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GENETIC DIVERSITY WITHIN GHANAIAN COWPEA GERMPLASM BASED ON SDS-PAGE OF SEED PROTEINS

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp is an important legume crop in Ghana and is, thus, among the candidate crops for the national crop improvement programme. However, utilisation of germplasm collections (largely local landraces) for crop improvement has been hampered by the existence of duplicates and genetically redundant accessions that are not noticeable by morphological markers. This study examined the genetic diversity within 60 Ghanaian cowpea germplasm on the basis of stored seed protein banding patterns by the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques. Protein banding patterns were scored for presence and and analysed for percent genetic similarities between pairs of accessions. Similarity index and simple matching coefficient indicated a high degree of homogeneity in banding patterns. A dendrogram constructed on the basis of a genetic distance matrix and by unweighted paired group method with arithmetic averages (UPGMA), using the NTSYS-pc programme, resolved the germplasm into three distinct clusters. With the exception of the BTB collections, accessions collected by different collecting teams were similar. The seed protein data could be used, together with other data, for the elimination of duplicate accessions and for the setting up of a core collection, to reduce maintenance cost and ensure efficiency in the use of the germplasm.

Key Words: Electrophoresis, molecular markers, NTSYS-pc programme

RÉSUMÉ

Le niébé (*Vigna unguiculate* L.) walp est une importante culture de légume au Ghana et ainsi fait partie des cultures sélectionnées pour le programme national d'amélioration des cultures. Néanmoins, utilisation des collections de germoplasmes [races locales] pour l'amélioration des cultures a été gênée par l'existence des copies et des accessions génétiquement superflues qui ne sont pas perceptibles par des marqueurs morphologiques. Cette étude a examiné la diversité génétique parmi les 60 semences du niébé sur la base du modèle en réserve de protéines de semences groupés par les techniques de gel électrophorèses de dodecyl-sulphate polyacrilamide de sodium [SDS-PAGE]. Les modèles de protéines groupées étaient marqués par la présence des analyses pour le pourcentage de similarités génétiques entre pairs d'accessions. L'indexe de similarité et le simple coefficient indiquaient un degré élevé dans les modèles groupés. Un dendrogramme a été construit sur la base de distance de matrice génétique et par une méthode de groupe des pairs non pesés avec une moyenne arithmétique [UPGMA], utilisant le programme NTSYS-pc, catégorisant la semence en trois groupes distincts. Avec l'exception des collections BTB, les accessions collectionnées par différentes équipes de collection étaient similaires. Les données de protéines de semences devraient être utilisées ensemble avec d'autre données pour l'élimination des acquisitions copiées et pour la création d'une collection noyau pour réduire le frais de maintenance et assurer la capacité dans l'utilisation de germoplasmes.

Mots Clés: Electrophorèse, marqueur moléculaire, logiciel NTSYS-pc

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.), Walp) is native to West Africa and is a major source of vegetable protein for both humans and livestock in developing countries, particularly in Africa and Asia (Ahenkora *et al.*, 1998). New cultivars have been developed which have led to the improvement of the crop, particularly in terms of overall yield and reaction to a range of diseases and pests (Asafo-Agyei *et al.*, 1999).

The national germplasm conservation centre in Ghana (Plant Genetic Resources Centre, Bunso) holds a collection of cowpea accessions that have largely been characterised only by morphological markers (Bennett-Lartey, 1992). Several new cultivars, introduced through the cowpea trade, bear no description and their origin remains uncertain. Because of the informal nature of the cowpea trade and exchange of genetic material, it is uncommon to find that morphologically similar cultivars do not bear the same name, while cultivars bearing the same name may not be identical morphologically. Ghana's germplasm bank holds accessions collected from all over the country. It is essential that these collections be properly characterised to help identify and eliminate duplicates and genetically redundant accessions. Duplicate and genetically redundant accessions increase the cost of handling and, most importantly, decrease the efficiency of evaluating collections (Toledo *et al.*, 1989; Greene and Pederson, 1996), and hence germplasm enhancement programmes.

Molecular fingerprinting techniques currently hold the best promise of identifying duplicates and achieving this objective. The use of gel electrophoresis of seed proteins by the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique is a reliable, yet relatively inexpensive way of developing genetic markers for identification and genetic analyses of several important agricultural commodities (Bushuk and Zillman, 1978; Wrigley *et al.*, 1982; Kreis *et al.*, 1983; Ferguson and Grabe, 1986). It is a direct multilocus approach involving the electrophoretic examination of proteins which are encoded genetically at more than one locus, and which display a substantial degree of molecular polymorphism. Analysis of seed proteins of grain legumes by the SDS-PAGE technique has also been useful in identifying and determining the inheritance patterns of insect pest resistance (Osborn *et al.*, 1986; Romero-Adreas *et al.*, 1986; Pusztai *et al.*, 1993; Fory *et al.*, 1996; Hartweek *et al.*, 1997). The objective of this study was to use the SDS-PAGE technique to characterise the cowpea accessions present in the germplasm pool in Ghana, and to conduct a genetic analysis to determine the extent of genetic variation in the collection.

MATERIALS AND METHODS

Sixty accessions, derived from different geographical locations and sources in Ghana (Table 1), and secured at the Plant Genetic Resources Centre (PGRC), Bunso, were used in this study. Ten single seeds of each accession were analysed.

Total soluble proteins were extracted by a modification of the procedure of Smith and Payne (1984). Single seeds were crushed and transferred to 150 µl of extraction buffer, which consisted of 1.7 ml extraction stock solution, 0.6 ml β-mercaptoethanol, and 1.0 ml dimethyl formamide. Stock solution contained 3 ml of 1M tris-HCl (pH 6.8), 6 ml distilled H_2O, 5 ml glycerol, 1g SDS, and 5 mg pyronin Y. Samples were digested at 50° C for 5 minutes and centrifuged at 10,000 rpm for 10 minutes.

Extracted soluble proteins were fractionated by one dimensional SDS-PAGE (Laemmli, 1970). Twelve percent of running and stacking slab gels were used; gels measured 16 cm x 14 cm x 0.15 cm. Electrophoresis was conducted at a constant current 25 mA until the Pyronin Y tracking dye reached the bottom of the gel. After electrophoresis, the gels were stained overnight in Coomassie brilliant blue - R250, followed by de-staining in methanol and acetic acid for 30 minutes. Gels were further de-stained in acetic acid until the background was clear enough for scoring. In order to eliminate differences in electrophoretic conditions as a cause of variation in the protein profiles, each cultivar protein sample was separated by SDS-PAGE at least twice.

Protein banding patterns having unambiguous resolutions were coded 0 or 1, respectively, depending on their absence or presence. Percent genetic similarities between pairs of cultivars

TABLE 1. Passport data on the *Vigna unguiculata* (cowpea) accessions derived from different locations in Ghana and secured at the Plant Genetic Resource Centre, Bunso

Accession No.	Collection No.	Vernacular name	Locality	Source of sample
3685	GJ 93/163	Asedua	Fukuokrom	Field
3670	GJ 93/031	Ayi	Fedafene	Farm Store
3794	GJ 93/266	Tia	Kandiga	Farm Store
3671	GJ 93/034	Ayi	Tedafenu	Farm Store
5343	OAA 96/030	Asedua	Manso Atwere	Field
5045	AMO 96/107	Yor	Sutapong	Farm Store
5039	AMO 96/038	Ekye	Abonse	Farm Store
3687	GJ 93/219	Waakye	Babafo	Market
4533	BTB 96/092	Ayi	Dabala	Market
4535	BTB 96/129	Ayi	Vidadakope	Garden
3679	GJ 93/106	Yor	Takunya	Field
4546	BYB 96/238	Ayi	Sanga	Field Store
5046	AMO 96/128	Adua Nsawa	Suminakese	Field
4771	GA 96/047	Bengne	Serekpere	Field
4527	BTB 96/043	Yor	Kasseh	Market
4772	GA 96/052	Bengne	Serekpere	Field
3666	GJ 93/023	Ayi	Juapong	Market
3711	GJ 93/315	Chibe	Kunkunde	Farm Store
4530	BTB 96/055	Yor	Kasseh	Market
4529	BTB 93/054	Yor	Kasseh	Market
3701	GJ 93/244	Sanji	Lahagu	Farm Store
4549	BTB 96/262	Ngwesem	Agormenya	Market
4531	BTB 96/056	Yor	Kasseh	Market
4542	BTB 96/213	Ayi (Achimota)	Kpando	Market
4767	GA 96/023	Ayi (Achimota)	Piise	Field
3708	GJ 93/303	Sega	Grupe	Farm Store
3673	GJ 93-74	Ayi	Bame Awumome	Farm Store
4526	BTB 96-042	Yor	Kasseh	Market
5049	AMO 96-164	Asedua	Okumanining	Field
5042	AMO 06-062	Yor	Ahabasu Gyasu	Farm Store
5047	AMO 96-129	Adua Nsawa	Suminakese	Field
3706	GJ 93-294	Bengne	Jenina	Farm Store
4542	BTB 96-154	Ayi	Ziope	Market
3674	GJ 96-075	Eweyi	Bame Anyinawase	Farm Store
4769	GA 96-045	Bianga	Wa	Market
5048	AMO 96-131	Adua Nsawa	Suminakese	Field
4534	BTB 96-106	Ayi	Nyinguto	Farm Store
4528	BTB 96-048	Yor	Kasseh	Market
5350	OAA 96-226	Asedua	Asare	Farm Store
5038	AMO 96-030	Vakli	Akuni No.2	Farm Store
5039	BTB 96-046	Vakli	Akuni NO.1	Farm Store
4765	GA 96-001	Bena	Siriyiri	Field
4547	BTB 96-.260	Yortsu	Agormenya	Market
4532	BTB 96-091	Ayiyibor	Dabala	Market
4548	BTB96-261	Otorhwii	Agormenya	Market
5050	AMO 96-.204	Asedua	Kokoben	Farm Store
4083	SO 96-060	Yor	Pimpinso	Farm Store
5041	AMO 96-061	Yor	Ahabasu Gyaesu	Farm Store
3668	GJ 93-027	Ayi	Juapong	Market
4778	GA 96-137	Gonja	Siriyiri	Field
4032	SO 96	Gonja	Siriyiri	Market
4026	SO 96-003	Adua nsawa	Owusukrom	Farm Store
4543	BTB 96-215	Ayi (Achimota)	Kpando	Market
5043	AMO 96-084	Asedua (Asontem)	New Tafo	Garden
4774	GA 96-074	Bengne	Nimorow	Field
5344	OAA 96-046	Asedua	Juaben	Field
5044	AMO 96-105	Yor	Sutapong	Field
5040	AMO 96-060	Yor	Ahabasu Gyaesu	Farm Store
3703	GJ 93-250	Tua	Loagri	Farm Store
5349	OAA 96-196	Asedua	Fetentaa	Farm Store

were derived by the Nei and Li (1979) similarity index and by the simple matching coefficient. Only reproducible bands occurring in high frequency in independent runs were used for scoring and analysis. A dendrogram based on the genetic distance matrix was constructed by the unweighed pair group method with arithmetic averages (UPGMA) cluster analysis. The distance matrix and dendrogram were both constructed using the NTSYS-pc version 1.8 (Rohlf, 1992).

RESULTS AND DISCUSSION

Figure 1 represents the electrophoretic patterns of seed proteins of the 60 cowpea accessions studied. Total protein extracts of cowpea seed, when subjected to SDS-PAGE, exhibited considerable homogeneity in banding patterns. Total seed storage proteins fell into five distinct groups, (Fig. 1), and each group of protein phenotypes consisted of several sub-units. This is consistent with results of previous studies. For instance, Pedalino *et al.* (1990) found that total protein extracts of cowpea seed is composed of numerous sub-units, which could be grouped into major and minor sub-units. They identified only two banding patterns (pattern A and pattern B) among the genotypes studied. The highest amount of polymorphism was concentrated in the molecular weight region of <42 kd. Based on the positions of standard proteins of known molecular masses and the types of legume storage proteins (Cooke, 1989), the most polymorphic region fell within the legumin and legumin/vicilin storage proteins regions. In the present study, however, little genotypic variation was found in the high molecular weight protein sub-units; in particular convicilins, showed little variation between genotypes (Fig. 1). The genetic heterogeneity observed in the accessions studied is found mainly in the low molecular weight region of the protein profiles, it also corroborates the findings of Pedalino *et al.* (1990).

Estimates of percentage genetic similarities (GS) between all possible pairwise combinations, indicated that GS ranged from 47.8 to 100% (data not presented). These estimates indicated that 97% of the pairwise combinations of accessions shared 60% or more of their protein bands in common. The highest GS values (GS = 100%) were found between 8 pairs of combinations (GJ93/244, BTB96/054, BTB96/262 , BTB96/054, BTB96/056, BTB96/054, BTB96/262, GJ93/244, BTB96/056, GJ93/244, BTB96/056; BTB96/262, SO96/060, AM96/204, AM96/030; OA96/226). This implies that, on the basis of their storage protein banding patterns, these pairs are genetically identical. This must have resulted from duplications in collections. The two most important factors responsible for this duplication are the collection strategy used and exchange of planting material. It may also have arisen from

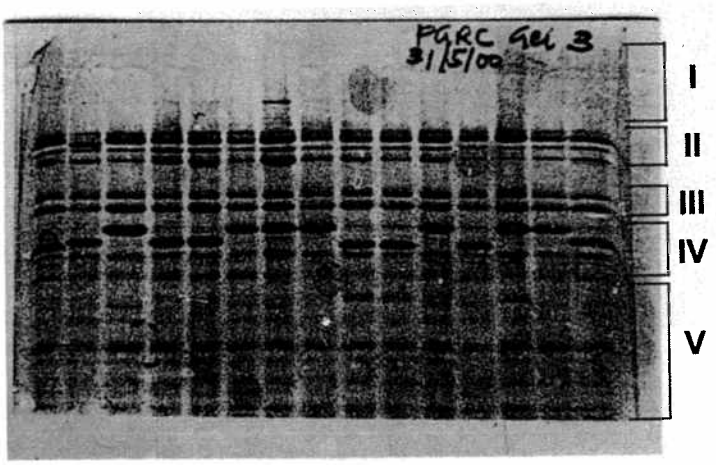

Figure 1. Seed storage proteins from some of the Ghanaian cowpea landraces separated by SDS-PAGE and stained with Coomassie Blue. From left, Acc No. 4534, 4543, 4770, 5042, 4769, 4547, 3706, 4548, 4541, 3674, 5047, 4528, 4532, 4765, and 5043.

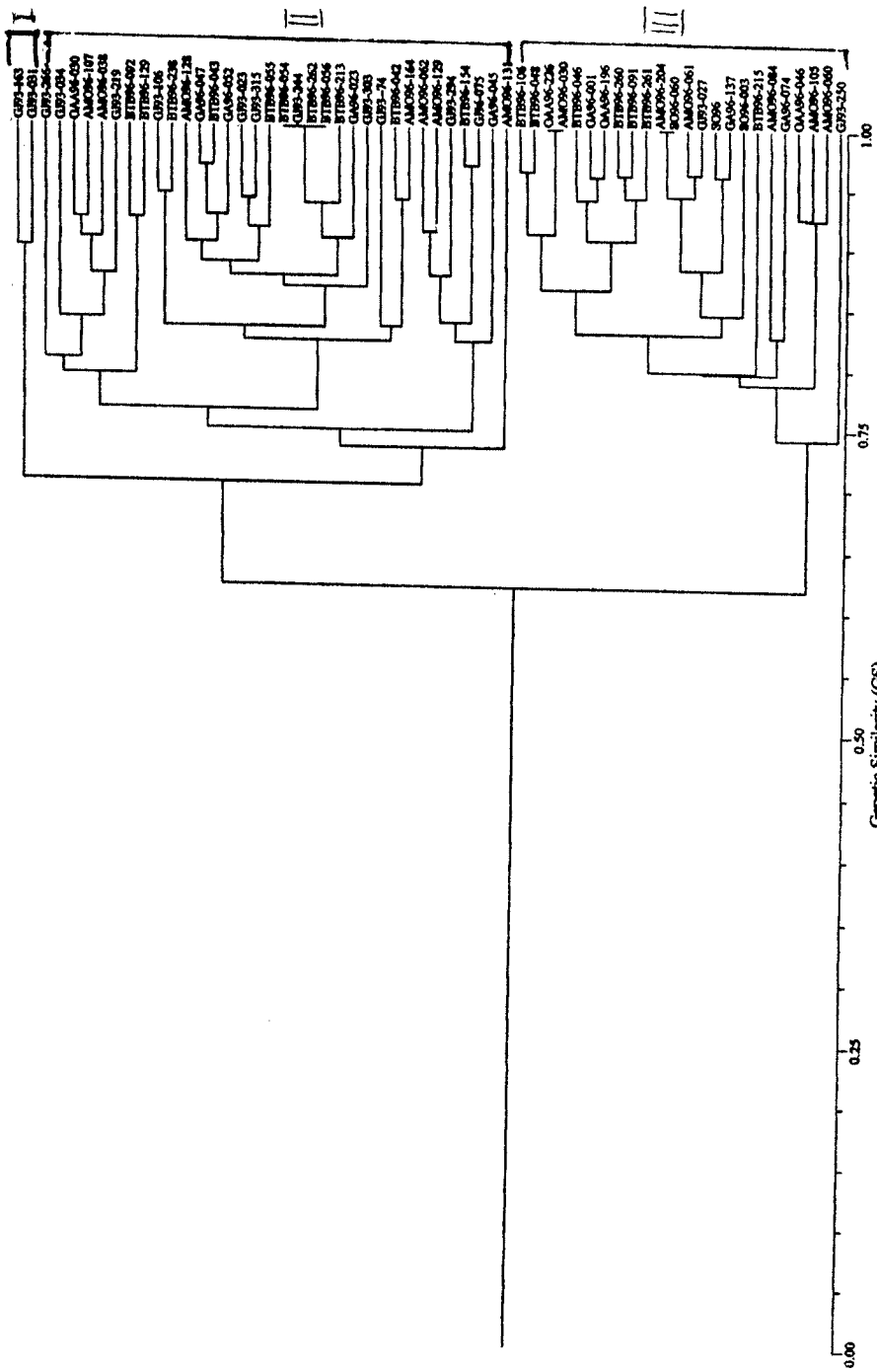

Figure 2. Dendrogram of the 60 cowpea accessions obtained from the PGRC, revealed by UPGMA cluster analysis of SDS-PAGE-based genetic similarity (GS) estimates.

exchange of genetic material between farmers as well as through cowpea trade. In Ghana, there are no barriers to the flow of genetic material since the seed industry and trade, in general, are not very well developed. Farmers commonly select their own seed for planting from the previous crop. However, informal exchange of seed between farmers is common. A good performing variety, therefore, stands the chance of featuring in most farmers' fields across the country as well as in the cowpea trade nationwide. The two most genetically dissimilar genotypes, GJ93/266 and AM96/060, had a percentage GS value of 47.8%.

A dendrogram constructed from the similarity data shows that the cultivars may be grouped into 3 clusters (Fig. 2). Accessions collected by different collection teams grouped with other accessions in a relatively indistinct manner. However, most of the BTB collections clustered together in the second cluster than the other collections. Furthermore, more ties were found among the BTB accessions. This may be because they were selected from the same fields or that they may have a similar pedigree. The smallest cluster comprised of GJ93/163 and GJ93/031, and these may also be products of similar pedigrees.

With the exception of GJ93/244 having a genetic similarity of 100% with "BTB96/054", "BTB96/262" and "BTB96/056", there were noticeable differences recorded between accessions collected in different years. The ties recorded between accessions collected within the same year imply possible duplication in collection.

The absence of large genetic differences between the accessions does not imply that the differences between the genotypes are not significant for germplasm enhancement purposes. This may be because of limitations inherent in the use of protein electrophoresis in investigating genetic phenomena at the molecular level. Murphy *et al.* (1996) states that enzymatic and non-enzymatic proteins do not always generate sufficient variability for studies of population structure, breeding biology and other intra-specific applications. This makes other methods such as DNA-RFLP more appropriate. For instance, Bunce *et al.* (1986) and Oppong-Konadu (1994) demonstrated greater genetic polymorphism at the B and C hordein loci in barley using the DNA-RFLP methods than that revealed by the SDS-PAGE. This is probably because changes in proteins result in shift mobility detectable by SDS-PAGE (Giles, 1981). The variation recorded, albeit appears to be small, therefore, gives an indication that there is appreciable level of variability between the accessions studied and, hence, ample scope for genetic improvement through breeding.

This study suggests the existence of duplicate germplasm in the cowpea collections in Ghana that need to be eliminated, to reduce maintenance costs and ensure efficiency in the use of the germplasm.

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