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Assessment of the genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm from Ghana using simple sequence repeat markers

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Abstract

Genetic diversity and phylogenetic relationships among 141 cowpea (*Vigna unguiculata* L. Walp.) accessions collected throughout the nine geographical regions of Ghana were evaluated using simple sequence repeat (SSR) molecular markers. Twenty-five primer combinations pre-selected by their ability to polymerase chain reaction amplify SSRs from a set of test cowpea germplasm were evaluated. Of these, 20 primer combinations gave reproducible polymorphisms among 97.2% of the cowpea accessions tested, with the remaining accessions being found to be genetically identical. The informative primer combinations revealed a total of 74 alleles at 20 loci with an average of 3.8 alleles detected per locus. Variation in heterozygosity among cowpea SSRs ranged from 0.01 to 0.84 with an average occurrence of 0.19. The polymorphism information content varied from 0.07 to 0.66 with an average of 0.38. The Ghanaian cowpea accessions clustered into five main branches, each of which was loosely associated with the geographical regions from which samples were obtained. Accession GH2288 was found to be the most divergent cowpea accession compared with all others including the outgroup IT84S-2049, a breeding line from Nigeria. Our results provide a framework for future studies aimed at the conservation and management of cultivated cowpea germplasm in Ghana, and a good starting point for the selection of parental lines for genetic improvement programmes.

Keywords: cowpea; genetic diversity; germplasm; microsatellite; phylogenetic structure; simple sequence repeat

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is the most important grain legume grown in sub-Saharan Africa (Ehlers and Hall, 1997; Timko *et al*., 2007; Timko and Singh, 2008). The majority (~64%) cowpea grain production, 12.5 million tons worldwide, takes place on low-input, subsistence farms in this region (Langyintuo *et al*., 2003). Referred to as the 'poor man's meat' because of its good protein quality and high nutritional value (Diouf and Hilu, 2005), cowpea hay is also critical in the feeding of animals during the dry season in many parts of West Africa (Tarawali *et al*., 2002). In addition, cowpea is a nitrogen-fixing plant, and helps restore soil fertility

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when used in rotation with cereal crops (Eloward and Hall, 1987; Sanginga *et al.*, 2003).

A major goal of cowpea breeding and genetic improvement programmes around the world is to combine desirable agronomic traits (e.g. time to maturity, photoperiod sensitivity, plant type and seed quality) with resistances to the major diseases, insect pests or parasites that afflict cowpea in agroecologically adapted cultivars (Timko *et al.*, 2007; Timko and Singh, 2008). At present, depending on the source of the trait being introgressed, a decade, more or less, is needed to breed a superior improved line using traditional selection and hybridization strategies. Leveraging emerging gene-based tools for tracking single genes and quantitatively inherited traits linked to important diseases and pest resistances and the establishment of breeder-friendly protocols for marker-assisted selection (MAS) and breeding can substantially reduce this time frame. The overall efficiency and effectiveness of cowpea improvement programmes can also be enhanced by knowledge of the genetic diversity available within local and regional germplasm collections (Hall, 2004; Hegde and Mishra, 2009).

Previous studies of diversity in wild and cultivated cowpea germplasm have used a variety of approaches including analysis of morphological and physiological traits (Fery, 1985; Perrino *et al.*, 1993; Ehlers and Hall, 1996), allozymes (Panella and Gepts, 1992; Pasquet, 1993, 1999, 2000; Vaillancourt *et al.*, 1993), seed storage proteins (Fotso *et al.*, 1994), and chloroplast DNA polymorphisms (Vaillancourt and Weeden, 1992); random amplified polymorphic DNA (RAPD; Mignouna *et al.*, 1998; Nkongolo, 2003; Fall *et al.*, 2003; Ba *et al.*, 2004; Diouf and Hilu, 2005; Xavier *et al.*, 2005; Zannou *et al.*, 2008); restriction fragment length polymorphisms (Fatokun *et al.*, 1993); amplified fragment length polymorphisms (Fatokum *et al.*, 1997; Fang *et al.*, 2007); DNA amplification fingerprinting (Simon *et al.*, 2007) and analysis of simple sequence repeats (SSRs Wang *et al.*, 2008; Ogunkanmi *et al.*, 2008; Uma *et al.*, 2009; Xu *et al.*, 2010) or sequence tagged microsatellite sites (Choumane *et al.*, 2000; Li *et al.*, 2001; Abe *et al.*, 2003; He *et al.*, 2003). Of these techniques, analysis of SSRs has proven to be particularly useful since these sequences, besides being abundant and distributed throughout eukaryotic genomes, are highly polymorphic, inherited codominantly and reproducible, with simple screening requirements (Dib *et al.*, 1996). SSRs have also been extensively used in genotype identification, seed purity evaluation and variety protection (Brown *et al.*, 1996; Senior *et al.*, 1998), pedigree analysis (Ayres *et al.*, 1997; Bowers *et al.*, 1999), and genetic mapping of simple and quantitative traits and MAS (Blair and McCouch, 1997; Chen *et al.*, 1997; Weising *et al.*, 1998).

Different forms of cowpea are cultivated in the savannah and forest regions of Ghana based on local preferences for growth characteristics and culinary properties (Quaye *et al.*, 2009). For the most part, farmers practise traditional methods of seed selection and conservation, and little effort has been made for germplasm characterization and breeding for genetic improvement within the country. In recent years, the Plant Genetic Resources Research Institute (PGRRI) located in Bunso, Ghana, has been collecting germplasm from farmers' field and conserving these accessions in the PGRRI genebank with the aim of tracking local diversity and facilitating breeding for improved varieties in Ghana in the face of changing biotic and abiotic factors affecting production of the crop. In this study, we developed paired primer combinations that amplify polymorphic SSRs from cowpea, and used these to examine the genetic diversity and relatedness of the cowpea germplasm being conserved at the PGRRI in Ghana. Our results provide a framework for future studies aimed at the conservation, improvement and management of cultivated cowpea in Ghana.

Materials and methods

Plant material

Seeds of 141 accessions of cowpea (*V. unguiculata* L. Walp.) collected from different localities in nine geographical regions of Ghana (Fig. 1; Table S1, available online only at http://journals.cambridge.org) were obtained from the PGRRI of Ghana at Bunso. The cowpea breeding line, IT84S-2049, developed at the International Institute for Tropical Agriculture (IITA, Ibadan, Nigeria), served as an outgroup in this work.

Plant growth and genomic DNA isolation

Cowpea seeds were germinated and grown on a moistened Whatman filter paper in Petri dishes held at 33°C under 12-h light photoperiods. Young leaves of 7-day-old seedlings were harvested, frozen immediately in liquid nitrogen and stored at −80°C until used for nucleic acid preparation. Total DNA was isolated from the leaf tissues using DNAzol ES® (MRC, Inc., Cincinnati, OH, USA) according to the manufacturer's protocol with slight modifications. The precipitated DNA was resuspended in 200 µl of 10 mM Tris–Cl, pH 8.0, and 1 mM EDTA buffer, and the concentration was determined spectrophotometrically on a ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The intactness of the DNA was accessed by electrophoresis through a 0.8% (w/v) agarose gel. Working solutions of 10 ng/µl were prepared for each of the samples.

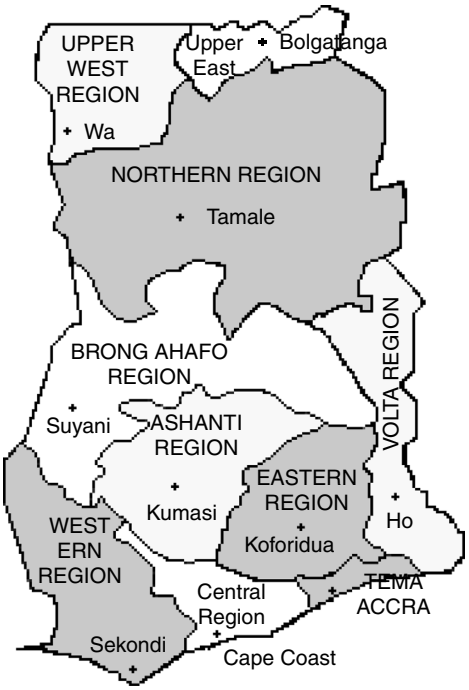

Fig. 1. Regional map of Ghana. The nine regions of Ghana from which cowpea accessions were collected for use in this study are shown.

SSR identification and polymerase chain reaction (PCR) amplifications

Cowpea gene-space sequence reads (GSRs) (Timko *et al.*, 2008) annotated for homology to pathogen and pest resistance genes were analyzed for the presence of SSRs (Chen *et al.*, 2007) using the Tandem Repeats Finder program (Benson, 1999). GSRs containing potential SSRs were tested for their ability to amplify a target sequence using a panel of highly divergent cowpea genotypes, and information on band size, composition and the primers for their amplification were loaded into relational tables found on CGKB database (http://cowpeagenomics.med.virginia.edu/CGKB). Twenty-five pairs of primers (Table 1) were commercially synthesized (Sigma-Aldrich, St Louis, MO, USA) and used to amplify SSRs in PCRs using 10 mM Tris–HCl, 1.5 mM $MgCl_2$ and 50 mM KCl (pH 8.3), 1 μM of each forward and reverse primer, 1 mM dNTPs, 0.5 units of Taq DNA Polymerase and 10 ng/μl template DNA in a final volume of 25 μl PCR mixture. The PCR amplifications were performed in an Eppendorf Mastercycler with the following settings: 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. The reaction ended with a 10-min final extension at 72°C, and the PCR products were stored at 4°C until analysis.

The PCR products were resolved for 1.5 h at 250 V on 6% (w/v) polyacrylamide gels in 0.5 × Tris/borate/EDTA buffer using a vertical gel electrophoresis apparatus (Model V16.2 or V16; Gibco BRL, Gaithersberg, MD, USA). The gels were stained with ethidium bromide and photo-documented with a digital camera (Alpha Innotech, Inc., San Leandron, CA, USA). The size of DNA bands in base pairs was determined using the 100 bp DNA standard (Invitrogen, Carlsbad, CA, USA), ladder standard marker, and a data matrix was generated for analysis.

Data analysis

The individual SSR fragments were scored for size and polymorphism. Amplified bands present across all genotypes (monomorphic bands or invariable markers)

Table 1. Sequences of oligonucleotide primer pairs used in PCR amplifications to detect SSRs in cowpea

Primer code	Primer sequences (5′–3′)
SSR-6217	5′ GGGAGTGCTCCGGAAAGT 3′
	5′ TTCCCTATGAACTGGGAGATCTAT 3′
SSR-6218	5′ GTGGAAGGAATGGGTCCAG 3′
	5′ AGGAAATTTGCATTCCCTTGT 3′
SSR-6243	5′ GTAGGGAGTTGGCCACGATA 3′
	5′ CAACCGATGAAAAAGTGGACA 3′
SSR-6258	5′ GGTTTCCTAGTTGGGAAGGAA 3′
	5′ ATTATGCCATGGAGGGTTCA 3′
SSR-6265	5′ CAGAAGCGGTGAAAATTGAAC 3′
	5′ GCATGTTGCTTTGACAATGG 3′
SSR-6277	5′ CACCCCCGTACACACACAC 3′
	5′ CACTTAAATTTTCACCAGGCATT 3′
SSR-6323	5′ CAAAGGGTCATCAGGATTGG 3′
	5′ TTTAAGCAGCCAAGCAGTTGT 3′
SSR-6336	5′ TGAAAACAACGATATGCAGAAG 3′
	5′ TCAGTCTTAGAATTGAGTTTTCTTCG 3′
SSR-6352	5′ GTTGTGAGCTTCCCCAGATG 3′
	5′ AATTTTGAACCCACCACCAG 3′
SSR-6353	5′ TCATGGGTTAAATTTGCTTCAA 3′
	5′ AAACCATGTGGTTGTTGCAC 3′
SSR-6356	5′ TGCAATATGGACCAGAAGAAA 3′
	5′ ATGCCCCAACAACAACATTT 3′
SSR-6370	5′ CAACTTCACAGCCCTCAA 3′
	5′ TTGAAGGTATGGCCTTTTGTTT 3′
SSR-6371	5′ TGCTCATCGTGCTTTGTCTT 3′
	5′ CACTTCAGACTTAGAGCGAAGAA 3′
SSR-6375	5′ GCTCGGATATGGTCCTGAAA 3′
	5′ TCAGTGTCAGCACCAT CCC 3′
SSR-6436	5′ CAGAATCCTTGTGAACCTG 3′
	5′ TTTCGCAATATGCCCTTTTC 3′
SSR-6451	5′ AAAGAGATACACATGCCTAACA 3′
	5′ GACCAACAGCGACTTTGAGC 3′
SSR-6587	5′ GATATAGAATAGCATATTTAACATATTAG 3′
	5′ GTTGAAAGTTTGATAGTAAAGTGG 3′
SSR-6603	5′ GAGAACTTCACGCACAATAG 3′
	5′ CGCGGTAGCATGATTGAATTTTG 3′
SSR-6608	5′ CTAAATTATAATATTCGTCGGTC 3′
	5′ GGTTAAGGAAAAGAGGGTAGG 3′
SSR-6613	5′ CTATTGGAATCTTGCCGTTG 3′
	5′ CTTTACCTTTATGCAAACCAATTC 3′

were not included in the statistical analysis because they are not informative. PowerMarker version 3.25 (http://www.powermarker.net) (Liu and Muse, 2005) was employed to carry out cluster analysis and construct dendrogram from the SSR data for 142 accessions of cowpea involving the unweighted pair group method with arithmetic mean based on Nei's genetic distances (Nei and Li, 1979). Allele frequency and heterozygosity, polymorphism information content (PIC) and genetic distances were calculated using PowerMarker version 3.25, and the pattern of distribution of cowpea accessions with respect to the geographical regions in Ghana was also aligned with the generated phylogenetic tree using Mega 4 (http://www.megasoftware.net/mega.html).

Results

Polymorphism of SSRs in cowpea germplasm

A set of 25 primer combinations pre-selected by their ability to PCR amplify SSRs in cowpea germplasm were used to examine the genetic diversity and phylogenetic relationships among 141 cowpea accessions (Table S1, available online only at http://journals.cambridge.org) collected throughout nine geographical regions of Ghana: the Northern zone, Central zone, Eastern, Upper Eastern and Upper Western regions, Volta, Greater Accra, Ashanti and Brong Ahafo regions (Fig. 1).

Twenty of the primer combinations reproducibly gave polymorphic DNA fragments following electrophoretic analysis of PCR amplification products. Five primer combinations generated monomorphic allelic amplification profile across all cowpea genotypes tested, and were excluded in the analysis. The sizes of amplified polymorphic DNA fragments (bands) ranged from 80 to 500 bp. The primer pairs, their sequence, allele number and frequency, gene diversity, heterozygosity and PIC are given in Table 2.

The informative SSRs were able to distinguish 138 (97.2%) accessions of the cowpea including those with the same local name, seed coat colour and growth habits independent of geographical regions from which samples were obtained. A total of 74 alleles at 20 loci could be scored. The number of alleles detected per primer pair varied from a minimum of 1 to a maximum of 6 with an average of 3.8. Gene diversity also ranged from 0.12 to 0.68 with an average of 0.44. Variation in heterozygosity among cowpea SSRs increased from 0.01 to 0.84 with an average occurrence of 0.19. The PIC varied from 0.07 to 0.66 with an average of 0.38 (Table 2).

Phylogenetic analysis

Based on their molecular profiles resolved using informative SSRs, the 141 cowpea accessions from Ghana clustered into five main groups, which we designated as groups A to E (Fig. 2). Groups A and E are the

Table 2. Number of alleles, gene diversity, heterozygosity and polymorphism information content for the primers used in this study

Primer code	No. of alleles	Allele frequency	Gene diversity	Heterozygosity	PIC
SSR-6217	5	0.42	0.64	0.08	0.57
SSR-6218	3	0.48	0.56	0.11	0.46
SSR-6243	4	0.35	0.71	0.44	0.66
SSR-6258	2	0.57	0.49	0.14	0.37
SSR-6265	4	0.54	0.60	0.82	0.53
SSR-6277	4	0.80	0.33	0.01	0.28
SSR-6323	5	0.83	0.29	0.06	0.27
SSR-6336	4	0.88	0.22	0.01	0.21
SSR-6352	2	0.69	0.43	0.01	0.34
SSR-6353	2	0.73	0.39	0.04	0.32
SSR-6356	2	0.96	0.07	0.01	0.07
SSR-6370	3	0.89	0.21	0.00	0.20
SSR-6371	4	0.51	0.10	0.51	0.40
SSR-6375	4	0.94	0.11	0.04	0.11
SSR-6436	5	0.45	0.68	0.06	0.62
SSR-6451	4	0.47	0.65	0.02	0.58
SSR-6587	4	0.64	0.50	0.02	0.42
SSR-6603	5	0.84	0.28	0.04	0.26
SSR-6608	4	0.44	0.62	0.84	0.54
SSR-6613	6	0.58	0.55	0.54	0.47
Mean	3.8	0.65	0.44	0.19	0.38

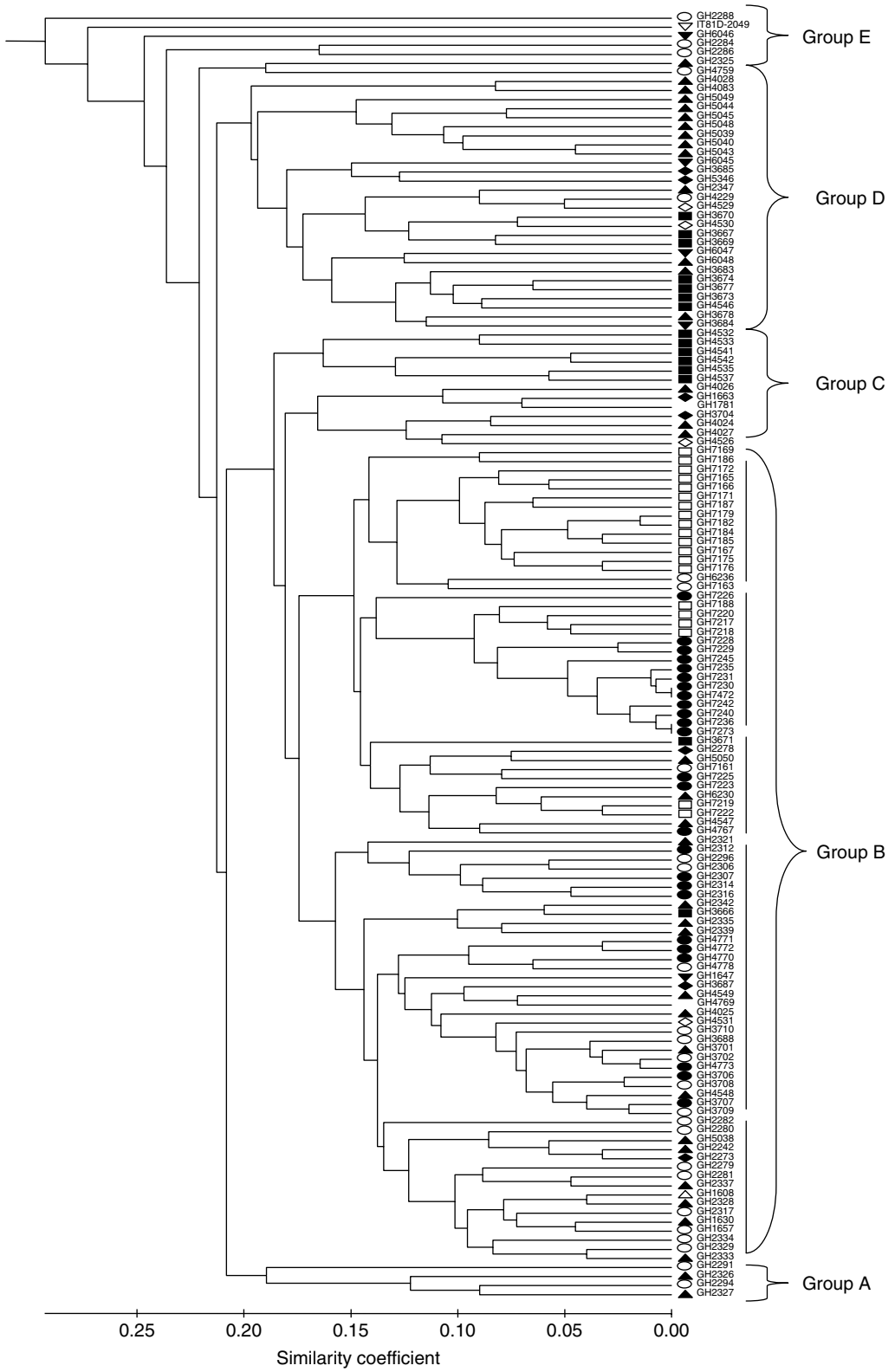

Fig. 2. Phylogenetic relationship among cowpea accessions in the PGRRI germplasm collection in Ghana. A dendrogram illustrating the relatedness of 141 cowpea accessions collected in Ghana and one outgroup is shown, which was generated using 20 informative SSR markers and the sequential clustering algorithm (UPGMA) based on genetic similarity (Nei and Li, 1979). The cowpea accessions are defined as in Table 1. Key to geographical regions are as follows: ▽, IT84S-2049 from IITA; ○, Northern; □, Upper Eastern; ●, Upper Western; ▲, Eastern; △, Central; ■, Volta; ◇, Greater Accra; ▼, Ashanti; ◆, Brong Ahafo.

two smallest groups, with the latter containing the accessions that are the most diverged. Group A consists of four accessions, two each from the Eastern and Northern regions that differ in seed colour and plant type. Group E consists of six Ghanaian genotypes and the outgroup IT84S-2049 from Nigeria. The Ghanaian genotypes were predominately collected in the Northern region of the country, and the majority are brown or mottled brown seeded types. Groups C and D made up cowpeas from mainly the Volta, Eastern, Ashanti and Northern regions of Ghana. Within each of these larger groups are distinct subclusters at different genetic distance levels that reflect regional origin. For example, clear subclusters of genotypes from Volta exist within both groups C and D. Group B contains the largest number of accessions, and could easily be broken into four or five subgroups. One subgroup contains the majority of genotypes collected from the Upper Eastern portion of the country, and the other subgroup is dominated by accessions representing the Upper Western portion indicating that the cultivars grown in these regions share many common characteristics. The other subgroups seem to cluster accession collected more broadly over the whole of Ghana, encompassing geographical regions of both the savannah and forest ecological zones.

Generally, genetic distances among cowpea genotypes are low, reflecting the initial bottleneck during domestication, and maintained by the inherent self-pollination mechanism in the crop. On the whole, the genetic distance among the cowpea accessions analyzed here varied from 0.00 to 0.68. Two pairs of accessions had genetic distances of 0.00 (GH7236 and GH7273; GH7230 and GH7472), suggesting that the members of these pairs may in fact be separately collected, but had identical germplasms. Accessions GH2325 and GH6046 had the highest genetic distance (0.68) among the accessions compared. The genetic distance between GH2325 and GH6046 was even greater than the largest distance between a Ghanaian genotype GH5039 and the outgroup IT84S-2049, which was measured to be 0.63. Of the accessions analyzed, GH2288 was the most genetically diverged (Fig. 2). It has genetic enrichment with 9.9% frequency of genetic distances exceeding 0.60 compared with 0.7% frequency of genetic distances exceeding 0.60 expressed by the outgroup IT84S-2049.

Discussion

Consistent with previous reports, genetic variability among the Ghanaian cultivated cowpea genotypes considered in this study was low. This is not surprising since it is well documented that cowpeas in general have a narrow genetic base due to the fact that a single domestication event is involved in the origin of this crop (Doebley, 1989; Pasquet, 2000; Coulibaly *et al.*, 2002; Ba *et al.*, 2004). The accessions used in this study were part of the germplasm collection at the PGRRI, and for the most part, these materials were collected from farmers' fields, open markets and agricultural stores. Traditionally, subsistence farmers in both the savannah and forest ecological regions of Ghana save seed and rely on their own experience to select and improve their varieties (Quaye *et al.*, 2009). Seed conservation limits the exchange of germplasm throughout the country, and also inhibits the integration of genotypes from other sources outside of Ghana into local breeding programmes. These factors surely contribute to limiting genetic diversity.

Conventionally, cowpeas grown in Ghana are identified by local names based on morphological characteristics alone (such as plant morphology, seed coat colour or other visible seed/pod characteristics). One could anticipate that this could lead to redundancy within the PGRRI collection if the same genotype were collected under different names depending on the ethnic group or locality responsible for its collection. It would also contribute to decreasing measures of diversity within the target population of samples. We found relatively few examples where such redundancy might exist. For example, accession GH7230, a red seeded type grown in the Upper Western part of Ghana called Bene, appears to be genetically similar to GH7472, a white seeded cowpea called Sompla grown in the same region. Similarly, accession GH7236, a mottled brown seeded variety also called Bene, is very genetically similar to GH7273, a white seeded cowpea called Sompla (Fig. 2).

The type of DNA markers employed can also affect the level of polymorphism revealed among genotypes. Previous studies aimed at looking at local genetic diversity of cowpea have used a variety of different molecular marker tools including isozymes, RAPDs, gene sequencing and SSRs. Li *et al.* (2001) used 12 cowpea-derived SSR primers to examine the genetic similarities and relationships among cowpea breeding lines developed at the IITA. In this study, the different primer combinations detected between 4 and 13 alleles among 48 wild cowpea lines with an average of 7.5 alleles per primer. Diouf and Hilu (2005) were able to resolve between 1 and 9 alleles per SSR primer combination in germplasm from Senegal. Uma *et al.* (2009) reported that 80% of the SSR primer combinations tested were able to detect polymorphisms within a collection of 83 Indian genotypes representing different geographical regions, pedigrees and morphological

characteristics, but did not report on the degree of allelic variability observed. In the present study, the 20 informative SSR primer combinations used to analyze the Ghanaian germplasm yielded one to six alleles per primer pair or locus with an average of 3.8. Our results are consistent with what has been observed in other legumes (e.g. chickpea from 1 to 8 (Winter *et al*., 1999), alfalfa from 9 to 14 (Mengoni *et al*., 2000), soybean from 11 to 26 (Rongwen *et al*., 1995) and yardlong bean from 2 to 6 (Li *et al*., 2001; Xu *et al*., 2010)). Therefore, we feel that the use of variations in SSRs is reflective of the extent of genetic variation present in the Ghanaian cowpea gene pool. On the whole, the SSR markers used could differentiate 97.2% of the 141 cowpea accessions.

Defining the exact genetic relationship among accessions is inherently difficult, and clearly, the ability to resolve subtle differences is enhanced with a greater number of markers and with a good distribution of markers throughout the genome. Nonetheless, we were able to roughly place the various cowpea accessions from Ghana into several main groups that more or less reflect their origins and relatedness. Not surprisingly, in some cases, there was a clear geographical origin component to the grouping. In other cases, the groups comprised accessions collected more broadly throughout the whole of the country. We found little if any evidence to suggest that clustering of any sort was related to reported phenotypic characteristics such as seed colour or plant growth type. The highest heterozygosity of 0.84 indicated by primer SSR-6608 may suggest variations in the genome of the cowpea accessions including hybrids under open field cultivation.

Edaphic and climatic factors can dramatically influence plant physiology and morphology, and therefore, classification schemes relying only on visible characteristics are inherently flawed. The use of molecular markers provides a much more reliable approach to distinguish cowpea genotypes for germplasm conservation, and for the identification of parental lines for use in breeding for genetic improvement. Here, we have demonstrated the utility of SSR markers for analysis of the currently available cowpea germplasm in Ghana. The results described here provide a solid foundation upon which working towards a molecular marker-based breeding programme for germplasm improvement in Ghana can be begun, and underscore the need for including new sources of germplasm into current breeding efforts.

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References

Abe J, Xu D, Suzuki Y, Kanazawa A and Shimamoto Y (2003) Soybean germplasm pools in Asia revealed by nuclear SSRs. *Theorical and Applied Genetics* 106: 445–453.

Ayres NM, McClung AM, Larkin PD, Bligh HFJ, Jones CA and Park WD (1997) Microsatellite and single nucleotide polymorphism differentiate apparent amylase classes in an extended pedigree of US rice germplasm. *Theoretical and Applied Genetics* 94: 773–784.

Ba FS, Pasquet RE and Gepts P (2004) Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution* 51: 539–550.

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research* 27: 573–580.

Blair M and McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the bacterial blight resistance gene, *xa-5*. *Theoretical and Applied Genetics* 94: 773–784.

Bowers J, Boursiquot J, This P, Chu K, Johansson H and Meredith C (1999) Historical genetics: the percentage of Chardonnay, Gamay, and other wine grapes of northern France. *Science* 285: 1562–1565.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez-Candelas F and Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical and Applied Genetics* 93: 190–198.

Chen X, Temnykh S, Xu Y, Cho YG and McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 95: 553–567.

Chen XF, Laudeman TW, Rushton PJ, Thomas TA and Timko MP (2007) CGKB: an annotation knowledge base for cowpea (*Vigna unguiculata* L.) methylation filtered genomic genespace sequences. *BMC Bioinformatics* 8: 129.

Choumane W, Winter P, Weigand F and Kahl G (2000) Conservation and variability of sequence tagged microsatellites sites (STMS) from chickpea (*Cicer aerietinum* L.) within the genus *Cicer*. *Theoretical and Applied Genetics* 101: 269–278.

Coulibaly S, Pasquet RS, Papa R and Gepts P (2002) AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. *Theoretical and Applied Genetics* 104: 358–366.

Doebley J (1989) Isozymic evidence and the evolution of crop plants. In: Soltis DE and Soltis PS (eds) *Isozymes in Plant Biology*. Portland, OR: Dioscorides Press, pp. 165–191.

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Mare S, Hazan J, Seboun E, Lathrop G, Gyapay G, Morisselte J and Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature* 380: 152–154.

Diouf D and Hilu KW (2005) Microsatellite and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal. *Genetic Resources and Crop Evolution* 52: 1957–1967.

Ehlers JD and Hall AE (1996) Genotypic classification of cowpea based on responses to heat and photoperiod. *Crop Science* 36: 673–679.

Ehlers JD and Hall AE (1997) Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research* 53: 187–204.

Eloward HOA and Hall AE (1987) Influence of early and late nitrogen fertilization on yield and nitrogen fixation of cowpeas under well-watered and dry field conditions. *Field Crops Research* 15: 229–244.

Fall L, Diouf D, Fall-Ndiaye MA, Badiane FA and Gueye M (2003) Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] varieties determined by ARA and RAPD techniques. *African Journal of Biotechnology* 2: 48–50.

Fang J, Chao C-CT, Roberts OA and Ehlers JD (2007) Genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. *Genetic Resources and Crop Evolution* 54: 1197–1209.

Fatokun CA, Danesh D and Young ND (1993) Molecular taxonomic relationships in the genus *Vigna* based on the RFLP analysis. *Theoretical and Applied Genetics* 86: 97–104.

Fatokun CA, Mignouna HD, Knox MR and Ellis THN (1997) AFLP variation among cowpea varieties. *Agronomy Abstracts*. Madison, WI: ASA, p. 156.

Fery RL (1985) The genetics of cowpea: a review of the world literature. In: Singh SR and Rachie KO (eds) *Cowpea Research, Production and Utilization*. Chichester/New York: John Wiley and Sons, Ltd, pp. 25–62.

Fotso M, Azanza JL, Pasquet R and Raymond J (1994) Molecular homogeneity of cowpea (*Vigna unguiculata*, Fabaceae) seed storage proteins. *Plant Systematics and Evolution* 191: 39–56.

Hall AE (2004) Breeding for adaptation to drought and heat in cowpea. *European Journal of Agronomy* 21: 447–454.

He C, Poysa V and Yu K (2003) Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theoretical and Applied Genetics* 106: 363–373.

Hegde VS and Mishra SK (2009) Landraces of cowpea, *Vigna unguiculata* (L.) Walp., as potential sources of genes for unique characters in breeding. *Genetic Resources and Crop Evolution* 56: 615–627.

Langyintuo AS, Lowenberg-DeBoer J, Faye M, Lambert D, Ibro G, Moussa B, Kergna A, Kushwaha S, Musa S and Ntoukam G (2003) Cowpea supply and demand in West Africa. *Field Crops Research* 82: 215–231.

Li CD, Fatokun CA, Ubi B, Singh BB and Scoles GJ (2001) Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite primers. *Crop Science* 41: 189–197.

Liu K and Muse S (2005) PowerMarker. An integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128–2129.

Mengoni A, Gori A and Bazzicalupo M (2000) Use of RAPD and microsatellite (SSR) variation to assess genetic relationships among population of tetraploid alfalfa, *Medicago sativa*. *Plant Breeding* 118: 1–7.

Mignouna HD, Ng NQ, Ikca J and Thottapilly G (1998) Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. *Journal of Genetics and Breeding* 52: 151–159.

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences (USA)* 76: 5269–5273.

Nkongolo KK (2003) Genetic characterization of Malawian cowpea (*Vigna unguiculata* (L.) Walp.) landraces: diversity and gene flow among accessions. *Euphytica* 129: 219–228.

Ogunkanmi LA, Ogundipe OT, Ng NQ and Fatokun CA (2008) Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. *Journal of Food Agriculture and Environment* 6: 253–268.

Panella L and Gepts P (1992) Genetic relationships with *Vigna unguiculata* (L.) Walp. based on isozyme analyses. *Genetic Resources and Crop Evolution* 39: 71–88.

Pasquet RS (1993) Variation at isoenzyme loci in wild *Vigna unguiculata* (L.) Walp. (Fabaceae, Phaseoleae). *Plant Systematics and Evolution* 186: 157–173.

Pasquet RS (1999) Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. *Theoretical and Applied Genetics* 98: 1104–1119.

Pasquet RS (2000) Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp. *Theoretical and Applied Genetics* 101: 211–219.

Perrino P, Laghetti G, Spagnoletti Zeuli PL and Monti LM (1993) Diversification of cowpea in the Mediterranean and other centers of cultivation. *Genetic Resources and Crop Evolution* 40: 121–132.

Quaye W, Adofo K, Madode YE and Abizari A-R (2009) Exploratory and multidisciplinary survey of the cowpea network in Tolon-Kumbungu district of Ghana: a food sovereignty perspective. *African Journal of Agricultural Research* 4: 311–320.

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U and Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. *Theoretical and Applied Genetics* 90: 43–48.

Sanginga N, Dashiell KE, Diels J, Vanlauwe B, Lyasse O, Carsky RJ, Tasawali S, Asafo-Adjei B, Menkir A, Schulz S, Singh BB, Chikoye D, Keatinge D and Ortiz R (2003) Sustainable resource management coupled to resilient germplasm to provide new intensive cereal-grain-legume-livestock systems in the dry savanna. *Agriculture, Ecosystystems & Environment* 100: 305–314.

Simon MV, Benko-Iseppon AM, Resende LV, Winter P and Kahl G (2007) Genetic diversity and phylogenetic relationships in *Vigna Savi* germplasm revealed by DNA amplification fingerprinting. *Genome* 50: 538–547.

Senior ML, Murphy JP, Goodman MM and Stuber CW (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science* 38: 1088–1098.

Tarawali SA, Singh BB, Gupta SC, Tabo R, Harris F, Nokoe S, Fernandez-Rivera S, Bationo A, Manyong VM, Makinde K and Odion EC (2002) Cowpea as a key factor for a new

approach to integrated crop–livestock systems research in the dry Savanna of West Africa. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM and Tamo M (eds) *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. Ibadan: IITA, pp. 233–251.

Timko MP and Singh BB (2008) Cowpea, a multifunctional legume. In: Moore PH and Ming R (eds) *Genomics of Tropical Crop Plants*. New York, NY: Springer Science + Business Media, LLC, pp. 227–258.

Timko MP, Ehlers JD and Roberts PA (2007) Cowpea. In: Kole C (ed.) *Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants*. vol. 3. Berlin/ Heidelberg: Springer-Verlag, pp. 49–67.

Timko MP, Rushton PJ, Laudeman TW, Bokowiec MT, Chipumuro E, Foo Cheung F, Town CD and Chen XF (2008) Sequencing and analysis of the gene-rich space of cowpea. *BMC Genomics* 9: 103.

Uma MS, Hittalamani S, Murthy BCK and Viswanatha KP (2009) Microsatellite DNA marker aided diversity analysis in cowpea [*Vigna unguiculata* (L.) Walp.]. *Indian Journal of Genetics and Plant Breeding* 69: 35–43.

Vaillancourt RE and Weeden NF (1992) Chloroplast DNA polymorphism suggests a Nigerian centre of domestication for the cowpea *Vigna unguiculata* (Leguminisae). *American Journal of Botany* 79: 1194–1199.

Vaillancourt RE, Weeden NF and Barnard J (1993) Isozyme diversity in the cowpea species complex. *Crop Science* 33: 606–613.

Wang ML, Barkley NA, Gillaspie GA and Pederson GA (2008) Phylogenetic relationships and genetic diversity of the USDA *Vigna* germplasm collection revealed by gene-derived markers and sequencing. *Genetic Resarch* 90: 467–480.

Weising K, Winter P, Huttel B and Kahl G (1998) Microsatellite marker for molecular breeding. *Journal of Crop Production* 1: 113–143.

Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC, Sahi S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ and Kahl G (1999) Characterisation and mapping of sequence-tagged microsatellites sites in the chickpea (*Cicer arietinum* L.) genome. *Molecular and General Genetics* 262: 90–101.

Xavier GR, Martins LMV, Rumjanek NG and Filho FRF (2005) Variabilidade genética em acessos de caupi analisada por meio de marcadores RAPD. *Pesquisa Agropecuaria Brasileira (Brasília)* 40: 353–359.

Xu P, Wu X, Wang B, Liu Y, Quin D, Ehlers JD, Close TJ, Hu T, Lu Z and Li G (2010) Development and polymorphism of *Vigna unguiculata* ssp. *unguiculata* microsatellite markers used for phylogenetic analysis in asparagus bean (*Vigna unguiculata* ssp. sesquipedialis (L.) Verdc.). *Molecular Breeding* doi:10.1007/s11032-009-9364-x

Zannou A, Kossou DK, Ahanchédé A, Zoundjihékpon J, Agbicodo E, Struik PC and Sanni A (2008) Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. *African Journal of Biotechnology* 7: 4407–4414.