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Esterase, Total Protein and Seed Storage Protein Diversity in Okra (*Abelmoschus esculentus* L. Moench)

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Abstract

Twenty-two accessions of okra (*Abelmoschus esculentus*), maintained at the Plant Genetic Resources Centre, Bunso, Ghana, were assayed for diversity in esterases, and total and storage proteins. A total of 34 reproducible and easily scorable bands were exposed with the number of bands per accession ranging from one to 21. All but nine of the bands were polymorphic. Storage proteins were the most diverse while esterases revealed the least diversity. Similarity matrices were calculated using the Jaccard coefficient, and input into cluster analysis. The phenogram produced by the UPGMA of the Jaccard similarity matrix from the pooled data of the esterases, and total and storage proteins revealed three major clusters at the 55% level of similarity. Accession 5 collected from Nyinguto was relatively distant from the other main clusters and separated at the 42% level of similarity. The second and third clusters comprised 11 and 10 accessions, respectively. It was observed that 18 out of the 22 accessions were distinct accessions. Similarity indices ranged from 29% to 100%. The wide range of similarity indices, coupled with the clustering of accessions, suggests useful variability in the collection for genetic conservationists and plant breeders.

Introduction

The cultivated okra (*Abelmoschus esculentus* L.) also known as 'Lady's finger', 'gumbo', belongs to the Malvaceae family. The crop is a native of Africa and is still found growing wild around River Nile as well as Ethiopia (Kochhar, 1986). The crop was taken to other parts of the world by the Portuguese (Sinnadurai, 1992). Okra is cultivated in the tropics and sub-tropics on a varying scale. India, Costa Rica, Nigeria and Ghana are some of the major producers of okra (NARP, 1993). In Ghana, the crop is planted either as a sole crop or an intercrop. In terms of tonnage, the Brong Ahafo, Northern, Volta, Greater Accra and Central regions are the bulk producers (NARP, 1993).

The crop is put to several uses locally. The immature green pods and fresh leaves are used as potherbs. Immature pods are steamed as 'baby okra', deep fried, pickled or canned. Dried fruits of okra, as slices or in powder form are often stored and used in stews and soups. The fresh okra fruits are high in vitamins A and C; and in calcium (NARP, 1993). Significant levels of carbohydrate, potassium, magnesium and other vitamins are also present (Norman, 1992). The protein content of okra seeds varies between 15% and 26%, and edible oil content of more than 14% has been reported (NARP, 1993). A mucilaginous preparation from the pod can be used as a plasma replacement or blood volume expander. Also, mucilage from the stem and roots is used for clarifying sugar juice in India and sizing paper in Malaysia and China. The stalks are used locally for bast fibre and fuel wood.

Notwithstanding the great value of the crop, information on characterization is either not accessible or simply unavailable. Characterization and evaluation of crops is done to provide information on diversity within or among crops. This permits the identification of unique entries (accessions) necessary for curators of gene banks and plant breeders.

The traditional approach to characteri-zation and evaluation is based on morpho-logical features. Though such phenotypic evaluations are important, the data is not understood at gene level. This is because most economic characters are polygenically inherited and there are considerable interactions between genotype and environment. It is, therefore, essential that genetic diversity within collections be assayed in the context of total available genetic diversity for each species. Molecular markers may extend and complement characterization based on morphological and agronomic descriptions, providing more accurate and detailed information than classical phenotypic data (Karp

et al., 1997). Molecular markers recognized for use in characterization includes isozyme, total protein, seed storage protein, RAPDs, AFLPs and Microsatellites (Burr *et al.*, 1983).

Isozymes have been widely used as protein markers (Bournival *et al*., 1989; Powell, 1992; Hongrun & Yun-Tzu, 1993; Isshiki *et al*., 1994; Karihaloo & Gottlieb, 1995; Lebot *et al*., 1998; Lioi *et al.*, 1998). Isozymes exhibit codominance at a locus making it possible to differentiate between heterozygotes.

Seed storage protein analysis represents a valid alternative and/or improved approach to varietal identification, which currently is based on morphological traits recorded in the field (AOSA. 1991; ISTA, 1993). Seed storage protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts *et al.*, 1986). Seed storage protein markers have been used to study crops including *Solanum* spp. (Mennella *et al*., 1999). Total protein markers are also used extensively in characterization (Gustine *et al*., 1996; Mollema & Cole, 1996). In this study, 22 okra accessions were characterized based on their seed storage protein, total protein and isozyme (esterase) patterns to determine their suitability for any crop improvement programme.

Materials and methods

The study was undertaken at the Department of Crop Science, University of Ghana, Legon-Accra. The 22 Okra accessions (GH 4487, GH 4488, GH 4484, GH 4490, GH 4491, GH 4493, GH 4496, GH 4502, GH 4504, GH 4505, GH 4506, GH 4508, GH4514, GH 4515, GH4518, GH 4519, GH 4520, GH4521, GH 4523, GH 3740, GH 3762, GH3763), collected from sites shown in Fig. 1, were received from the Plant Genetic Resources Centre, Bunso. Okra seeds were used in the seed storage protein analysis. Four week old seedlings were raised for the total protein and isozyme studies.

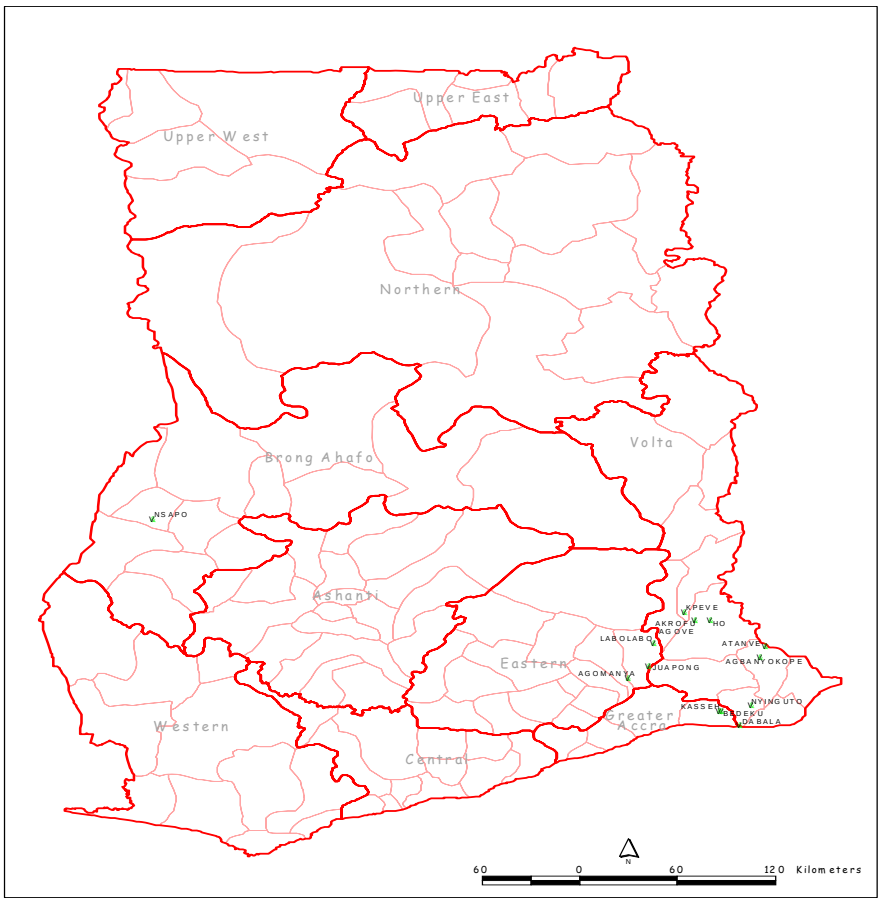

Fig. 1. Map of Ghana showing collecting sites of the 22 accessions of okra

Isozyme studies

Leaves of 22 accessions of okra were assayed for esterase. A modified protocol of the method described by Laemmli (1970) was used. Enzyme extracts were obtained from 0.2 g of young leaves of 4 week old seedlings. The leaves were thoroughly washed in distilled water and then macerated in ceramic mortar using pestles in 800 ml of extraction buffer. The homogenate was centrifuged at 10,000 g for 6 min. and an aliquot of 15 µl of the supernatant of each accession was taken for electrophoresis in 12.5% polyacrylamide gel. The gels were viewed on a light box after staining with the appropriate enzyme substrate.

Total protein studies

Electrophoretic study of total protein variation was carried out using 12.5% polyacrylamide gels. Twenty-two accessions were screened for total protein banding pattern using a modification of the method described by Agueguia *et al*. (1994). Young leaves (0.2 g) of plants were washed with distilled water and macerated in 800 µl of tris citric acid buffer of *p*H 8.0 using a pestle and mortar. The extract was transferred into eppendorf vials and centrifuged at 15000 rpm for 5 min. The supernatant was collected and 15 µl electrophoresed in 12.5% polyacrylamide gels. Gels were stained with Coomassie brilliant blue.

Seed storage protein studies

The seed storage proteins were analysed according to the method outlined by Agueguia *et al.* (1994) with few modifi-cations. Eight seeds per accession were used for sample preparation. Seeds were soaked overnight and seed

coat removed. The seeds were oven dried for 2 days at 60 °C and ground using an analytical pulveriser seed mill and a mortar and pestle. Half gramme of each sample was taken and defatted by soaking in 5 ml petroleum ether (boiling point 60–80 °C) for 30 min. with occasional agitation. The sample was decanted through a Whatman No. 1 filter paper and washed two times with fresh petroleum ether at 5 min intervals. The samples were folded within the filter paper, clipped and dried in an oven at 50 °C for about 18 h. A sub-sample weighing 0.5 g was taken and soaked in 0.45 µl of distilled water for 30 min. and centrifuged for 5 min. at 15,000 p.m. Ten µl of 2-Mercaptoethanol was added to an aliquot of 100 µl, boiled for 5 min., cooled on ice for 15 min. and 15 µl aliquot electro-phoresed on 12.5% sodium dodecyl sulphate polyacrylamide gels. Gels were stained with Coomassie brilliant blue.

Statistical analysis

For each okra accession, the band position of a distinct marker on the gel was scored as either present (1) or absent (0). Only polymorphic bands were included in the binary data set, and similarities were calculated using Jaccard's coefficient (Jaccard, 1901): $S_{ij} = a/(a+b+c)$, where S_{ij} is the similarity between two individuals i and j, a is the number of bands present in both i and j, b the number present in i but not in j, and c the number present in j but absent in i. Cluster analyses using the UPGMA (unweighted pair-group method with arithmethic averages; Sokal & Michener, 1958) were carried out on the similarity matrices using the software Genstat 5 statistical package (Genstat 7 Committee, 2000) and phenograms (dendrograms) constructed.

Results and discussion

A total of 34 bands were scored in the 22 accessions for the three marker systems. Esterases revealed five bands, total and storage proteins showed 13 and 16 bands, respectively.

Jaccard's similarity coefficient values ranged from 0.29 to 0.95. The phenogram produced by UPGMA of the Jaccard similarity matrix from the pooled data of the three marker systems from the 22 samples is shown in Fig. 2. Three major clusters were identified from the phenogram. The first major cluster which was a single accession, GH4491, collected from Nyinguto was relatively distant from the other accessions. It separated from the two major clusters at the 42% level of similarity. The second major cluster comprised 11 accessions (GH4488, GH4490, GH4496, GH4502, GH4504, GH4505, GH4506, GH4508, GH4514, GH4487 and GH4493) collected from six different localities.

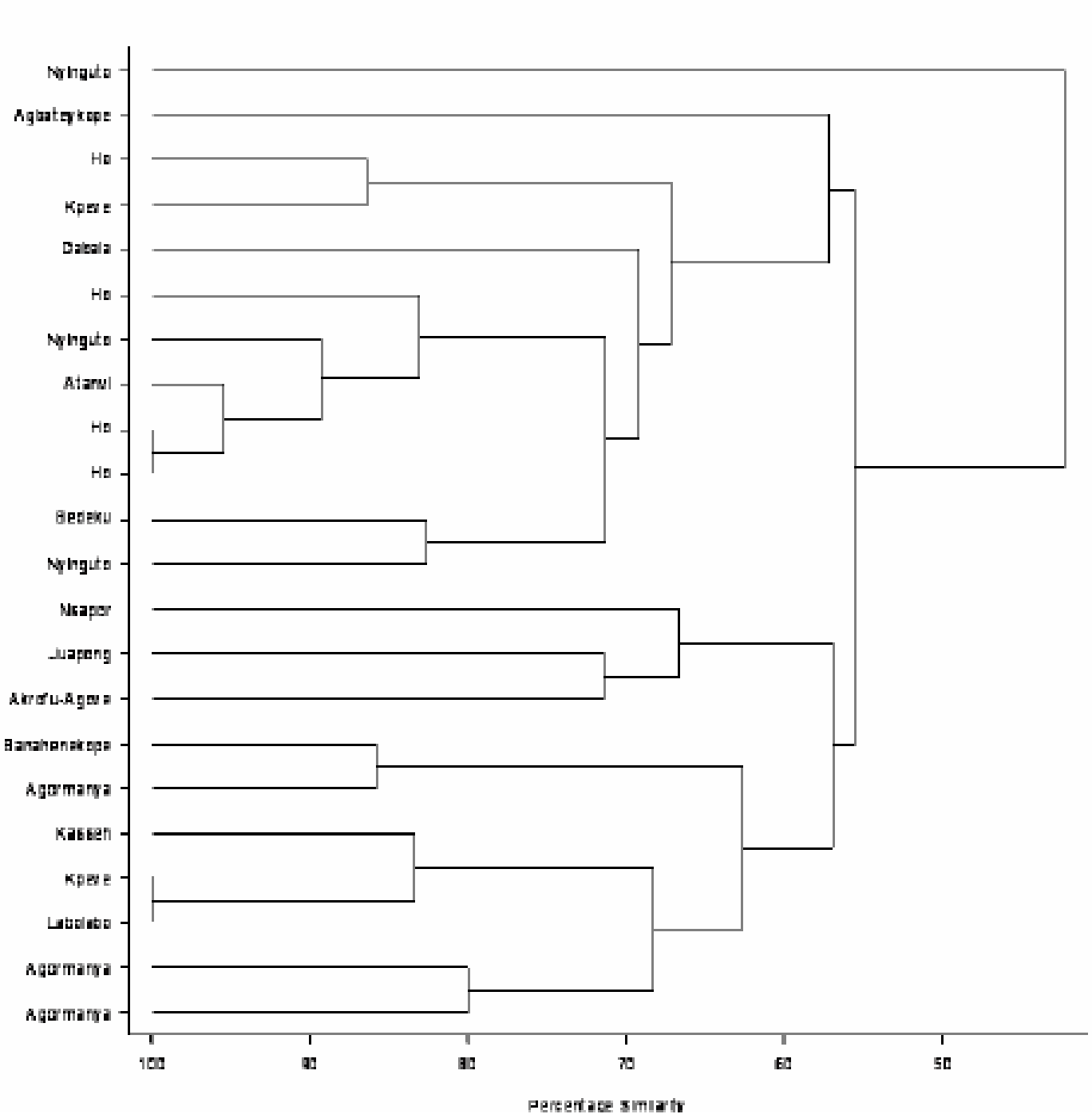

Fig. 2. Phenogram from the combined isozyme, total and seed storage protein data for the 22 accessions of okra (GH 4487 from Bedeku, GH 4488 from Agbateykope, GH 4484 from Banahenekope, GH 4490 from Dabala, GH 4491, GH 4493 and GH 4496 from Nyinguto, GH 4502 from Atavi, GH 4504, GH 4505, GH 4506, GH 4508 from Ho, GH4514 and GH 4515 from Kpeve, GH4518 from Labolabo, GH 4519 from Juapong, GH 4520, GH4521 and GH 4523 from Agomanya, GH 3740 from Akrofu-Agove, GH 3762 from Nsapor, and GH3763 from Kasseh)

There were two subdivisions; accessions GH4488, GH4508, GH4514, GH4490, GH4506, GH4496, GH4502, GH4504 and GH4505 comprised one subcluster, and GH4487 and GH4493 comprised the other subcluster. The third major cluster was subdivided into two subclusters at the 56% level of similarity. Subcluster 1 comprised three accessions (GH3762, GH4519 and GH3740 collected from Nsapor, Juapong and Akrofu-Agove, respectively). Sub-cluster 2 was made up of seven accessions (GH4484, GH4521, GH3763, GH4515, GH4518, GH4520 and GH4523)). It was evident from the clustering that all of the accessions collected from the same locality clustered in a group. The only exception was GH44.

Information on genetic relatedness among genetic resources of crop plants is useful not only for breeding purposes but also for the conservation of germplasm. Using the three marker systems, 18 of the 22 accessions were distinguished. Irwin *et al*. (1998) reported that closely related accessions are normally located within 80–90% similarity. Crosses between accessions with similarity indices of 80–100% may, therefore, not be recommended. Following the recommendation of Irwin *et al*. (1998), a cross between accession GH4491 from Nyinguto and any of the 22 accessions may generate an array of genotypes from which useful agronomic types may be selected.

The utility of molecular markers, for example storage protein patterns, is in their linkage to genes of economic importance (Brown *et al.,* 1990). Polymorphic bands can be followed and examined for possible association to economically important traits such as disease resistance, nematode, parasitic weeds and drought resistance. Total protein and esterase markers have also been used extensively in germplasm characterization. The extent of relatedness among the accessions studied under total protein was quite similar to that of the seed storage proteins suggesting the reliability of the method.

All accessions from Ho clustered in one major group as well as the accessions from Agormanya. Two of the accessions from Ho were identical suggesting that they may be duplicates. Also, one of the Kpeve accessions (GH4515) and GH4518 from Labolabo were also identical. Local germplasm of crops move from one town to the other through the normal farmer to farmer diffusion. This sometimes leads to change in the local names of varieties, and there are several examples of the same varieties bearing different names, and also different varieties of okro bearing the same name in Ghana. Only proper characterisation can resolve the confusion in nomenclature.

One major importance of the integration of markers to study diversity is that it allows for better discrimination among accessions than a single method. However, although protein electrophoresis provides a means of estimating levels of genetic variation, much variation may still remain undetected at the level as only parts of the genome which are expressed can be detected. The use of a range of currently available DNA-based markers complements variability detected at both the morphological and protein levels. The results, nonetheless, provide useful information for gene bank curators and plant breeders.

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