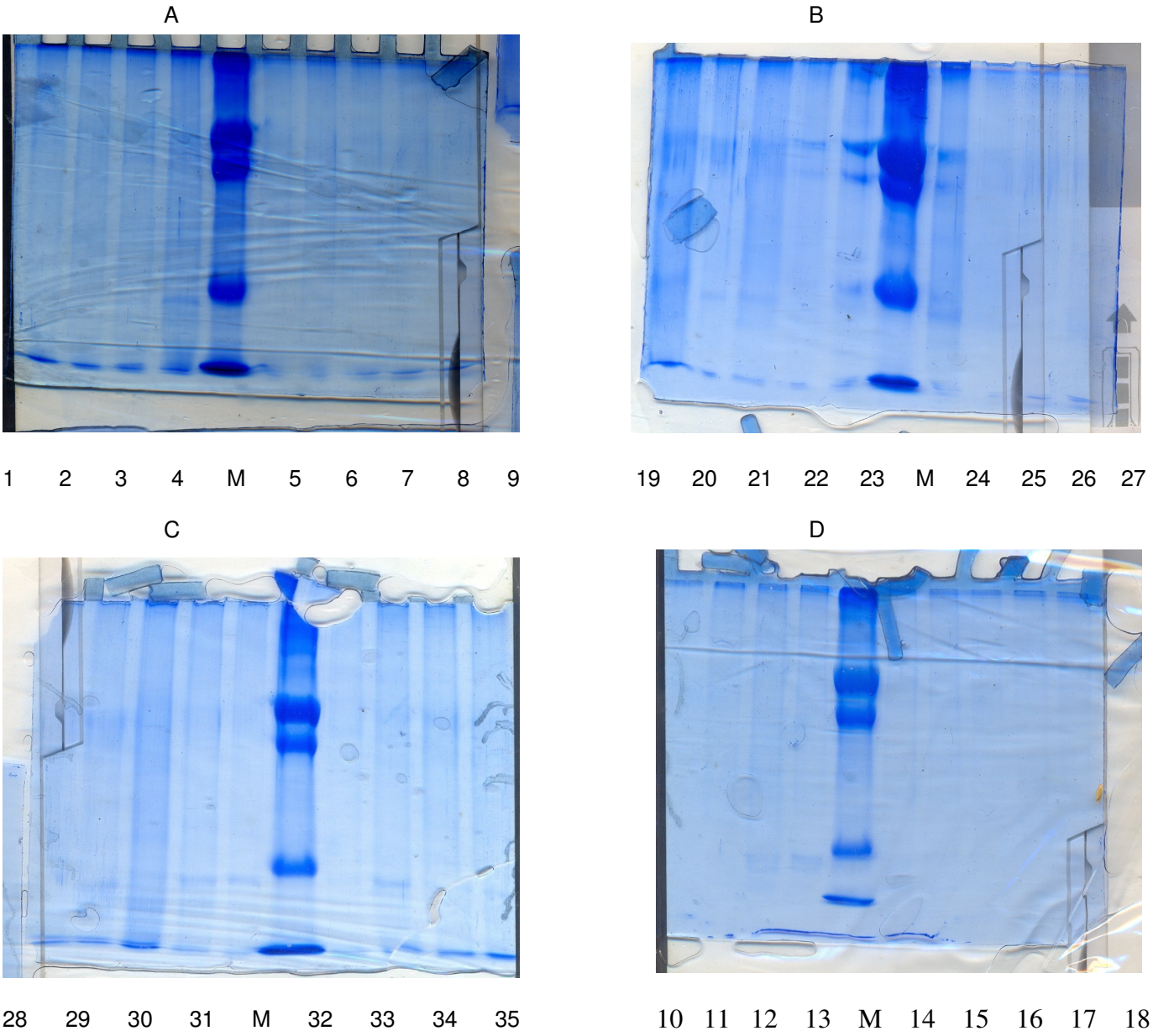

Fig. 1.0 : Electrophoretic Patterns of Protein Bands of Proteins of fresh leaves of 35

The migration was from the top to bottom

A. Protein bands of cultivars (1-9) with marker proteins at the centre;
B. Protein bands of cultivars (19-27) with marker proteins at the centre;
C. Protein bands of cultivars (10-18) with marker proteins at the centre; and
D. Protein bands of cultivars (28-35) with marker proteins at the centre

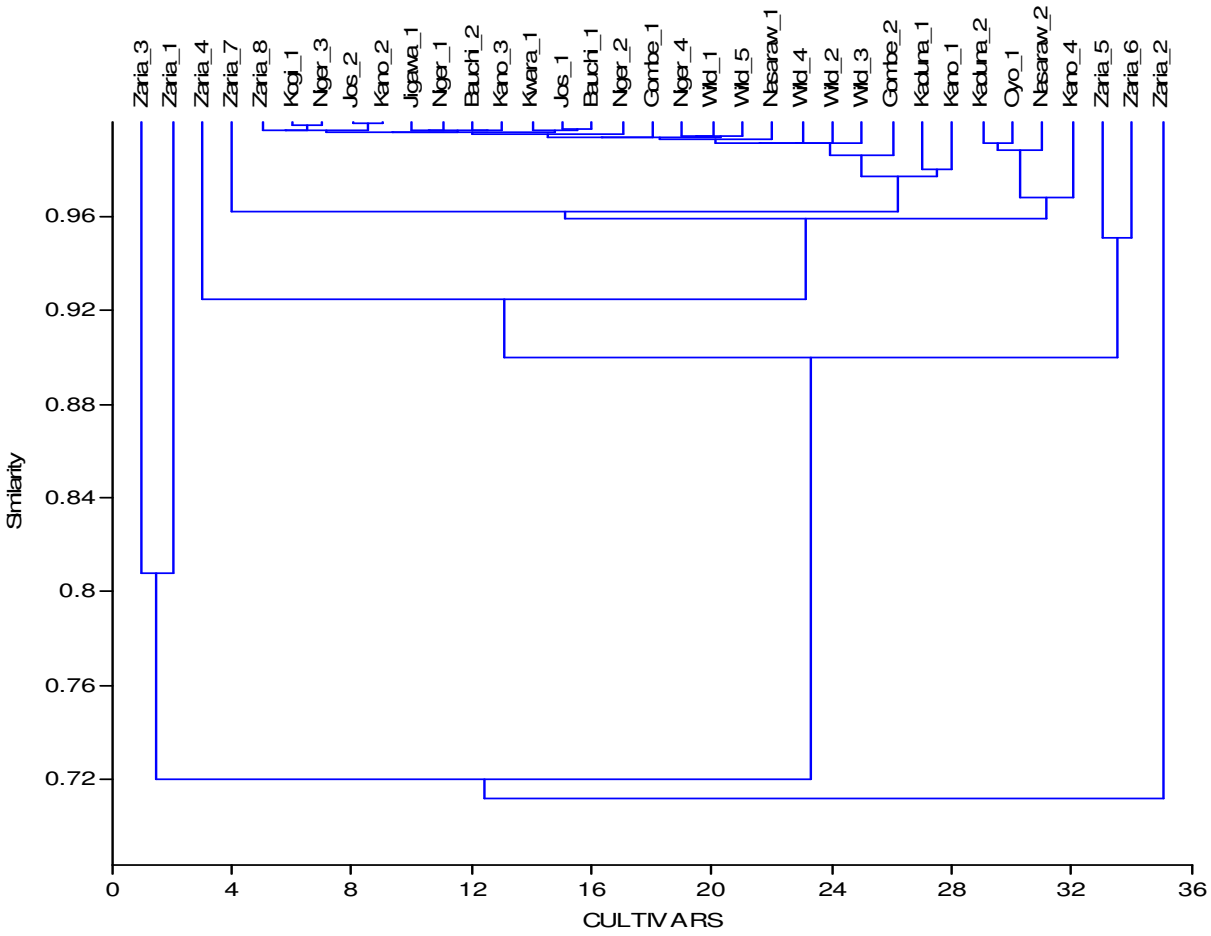

Fig. 2.0: DENDROGRAM SHOWING GENETIC RELATIONSHIP AMONG THE 35 SORGHUM COLLECTIONS

Overview of the 35 collections of *Sorghum* and the site of collection.

S/No	Name	National Code/ Site of collection	Pericarp color
1	Zaria 1	Samsorg 5	Light Yellow
2	Zaria 2	Samsorg 7	Light Yellow
3.	Zaria 3	Samsorg 8	Light Yellow
4.	Zaria 4	Samsorg 9	Red Color
5.	Zaria 5	Samsorg 14	Light Yellow
6.	Zaria 6	Samsorg 22	Light Yellow
7.	Zaria 7	Samsorg 23	Light Yellow
8.	Zaria 8	NSSH 91001	Light Yellow
9	Kwara 1	Ilorin	Light Yellow
10.	Kaduna 1	Birnin Yero Mkt.	White Color
11.	Kaduna 2	Birnin Yero Mkt.	Yellow Color
12.	Nasarawa 1	Keffi Market	Light Yellow
13.	Nasarawa 2	Keffi Market	Red Color
14.	Jos 1	New Market	White Color
15.	Jos 2	New Market	White Color
16.	Kogi 1	Lokoja Market	Yellow Color
17.	Oyo 1	Ogbomoso Market	Red Color
18.	Gombe 1	Farmers Purchase	White Color
19.	Gombe 2	Farmers Purchase	White Color
20.	Jigawa 1	Jigawa Market	White Color

21.	Bauchi 1	Muda Lawal Market	Red Color
22.	Bauchi 2	Muda Lawal Market	White Color
23.	Niger 1	Mokwa Market	Light Red Color
24.	Niger 2	Mokwa Market	White Color
25.	Niger 3	Mokwa Market	White Color
26.	Niger 4	Mokwa Market	Yellow/ Red
27.	Kano 1	Kano Market	Yellow
28.	Kano 2	Kano Market	White/ Red
29.	Kano 3	Kano Market	Yellow
30.	Kano 4	Kano Market	White
31.	Wild Type 1	W1 / Ibadan	Yellow
32.	Wild Type 2	W2 / Ibadan	Yellow
33.	Wild Type 3	W3 / Ilesha	Yellow
34.	Wild Type 4	W4 / Ilesha	Yellow
35.	Wild Type 5	W5 / Ibadan	Yellow

A great deal of attention and efforts have been paid to the development of biochemical methods for cultivar characterization, variety discrimination and identification (Gardiner *et al*., 1986; Cai and Bulletin 1992; El-Akkad and El-Kariem 2002, 2003; Oladipo *et al*., 2008). The most widely applied and versatile technique used for laboratory cultivar characterization has been the analysis of seed and leaf proteins and enzymes by various forms of electrophoresis. Cultivars of many crops have been analyzed and identified. Protein patterns have been successfully employed to resolve the taxonomic and evolutionary problems and complexicity of several crop plants (Cooke 1984, Sammour 1989, Khan 1992, Das and Makherjee 1995). The most commonly used proteins for cultivar discrimination are (a) seed storage proteins which are known to be polymorphic with respect to either size or charge or both; and (b) seedling or leaf enzymes/isoenzymes of various types (Gottlieb 1972). The proteins, the products of structural genes and represent a large number of gene products which serve as stable genetic makers (Aiken and Gardiner 1991, Rogi and Javomik 1996).

In Table 1.0, is the summary of soluble leaf protein concentrations in the homogenates of fresh leaves of *S. bicolor*. Of the 35 accessions, the average protein concentration was 21.8 ± 1.0 mg/g fresh leaf. The mean protein concentrations from North Central, North West, North East and South West (Wild) were 18.4 ± 0.8 mg/g; 22. ±1.8 mg/g; 22.0 ± 0.8 mg/g and 23.0 ± 1.0 mg/g fresh leaf respectively. The results revealed slight variations in the protein concentrations among the cultivars from the agro-ecological zones. However, the protein concentrations of the leaf seedling of cultivar from North Central zone was statistically lower than other zones. Studies have revealed that proteins are the products of structural genes and could also be influenced by environmental factors, application of fertilizers and other external conditions (Cai and Bulletin 1992; Gottlieb 1972).

The electrophoretic pattern of protein analyses of fresh leaves of *Sorghum bicolor* (cultivated and wild) are shown in Fig. 1 (a-d) and Table 2.0. In total, 141 protein bands were detected with molecular weights ranging from 190kD to 10kD. The proteins with molecular weights of 22kD and 11kD were common to all the cultivars. Moreover, proteins with molecular weights 190kD constituted 21%, 182 kD (4%); 132 kD (7%); 130 kD (2%); 32kD (6%); 26kD (0.7%); 22kD (21%); 21kD (4%), 11kD (24%) and 10kD (0.7%). It is clear from Table 2.0 that there was a slight variation in the number and positions bands from one cultivar to another.

The proportion of differentiated leaf protein (protein bands found in one but not in the other cultivars) Pp was employed to evaluate the differences between the cultivars. Analysis of Pp bands revealed that proteins with molecular weights 190kD and 11kD were common to all cultivars. The cultivars from North Central gave average of 3 bands, North East 4 bands, North West 4 bands, South West 3 bands and wild collections 5 bands (Table 3.0). The Pp among the cultivars was too low to make any appreciable comparison, North Central (0.10); North East (0.06); North West (0.24); and wild collections (0.19) which implied that there was a little genetic variation amongst the cultivars due to low proportion of differentiation (Fig. 2.0).

The overall summary of the electrophoretic run of *sorghum* proteins on SDS-PAGE revealed the presence of 7 bands in all, 4 prominent bands common to majority of the *sorghum* collections and 3 other protein bands. The presence of common bands among the accessions depicted two things. First, it is an evidence of an evolutionary origin of *Sorghum bicolor*, L., and secondly, the proteins are under the control of the same gene (Paterson et al., 2009). These might be adaptive genes which have evolved, dispersed and fixed in the species over evolutionary time. The numerous inter and intra specific bands reflect some level of affinity among the species. This is in an agreement with the observations of Illoh *et al*. (1993), that the concept of biochemical distances among species of known genetic relationship are measures of affinity. The diversity of protein bands are indicative of genetic diversity and may be useful in delimitation of the sorghum accessions . The protein bands pattern demonstrated a close relationship among the wild and cultivated sorghum

cultivars across agro-ecological zones. The protein bands pattern reveal a rather low genetic diversity among the collections of sorghum signifying a higher extent of genetic similarity among them, due to an appreciable number of similar proteins in the accessions.

CONCLUSION

The diversity of protein bands are indicative of genetic diversity and may be useful in delimitation of the sorghum accessions. The protein bands pattern demonstrated a close relationship among the wild and cultivated sorghum cultivars across agro-ecological zones. The protein bands pattern reveal a rather low genetic diversity among the collections of sorghum signifying a higher extent of genetic similarity among them, due to an appreciable number of similar proteins in the accessions.

The results revealed that geographical zones have not much effect on the protein concentrations and protein band patterns among the collections of *sorghum*. There were not many differences among these *Sorghum* collections despite their varied geographical zones. According to the observations of El-Akkad and El-Kariem (2002, 2003) that due to proximity, easy transportation and access within a particular country or geographical zones, there is likely to be proximity and low variations among germplasm collections within such a region.

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