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HETEROGENEITY IN THE SEED GLOBULIN AND ALBUMIN FRACTIONS FROM AFRICAN YAM BEAN *SPHENOSTYLIS STENOCARPA* (HOECHST. EX A. RICH) HARMS

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ABSTRACT

Successful fractionation of albumin, globulin and vicilin fractions from dry seeds of African yam bean (*Sphenostylis stenocarpa*) was achieved using established procedures for preparation of legume seed proteins. The resulting polypeptides were separated by native polyacrylamide gel electrophoresis under both reducing (in the presence of 2-mercaptoethanol [2-ME]) and non-reducing (without 2-ME) conditions. Based on this analysis, it was possible to establish similarities and differences among 26 different accessions of African yam beans collected from local markets in eastern Nigeria.

Key Words: African yam bean, albumins, fingerprinting, germplasm, globulins, Nigeria

RÉSUMÉ

Un fractionnement réussi de l'albumine, du globuline et de la viciline des graines sèches de haricot igname d'Afrique (*Sphenostylis stenocarpa*) a été réalisé par des procédures établies pour la préparation des protéines des graines des légumineuses. Les polypeptides qui en résultent ont été séparés par un gel d'électrophorèse de polyacrylamide natif sous les conditions de réduction (en présence du 2-mercaptoethanol [2-ME] et de no-réduction (sans 2-ME). Sur base de cette analyse, il a été possible d'établir des similarités et des differences entre les 26 accessions des haricots igname d'Afrique collectées dans les differents marchés lacaux de l'Est du Nigeria.

Mots Clés: Haricot igname d'Afrique, albumines, empreinte des doigts, germoplasme, globulines, Nigeria

INTRODUCTION

The African yam bean (AYB) (*Sphenostylis stenocarpa* Hoechst. Ex A. Rich) is by far economically the most important species within the genus *Sphenostylis* E. Meyer (Leguminosae, Papilionoideae; Phaseoleae) (Potter, 1992). It is also the most widely distributed and morphologically variable *Sphenostylis* species. The plant is found growing either wild or in cultivation in much of central (Gabon, Congo) and western (Nigeria, Cameroon, Togo, Ghana, Ivory Coast) Africa, especially in southern Nigeria (Okigbo, 1973). It is also reportedly cultivated in eastern (Kenya, Tanzania, Ethiopia) and southern (Mozambique, Zambia, Zimbabwe and Angola) Africa (Potter, 1992; Potter and Doyle, 1992). In West Africa, AYB is only cultivated for its edible seeds, unlike in other parts of Africa where the crop is grown mainly for its edible tubers (National Academy of Sciences, 1979).

Currently, very little has been done to make AYB easier to grow, and for it to be more productive and more nutritious. For example,

little is known about the natural varietal differences that exist within this species. Without this knowledge, genetic improvement cannot be carried out. There is also need for more germplasm collections to be made throughout Africa to ensure that valuable genotypes are not lost. Presently, the International Institute of Tropical Agriculture (IITA) holds at least 157 accessions of AYB. These include some accessions collected between 1995 and 1998 from eastern Nigeria, mainly Umuahia, Enugu, Nzukka and Umueze. These latter accessions have been collected and distinguished mainly on the basis of seed colour and are currently under evaluation with a particular focus on their potential usefulness as sources of insecticidal proteins and genes which can be deployed to control cowpea pests (Colucci *et al.*, 1999; Machuka *et al.*, 1999; Omitogun *et al.*, 1999). However, one problem that has been experienced is the lack of information regarding the genetic identity of AYB accessions now available in IITA genebank.

Among the techniques now in common use for genetic fingerprinting of germplasm (Brown and Kresovich, 1996), electrophoresic separation of proteins other than isozymes is probably the least used. However, two cases have been reported where sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE of protein have been used to distinguish *Vigna* species (Rao *et al.*, 1992; Piergiovanni, 1998). This paper reports the similarities and differences in the profiles of albumin, globulin and vicilin seed polypeptide fractions from 26 AYB accessions.

MATERIALS AND METHODS

Plant materials. Based on differences in seed colour, 26 different AYB collections were obtained from local markets (Umuahia, Enugu, Nzukka and Umueze) in eastern Nigeria by the present author and Dr. Louis Jackai, formerly of IITA's Legume Entomology Unit. Most of these collections have been successfully multiplied at IITA and assigned accession numbers according to standard procedures established by IITA's Genetic Resources Unit. These and other collections which have not yet been multiplied but which were used in this study are listed in Table 1. For convenience, the word "accession" is used to refer to all the accessions and collections described above.

Fractionation of albumin and globulin fractions. The procedure for fractionation of albumin and globulin fractions was used as described by Khan *et al.* (1980). AYB seed meal was extracted in 0.1 M potassium phosphate buffer, pH 8.0, containing 0.4 M NaCl. The slurry was filtered through miracloth and centrifuged at 5, 000 g for 10 min at 4 °C. Albumins and globulins were separated by dialysis of the extract against 33 mM sodium acetate buffer, pH 4.5, overnight at 4 °C. The extract was then centrifuged at 23, 000 g for 20 min at 4 °C, and the precipitated globulins re-suspended in distilled water and lyophilised. The supernatant solution containing the albumin fraction was also lyophilised.

Fractionation of vicilins. Fractionation of vicilins from AYB was carried out as described by Yunes *et al.* (1998). Ground seed meal (0.5 g/ml buffer) was extracted for 30 min at 4 °C with 50 mM sodium tetraborate buffer, pH 8.0. The slurry was centrifuged 10, 000 x g for 30 min at 4 °C and the resultant supernatant precipitated to 80 % saturation with ammonium sulphate. After further centrifugation (10, 000 x g for 30 min at 4 °C), the pellet formed was resuspended in water, dialysed extensively against distilled water and lyophilised.

Acrylamide gel electrophoresis. SDS-PAGE was carried out using 12 % acrylamide gels (Laemmli, 1970) whereas native (without SDS) PAGE utilised 7.5 % gels. Reduction was performed with 5 % 2-mercaptoethanol (2-ME) added to the protein samples prior to loading. Gels were stained with Coomassie Brilliant Blue R-250 and calibrated using the mid-range protein molecular weight standards (Sigma, St. Louis or Promega, Madison, USA).

RESULTS AND DISCUSSION

Successful fractionation of albumin, globulin and vicilin fractions from dry seeds of AYB was achieved using established procedures for preparation of seed proteins from legumes (Khan *et al.*, 1980; Yunes *et al.*, 1998). Using SDS-

PAGE under reducing conditions (in the presence of 2-ME), one major cluster of bands consisting of four (27, 29, 32 and 34 kDa) polypeptides was obtained (Fig. 1, track A). Based on previous observations (Machuka *et al.*, 1999), it is likely that the four bands are lectin subunits. It is also interesting that the ammonium sulphate precipitation procedure employed by Omotogun *et al.* (1999) to obtain lectin-enriched extracts from AYB yields essentially the same albumin profile as the potassium phosphate based procedure used in this study. This suggests that lectins are highly abundant in AYB seeds and probably constitute the main bulk of seed albumins in this crop. In the common bean (*Phaseolus vulgaris*), lectins alone account for up to 50 % of the total seed protein (Sharon and Lis, 1990). Two other prominent polypeptides (12 and 16 kDa, respectively) were also present in the albumin fraction. All 26 AYB accessions have the same albumin profile under reducing SDS-PAGE.

As with albumins, all accessions had identical globulin and vicilin patterns under reducing SDS-PAGE, as shown in Figure 1 (tracks G and V, respectively). The three major globulin polypeptides were resolved at 24, 34 and 55 kDa, respectively. One broad vicilin band (22-25 kDa) was observed, in addition to three other faint bands. These observations appear to be in line with what is known to date about the separation of legume seed storage proteins (SSPs) on SDS-PAGE gels, both quantitatively and qualitatively

TABLE 1. List of African yam bean (*Sphenostylis stenocarpa*) accessions and collections and their assigned protein profile types resolved on native polyacrylamide electrophoresis gels*

IITA Accession No. or Collection Code†	Albumin types		Globulin types		Vicilin types	
	Native + 2-ME	Native - 2-ME	Native + 2-ME	Native - 2-ME	Native + 2-ME	Native - 2-ME
TSs 140	H	H	A	A	E	E
TSs 141	H	H	B	B	E	E
TSs 142	H	K	D	D	C	C
Agbani 98-4-4	J	J	C	D	A	A
Agbani 98-4-5	K	G	D	D	C	C
TSs 143	J	J	E	E	F	F
TSs 144	J	J	C	C	A	A
TSs 145	K	K	D	D	B	B
TSs 146	J	J	A	A	A	A
Agbani 95-4-17	K	K	B	C	B	B
TSs 147	J	J	E	E	B	E
TSs 148	J	J	D	A	A	A
Enugu 95-3-1	J	J	A	A	A	A
TSs 149	J	J	E	E	E	E
TSs 150	J	J	A	A	A	A
TSs 151	J	J	A	A	A	A
TSs 152	K	G	C	C	C	B
TSs 153	J	J	A	A	A	A
TSs 154	J	J	D	D	D	D
TSs 155	K	K	B	B	B	B
TSs 156	J	J	D	D	D	D
Umuahia 95-1	K	H	C	B	C	C
Umuahia 95-1-1	J	J	D	C	F	F
TSs 158	H	J	E	E	E	E
TSs 159	H	H	C	C	C	C
TSs 157	J	J	D	A	A	A

* Accessions with differences in protein type based on native PAGE with and without 2-mercaptoetanol (2-ME) are indicated by small fonted upper case letters

† TSs, T=IITA; Ss = *Sphenostylis stenocarpa*

(Gatehouse *et al.*, 1980; Bewley and Black, 1994). In legumes, globulins are the most abundant class of SSPs, accounting for up to 80 % of the total seed protein, with albumins constituting the remainder (Derbyshire *et al.*, 1976; Bewley and Black, 1994). The globulins in turn are made up of vicilins (also called 7S globulins) and legumins (11S globulins), vicilins probably being the easier to characterise, as they are made up of fewer, low molecular weight, subunits following post-translational modifications (Shewry *et al.*, 1995).

Analysis of AYB globulins (including vicilin) on native PAGE gels produced 6 variable profiles among accessions. These profiles are summarised in Figure 2 (type A-F). Examples of each profile are shown in Figure 3 (globulins) and Figure 4

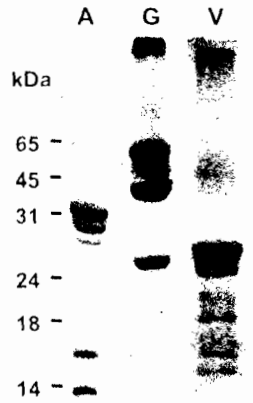

Figure 1. SDS-PAGE (12 % gel) under reducing conditions, of seed albumin (A), globulin (G) and vicilin (V) preparations from *Sphenostylis stenocarpa* (Accession TSs 140). Molecular weights of polypeptides are indicated.

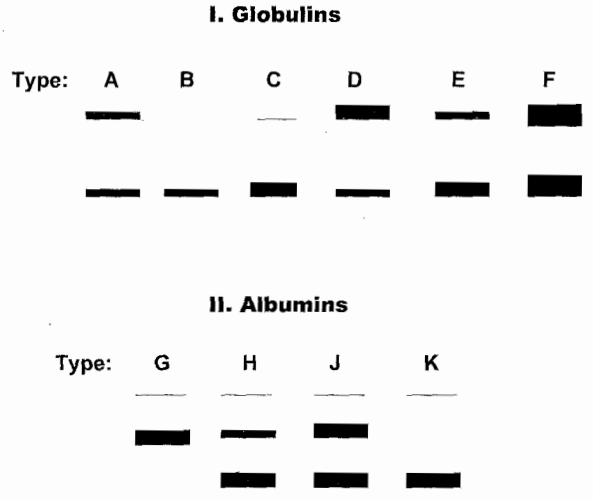

Figure 2. Patterns of globulin and albumin polypeptides obtained on native PAGE gels (7.5 %). Details are described in the text.

(vicilins). Among globulins, the bands were distributed into two groups of low (G-LMW) and high (G-HMW), molecular weight. The G-HMW group consisted of three main bands that were identical in all accessions, whereas the G-LMW group was composed of five profiles of bands, namely A, B, C, D and E (Figs. 2 and 3, Table 1). Native PAGE under reducing and non-reducing conditions resulted in variation in band profiles in six accessions (Agbani 98-4-4, Agbani 95-4-17, TSs 148, Umuahia 95-1, Umuahia 95-1-1 and TSs 157) (Fig. 3, Table 1). Similar analysis of vicilin fractions gave rise to only two major bands that were the same under both reducing and non-reducing conditions (Figs. 2 and 4). Although some of the patterns were similar to globulin

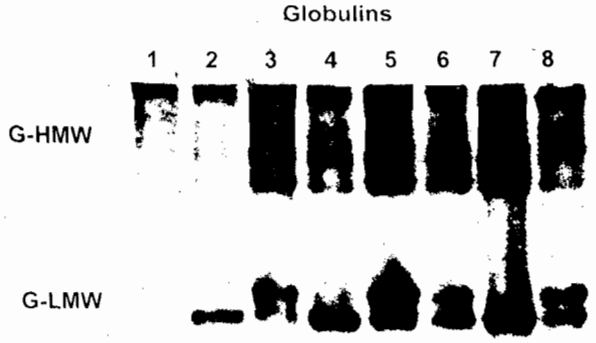

Figure 3. Representative patterns of globulin polypeptides resolved on native PAGE gels (7.5 %) under reducing conditions. Accession numbers are indicated. Letters of band profiles are as designated in figure 2 and Table 1. Details are described in the text.

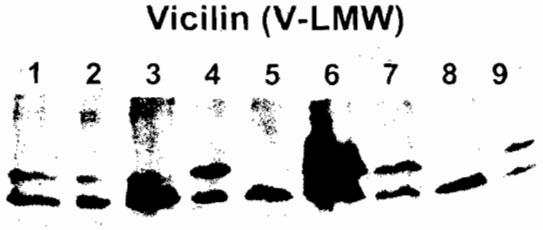

Figure 4. Representative patterns of vicilin polypeptides resolved on native PAGE gels (7.5 %) under non-reducing conditions. Accession numbers are indicated. Letters of band profiles are as designated in Figure 2 and Table 1. Details are described in the text.

types, two accessions (TSs 143 and Umuahia 95-1-1) had a unique pattern, characterised by two thick bands of vicilins designated type F in Figures 2 and Figure 4. The distinctions between globulin and vicilin types among accessions may be related to differences in the abundance of globulin (including vicilin) polypeptides. Therefore, differences between these two fractions on native gels are likely to result from the presence of other polypeptides in the globulin fraction. Another reason for the observed differences may lie in the fact that vicilins lack disulfide linkages, unlike the 11S legumins, which probably largely constituted the G-HMW group (Derbyshire *et al.*, 1986).

A summary of the albumin types (G-K) resolved by native PAGE is given in Figure 2 (G-K), with examples shown in Figure 5. As with the globulins, the albumins were also resolved into two major groups of low (A-LMW) and high (A-HMW) molecular weight. The A-HMW group consisted of one main band with molecular weight of approximately 122 kDa. This band was present in all 26 accessions, and corresponds to the previously reported galactose-specifc hololectin (Machuka *et al.*, 1999) that was confirmed to be present in all AYB accessions (data not shown). However, the three bands in the A-LMW group were variable, giving rise to A-LMW types G, H, J and K shown in Figure 2.

The most common band type in the A-LMW group was J, which was detected among 15 accessions. However, differences in A-LMW band types were observed in five accessions (TSs 142, Agbani 98-4-5, TSs 152, Umuahia 95-1 and TSs 158), between reducing and non-reducing native PAGE conditions (Table 1).

The results described above show that variation in the seed albumin and globulin polypeptides can be used to resolve differences and similarities among the 26 AYB accessions. Although globulins have been previously used for characterisation of genetic resources (Rao *et al.*, 1992; Piergiovanni, 1998), this is the first report in which albumins have been shown to exhibit within species variation. The differences detected among accessions were not specific to the locality from where the seeds were obtained. This is to be expected since exchange of pollen and seed materials in eastern Nigeria is most likely to be unrestricted.

CONCLUSIONS

This paper reports procedures to characterise and compare African yam bean germplasm using native PAGE of seed proteins. These methods may be applied to characterise *Sphenostylis* species, including *S. stenocarpa*, from more widespread geographical locations, such as central,

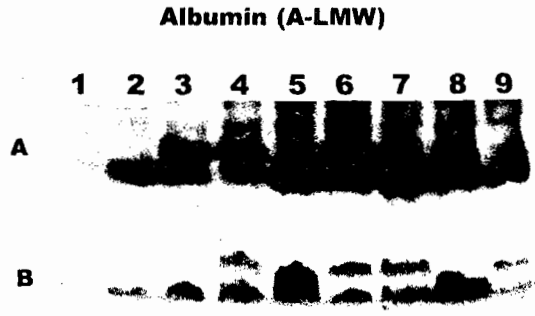

Figure 5. Representative patterns of albumin polypeptides resolved on native PAGE gels (7.5 %) under reducing (A) and non-reducing (B) conditions. Accession numbers are indicated. Letters of band profiles are as designated in Figure 2 and Table 1. Details are described in the text.

eastern, southern, and western Africa. For practical application of these procedures, the differences between reducing and non-reducing native PAGE are probably not significant. However, it may be expected that observed variations probably reflect not only genetic, but also environmental factors, even for collections from the same location.

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