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Assessing the genetic diversity of 48 groundnut (*Arachis hypogaea* L.) genotypes in the Guinea savanna agro-ecology of Ghana, using microsatellite-based markers

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Groundnut (*Arachis hypogaea* L.) is the most important grain legume in Ghana. However, its production is constrained by a myriad of biotic and abiotic stresses which necessitate the development and use of superior varieties for increased yield. Germplasm characterisation both at the phenotypic and molecular level is important in all plant breeding programs. The aim of this study was to characterise selected advanced breeding groundnut lines with different phenotypic attributes at the molecular level using simple sequence repeats (SSR) markers in Ghana. A total of 53 SSR markers were screened and 25 were found to be polymorphic with an average polymorphic information content (PIC) value of 0.57. Of the 48 groundnut genotypes studied, 67% showed very close relationship (~100% similarity) with one or more genotypes among themselves. In fact, there were 14 instances where two to three genotypes within the same sub-cluster exhibited 100% similarity even though they displayed different phenotypic attributes. The remaining 33% of the groundnut genotypes were distant from each other and could therefore serve as effective parental material for future work. In this study, the SSR-based markers were found to be quite discriminatory in discerning variations between and among groundnut lines even where the level of variation was low. Microsatellite-based markers therefore represent a useful tool for dissecting genetic variations in cultivated crops, especially groundnut.

Key words: Phenotypic traits, DNA extraction, PCR amplification, simple sequence repeats (SSR) markers, alleles, polymorphic information content, Jaccard's similarity coefficient.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in the world. Currently, China, India and Nigeria account for largest groundnut production in the world, with Ghana ranking 12th in land area under groundnut cultivation (FAO, 2014). Groundnut is therefore the most important grain legume in Ghana (MoFA-SRID, 2011) and is grown in all agro-ecologies of the country with the three northern regions accounting for 70% of production (Tsigbey et al., 2003). The grain, fodder, processed oil and cake serve as a major source

of cash income for smallholder famers. Groundnut grain contains 20 to 30% protein and 40 to 55% oil (Asibuo et al., 2008) and is therefore important for nutritional security. As a nodulated legume, groundnut can contribute substantial amounts of symbiotic N to cropping systems, which ranges from 43 to 171 kg N ha^{-1} in Zambia, Ghana and South Africa (Dakora et al., 1987; Nyemba and Dakora, 2010; Mokgehle et al., 2014). In Ghana, the haulms serve as high-protein fodder for livestock during the dry season (Martey et al., 2015). Despite the importance of groundnut to food and nutritional security and its contribution to soil fertility, increased production is constrained by a variety of factors. The yields obtained on farmers' fields are less than 1.0 t ha^{-1} due largely to biotic and abiotic stress (Naab et al., 2005; 2009). For example, foliar diseases such as early and late leaf spot caused by *Cercospora arachidicola* S. Hori and *Cercosporidium personatum* Berk. and Curt., respectively, are known to reduce groundnut yields on farmers field (Naab et al., 2009). Groundnut rosette virus and insect pest damage can also reduce groundnut yield (Padi, 2008). Abiotic factors such as low phosphorus and calcium in soils, as well as erratic rainfall, can cause poor yields of groundnut in Ghana (Abubakari et al., 2012; Rademacher-Schulz et al., 2014). The use of improved genotypes is a cost-effective and environmentally-safe approach to increasing yields in fields of resource-poor farmers (Holbrook and Stalker, 2003). Therefore, developing groundnut genotypes with tolerance to both biotic and abiotic stress has the potential to achieve higher yields on farmers' fields (Holbrook and Stalker, 2003). Traditionally, morphological characters have been used to describe traits such as seed distinctiveness, uniformity and stability of genotypes (Holbrook and Stalker, 2003). However, this method is sometimes influenced by the environment and is labour intensive. In recent times, molecular markers (especially DNA-based markers) have been employed as an alternative to the use of morphological traits. Even then, using a combination of the two (that is, morphological characters and DNA markers) has been found to offer a more comprehensive characterisation of plant genotypes (Holbrook and Stalker, 2003). Many DNA-based molecular markers have been used to characterise groundnut. These include restriction fragment length polymorphism (RFLP), amplified fragment length polymerphism (AFLP), sequence characterised amplified regions (SCARs), random amplified polymorphic DNA (RAPDs), and simple sequence repeats (SSR) or microsatellites (Kochert et al. 1991; Garcia et al. 1996; He and Prakash 1997; Hopkins et al., 1999; Cuc et al., 2008; Carvalho et al., 2010). More recently, single nucleotide polymorphism (SNP) has also been applied to the characterization of groundnut (Barkley et al., 2011; Nagy et al., 2012). However, SSR markers appear to have wider application because of their presence in genomes of all living organisms, their high level of allelic variation, their co-dominant way of inheritance and their potential for automated analysis (Rakoczy-Trojanowska and Bolibok, 2004). SSR markers have thus remained the common routine tool used in the breeding and genetic analysis of groundnut (Pandey et al., 2012a). In this study, 48 groundnut genotypes, comprising advanced breeding lines and farmer varieties that exhibited varying levels of resistance to drought, foliar diseases and aflatoxin contamination, were screened with 53 SSR markers in order to assess (i) the genetic diversity among the groundnut genotypes, and (ii) their potential as parental material in future groundnut breeding programs.

MATERIALS AND METHODS

Plant materials

In this study, a total of 48 groundnut genotypes exhibiting varying levels of resistance to drought, foliar diseases and aflatoxin contamination were used. These included 45 advanced breeding lines supplied by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and three commonly-grown varieties used by farmers in Northern Ghana (Table 1).

Plant DNA extraction

Total plant genomic DNA was extracted from young newly emerged leaves of 12-day-old plants using a modified CTAB protocol (Mace et al., 2003). The quality and quantity of DNA were estimated by running the extracted DNA on a 0.8% agarose gel stained with ethidium bromide. The DNA samples were diluted to 5 ng/µl prior to use in PCR analysis.

PCR amplification

Polymerase chain reaction was performed with the 53 SSR-based primers as described by Pandey et al. (2012b). The reaction was conducted in a 10 µl reaction volume containing 5 ng of genomic DNA, 0.5 µmoles of each primer, 1.0 µl 10X PCR buffer, 0.25 mM of each dNTPs, 2 mM $MgCl_2$ and 1.0 U Taq DNA Polymerase (Sib enzyme, Russia). Touchdown PCR amplification was performed on an ABI Thermal Cycler (GeneAmp PCR system 9700) with an initial denaturation step (94°C for 3 min), and five cycles of

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Abbreviations: PIC, Polymorphic information content; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **SCARs,** sequence characterised amplified regions; **RAPDs,** random amplified polymorphic DNA; **SSR,** simple sequence repeats **SNP,** single nucleotide polymorphism.

Table 1. Biological traits of groundnut germplasm used in this study and their sources.

Genotype	Pedigree	Phenotypic trait	Source
ICG 6222	Germplasm line (PI 262060, landrace, Brazil)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 03315	(ICGV 91284 x ICGV 87846)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 91177	(ICGV 86012 x ICGV 86407)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 91324	(U4-7-5 x PI 337394F)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 94379	(UF 71513-1 x U1-2-1)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 03323	(J 11 x ICGV 87350)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 92305	(NCAc 343 x ICGV 86309)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 91328	(J 11 x U4-7-5)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 92302	(Ah 7223 x U1-2-1)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 91341	(UF 71513-1 x U1-2-1)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 91317	(U4-7-5 x JL 24)	Aflatoxin tolerance	ICRISAT, Mali
ICGV 86124	[JL 24 x (Dh. 3-20 x Robut 33-1) F_8]	Drought tolerance	ICRISAT, Mali
ICGV 91114	(ICGV 86055 x ICGV 86533)	Drought tolerance	ICRISAT, Mali
ICGV 99247	(ICGV 87354 x SANGDI)	Drought tolerance	ICRISAT, Mali
ICGV 97188	(ICGV 86887 x ICGV 87121)	Drought tolerance	ICRISAT, Mali
ICGV 02313	(ICGV 87290 x ICGV 87846)	Drought tolerance	ICRISAT, Mali
ICGV 02171	[(ICGV 88145 x ICGV 87110)F1 x ICGV 88312]	Drought tolerance	ICRISAT, Mali
ICGV 00369	(ICGV 86300 x TAG 24)	Drought tolerance	ICRISAT, Mali
ICGV 99240	(ICGV 87290 x ICGV 87846)	Drought tolerance	ICRISAT, Mali
ICGV 03056	(ICGV 99160 x ICGV 99240)	Drought tolerance	ICRISAT, Mali
ICGV 99249	(ICGV 87846 x TAG 24)	Drought tolerance	ICRISAT, Mali
ICGV 00350	(ICGV 87290 x ICGV 87846)	Drought tolerance	ICRISAT, Mali
ICGV 00308	(ICGV 95244 x ICGV 96223)	Drought tolerance	ICRISAT, Mali
ICGV 00362	(ICGV 86300 x ICGV 92242)	Drought tolerance	ICRISAT, Mali
ICGV 03196	(ICGV 96342 x ICGV 98266)	Early maturity	ICRISAT, Mali
ICGV 03206	(ICGV 98191 x ICGV 93382)	Early maturity	ICRISAT, Mali
ICGV 03181	(ICGV 95319 x ICGV 92206)	Early maturity	ICRISAT, Mali
ICGV 02022	(ICGV 94361 x ICGV 92267)	Early maturity	ICRISAT, Mali
ICGV 03157	(ICGV 93470 x ICGV 96403)	Early maturity	ICRISAT, Mali
ICGV 03184	(ICGV 95319 x ICGV 92206)	Early maturity	ICRISAT, Mali
FLEU II	Unknown	Early maturity	ICRISAT, Mali
ICGV 03166	(ICGV 87378 x ICGV 96342)	Early maturity	ICRISAT, Mali
ICGV 03169	(ICGV 87378 x ICGV 96342)	Early maturity	ICRISAT, Mali
ICGV 03179	(ICGV 96300 x ICGV 96352)	Early maturity	ICRISAT, Mali
ICGV 02144	(ICGV 97052 x U 4-7-5)	Early maturity	ICRISAT, Mali
ICIAR 19 BT	ICGM/754 x ICGV 87922	Early maturity	ICRISAT, Mali

Table 1. Contd.

Genotype	Pedigree	Phenotypic trait	Source
ICGV 03207	(ICGV 98191 x ICGV 93382)	Early maturity	ICRISAT, Mali
ICGV 03194	(ICGV 98247 x ICGV 98262)	Early maturity	ICRISAT, Mali
ICG 7878	Germplasm line (NC Ac 10811-A)	Foliar disease tolerance	ICRISAT, Mali
ICGV 01276	(ICGV 92069 x ICGV 93184)	Foliar disease tolerance	ICRISAT, Mali
ICGV 99029	(ICGV 94118 x ICGV 93427)	Foliar disease tolerance	ICRISAT, Mali
ICG (FDRS) 4	ICGV 87157 (Argentine x PI 259747)	Foliar disease tolerance	ICRISAT, Mali
ICGV 00064	{[ICGV 88312 x (B4 x ICGV 86885)] x [(JL 24 x ICG(FDRS) 4) x JL 24]}	Foliar disease tolerance	ICRISAT, Mali
ICGV 00068	(ICGV 92069 x ICGV 94088) F2-SSD-SSD-B2-B1-B1(VB)	Foliar disease tolerance	ICRISAT, Mali
ICGV-IS 08837	(Agentine x PI 129747) F_3	Foliar disease tolerance	ICRISAT, Mali
CHINESE	Unknown	Early maturity	SARI, Ghana
NKATIESARI	F-mix X ICG (FDRS) 20	Foliar disease tolerance	SARI, Ghana
SUMNUT 22	Unknown	Foliar disease tolerance	Nigeria

denaturation (94°C for 20 s), annealing (65°C for 20 s with a decrease in 1°C for each cycle), and extension(72°C for 30 s). This was followed by 35 cycles of 94°C for 20 s with a constant annealing temperature of 59°C for 20 s and 72°C for 30 s, followed by a final extension of 72°C for 20 min. PCR products of four different fluorescence dye-labelled primers were mixed with 0.2 µl of Gene scan LIZ Size standard (Applied Biosystems, California, USA) and 9.3 µl of Hi-Di™ formamide (Applied Biosystems, California, USA). The DNA fragments were denatured and size-fractioned using capillary electrophoresis on ABI 3730xl genetic analyzer (Applied Biosystems, California, USA). Allele size estimation was performed using GeneMapper v 4.0 genotyping software (Applied Biosystems, California, USA). The software PowerMarker version 3.2 (Liu and Muse, 2005) was used to analyse major allelic frequencies, polymorphic information content (PIC) of markers, and gene diversity. DARwin version 5.0 (Perrier and Jacquemound-Collet, 2006) was used to assess genetic diversity, and NTSYSpc version 2.1 (Rohlf, 1992) to generate a dendrogram for assessing genotypic relatedness among the test groundnut material.

RESULTS AND DISCUSSION

A total of 53 SSR markers with PIC values ≥ 0.5 were selected and used in this study (Table 2). Twenty five (25) of the 53 SSR markers (47%) successfully amplified polymorphic fragments in all the 48 groundnut genotypes tested (Table 3). These 25 SSR markers amplified a total of 164 alleles (Table 3). However, the number of alleles per marker was highly variable, and ranged from two for marker GM1357 to 21 for GM1577, with an average of 6.56 alleles per marker. Major allele frequency per marker also ranged from 0.13 to 0.91, which indicated the presence of allelic variants. Marker GM1577 (0.13) recorded the lowest allele frequency, with GM2053 (0.91) and S003 (0.91) showing the highest (Table 3). Large variability was observed between and among the markers for gene diversity, which ranged from 0.172 to 0.929. Markers GM2053 and S003, which were found to have the highest major allele frequency, revealed the lowest gene diversity. In contrast, marker GM1577 which showed the lowest major allele frequency, exhibited the highest gene diversity. Although, all the markers selected for use in this study had a PIC value ≥ 0.5 Pandey et al. (2012a), the PIC values obtained here ranged from a low 0.16 for markers GM2053 and S003 to 0.92 for GM1577, yielding a PIC mean value of 0.57. The observed variation in PIC values in this study could be attributed to genotypic differences in the groundnut material used. To assess the diversity among the 48 groundnut genotypes, Jaccard's similarity coefficient was calculated using NTSYSpc v. 2.1 and a dendrogram generated based on the unweighted pair-group method with arithmetic mean (UPGMA) procedure (Figure 1). Overall, two major clusters were formed at 72% coefficient of similarity. Cluster I consisted mainly of genotypes that matured at 85 to 90 days after sowing (early maturity) with the exception of NKATIESARI, which matured at 110 days after planting (medium maturity). Cluster II showed no clear-cut demarcation as it contained both early- and late-maturing genotypes. However, the majority of the genotypes in cluster II exhibited tolerance to foliar diseases and aflatoxin contamination. Two sub-clusters (sub-cluster IA and IB or IIA and IIB) were formed within each cluster at 75% coefficient of similarity. Sub-cluster IA contained two early-maturing genotypes and IB

Table 2. Description of SSR markers used in this study and their sources.

Primer	Forward sequence	Reverse sequence	Repeat motif	Number of repeat units	Allele size (bp)	Reference
GM1937	TTCATCCTCTGCTTCCTTTGA	TGACCAAACCCATCATCATCT	(TC)12	4	107	Guo et al., 2012
GM1043	GAATTCAGCTTGTGGATTGGA	TTGTTGTTGTTAGGCCACCAC	(TGA)6	4	258	Guo et al., 2012
GM1357	ACGCAATGCACATCCTTTAGA	CAGAAACAGGTGAAGGAGCTG	(TTC)6	5	103	Guo et al., 2012
GM1483	GCTGTTACATGGGCATCATTT	TCATCAGAGACCCAAGATCCA	(TCA)13	5	113	Guo et al., 2012
GM2444	CCCTGTTACACACAAGCCATT	TGAGCAAGTGTTAGCCATGAA	(CAA)10	5	268	Guo et al., 2012
GM1477	GTTTGTGTTTGTGCCGAACTT	CAAGCAACCCTTGATGTGTTA	(TTTG)5	5	408	Guo et al., 2012
GM1834	GAAGCAAGAAACCAACCAAGTC	GTGATAAAGCGGCCACAATAG	(CAT)9	5	104	Guo et al., 2012
GM2103	GCAACATGCCCTTAGACATACA	GCTTTCTCTTCTCGCTTCCTC	(TGA/TGA)	5	294	Guo et al., 2012
GM1489	GGAAGATGTGGTTGCAAATTC	CTCCCAGCTATCAACTTCACG	(AT)35	4	408	Guo et al., 2012
S046	ATGGCGAATCGGAGGGTAGGTT	TCCAATCGTGCGTTTCAATCATCT	GAA	4	272	Wang et al., 2007
S108	GCTTACATTACACGTCATCTC	CCGAACTTACAGTTAGGAG	(TC)14(AC)15	4	221	Wang et al., 2007
S003	GCACCAATTTTGTCCCTGAT	AAGGGGTTTGCACGTAAATG	TCT	5	188	Wang et al., 2007
S021	AGTCCTACTTGTGGGGGTTG	TCCCTTTTGCAGTGAAATCC	CT	5	240	Wang et al., 2007
GM2730	GGAGACGAGTTGTTGTTACCG	GGAAAGAGACCCCATCAACTC	(ATG)6	4	100	Guo et al., 2012
S073	AGTCCACTGAACCGAACACCAATC	TCCCTACCACCGAACGAAACAAT	AAG	4	335	Wang et al., 2007
GM2084	CGCAGAAATGAACCGAAATTA	GGATGCATTCTTCTTCCTCCT	(ATG/TGA/AACAAT)	5	418	Guo et al., 2012
S076	ATACTGATAGATAGGGTCGAAGGAGAG	CAACGAAAGAAAAATAAGGACATAGTG	GA	5	305	Wang et al., 2007
S118	TATATGATGCTTGATTGAGACT	CATGTAGAAGGCTTGGAGGGTAT	(AAT)6	5	271	Wang et al., 2007
GM1959	GTGTTCTCAGCCATCTTTTCG	GTGAAGGTGTTGTGAATGCAG	(TC)22	6	158	Guo et al., 2012
GM2053	ACAAGGAAAACCCATCCAATC	ACGTGATGGATTCTTGTGGAG	(TCT)5, (TGATTC)3	4	405	Guo et al., 2012
GM995	GAGGAAGCGGATTAGTTGAGG	GAAAGCTACCGCTGTCAAGTG	(TG)8	5	408	Guo et al., 2012
S084	CAGCCAATATGTCACAACCCTAAT	CTCCCACTACAAATCTCCAATCAAT	GA	4	265	Wang et al., 2007
GM1533	CCATTAGCTGAAGGCATTGAA	CAAGATGGTGACGAGAAGAGC	(TTC)7	5	250	Guo et al., 2012
GM2638	ATGCTCTCAGTTCTTGCCTGA	CAGACATAACAGTCAGTTTCACC	(TC)14	5	107	Guo et al., 2012
S024	GGCAATGCACACGCTACTCT	CGTGAGGCGTGAGAGTTCAT	GA	5	217	Wang et al., 2007
S086	TCCATGAGGGGTTATAGGTGTTT	GGGTGTATTTCTGAAGTTCCATTATC	TTC	5	132	Wang et al., 2007
GM1911	CAGCTTTCTTTCAATTCATCCA	CACTTCGTGTTCTTCCTGCTC	(AG)25	5	136	Guo et al., 2012
S070	CCTTTCCCATTCCATTAGC	GTCCGAGTTGAGGAACAACAA	TTG	5	258	Wang et al., 2007
S080	GGCGTCCCATTGCTTAC	AGAATGCGTTGATGTTATGAA	TCT	5	225-267	Wang et al., 2007
S113	TTGCATGTAGGAAAGAAAGATT	TTGGATGTGGTGGTGATGT	(TC)9	5	263	Wang et al., 2007
GM1577	GCGGTGTTGAAGTTGAAGAAG	TAACGCATTAACCACACACCA	(GA)13	5	278	Guo et al., 2012
GM2602	ACTCGATTGGGAACTGAGGAT	TCTCGTCTCTGCCATTAGTACC	(GAA)7	6	97	Guo et al., 2012
GM2348	ACACAAGAACCACCAAAAGCA	CAGCGCCATTTCTCAACTATC	(CA)8	4	142	Guo et al., 2012
GM1991	GAAAATGATGCCGAGAAATGT	GGGGAGAGATGCAGAAAGAGA	(TC)12	6	122	Guo et al., 2012
S093	TTGGGGAAATACAGAATAACG	CTCCCACATCCCCACCAT	(TG)10(AG)14	5	162	Wang et al., 2007

Table 2. Contd.

Primer	Forward sequence	Reverse sequence	Repeat motif	Number of repeat units	Allele size (bp)	Reference
S052	CCCTGAGAATGAAAGAAAGAAACA	CAACCGCAGCGACGATAGATG	GA	6	142	Wang et al., 2007
GM822	CACGGAACCCAGATAAACTGA	ATCACCATCACCATCGTTGTC	(ACG)5	5	409	Guo et al., 2012
GM1996	CATCCCATCATTTTCCCTCTT	TACAGTGAAGGTGGGATCCTG	(CAA)19	6	150	Guo et al., 2012
GM1864	CAACACACCCAGTCACTCTCTC	TCCTTTCTGATGTTCTGTGTGTG	(CT)15	5	106	Guo et al., 2012
GM1502	TTCCTTTACACACACGCACAC	TGGAGGAAATGTAGGGAAAGG	(TC)19	7	424	Guo et al., 2012
GM2504	ACATCAATCCCTGCCTACCTC	TCGGATTCTGTTACCACCTCA	(AT)18	7	307	Guo et al., 2012
S023	CTGGAAGTGGTCCTGTTGGT	GCTGCTCCTGTCTCTGGAAT	TGT	6	188	Wang et al., 2007
GM2637	ATGCTCTCAGTTCTTGCCTGA	AAGGAGCCAGCTAGCTACATAGT	(GA)12	6	131	Guo et al., 2012
S038	GGCAGCGAAGCACCCATTGTTA	GTAGGGTTGCGTTTCGTTTTCTTATCG	GA	8	212	Wang et al., 2007
GM2009	CAAACGCATACACCCCATAAC	TTTGGTTCTCGTTTGTGTTTT	(AG)16	7	107	Guo et al., 2012
S083	CTTGAACTTATTTTTGGTGGGTGAAC	CAAGGGAGAATGAAGAATGCTAAG	(CA)15TA(CA)11	6	247	Wang et al., 2007
GM1986	GCTGCTGCAAGTCTTAAGGAA	AAAGTGTCAGGTGCAAAGCAT	(TA)14	6	133	Guo et al., 2012
GM2605	ACTGCTGCCATGGTTGAGTTA	TTTCGCACTTTCTCAGTTTCC	(CT)8	7	104	Guo et al., 2012
S001	TGGACTAGACAAGGAACAACCA	GAGCCATGAGCACACAACAC	CT	8	202	Wang et al., 2007
S019	GCTCCACTAGTGCCGAAATC	CAGACACCCGGAGGCTTA	CT	9	103	Wang et al., 2007
GM744	TGATGCCTGAGAGACTTTGGT	GACTCCTTCACCTCCCTAAGC	(GAA)5	8	408	Guo et al., 2012
GM840	GCAGCATACAAGCAATCCACT	TTTGCCATTTGCTGTTCTACC	(GT)9	9	415	Guo et al., 2012
S009	CGCTGTCCTTATCGAACCAT	CTCTCACTCGCGCTTTCTCT	AG	11	125	Wang et al., 2007

Table 3. Summary statistics and polymorphism of markers used in this study, calculated using PowerMarker v.3.25. Map position and distances were obtained from the integrated consensus map of cultivated groundnut (Shirasawa et al., 2013).

Marker	Major allele frequency	Allele number	Gene diversity	PIC	Map position	Distance (cM)
GM1357	0.56	2.00	0.49	0.37		
GM1483	0.56	3.00	0.56	0.47	B03, B10	40.21, 3.239
GM1502	0.82	5.00	0.31	0.29	B03	38.452
GM1577	0.13	21.00	0.93	0.92	A05	53.751
GM1834	0.52	4.00	0.54	0.43		
GM1864	0.31	4.00	0.74	0.69		
GM1911	0.31	8.00	0.79	0.77	A09	83.224
GM1937	0.52	7.00	0.67	0.64	A07	74.214
GM1959	0.21	13.00	0.87	0.86	B04	64.853
GM1986	0.29	13.00	0.84	0.82	A07, B08	84.217, 73.894
GM1991	0.18	12.00	0.90	0.89	B06	92.887

Table 3. Contd.

Marker	Major allele frequency	Allele number	Gene diversity	PIC	Map position	Distance (cM)
GM1996	0.63	5.00	0.55	0.50	B03	63.726
GM2053	0.91	3.00	0.17	0.16	B03	74.424
GM2084	0.23	17.00	0.89	0.88	B05	21.271
GM2348	0.63	5.00	0.53	0.47		
GM2637	0.24	8.00	0.83	0.81	A04	88.62
S3	0.91	3.00	0.17	0.16		
S21	0.33	4.00	0.73	0.68		
S23	0.78	3.00	0.36	0.32		
S38	0.54	4.00	0.61	0.54		
S70	0.52	5.00	0.64	0.59		
S80	0.50	6.00	0.66	0.61		
S83	0.56	3.00	0.57	0.49		
S84	0.54	3.00	0.59	0.52		
S93	0.57	3.00	0.53	0.44		
Mean	0.49	6.56	0.62	0.57		

20 genotypes belonging to minor clusters. Significant among them was a cluster that contained the single genotype ICIAR 19BT, and shared 76% similarity with the others. The remaining genotypes within sub-cluster IB showed similarities ranging from 90 to 100%. Sub-cluster IIA and IIB shared a similarity coefficient of 75%. Sub-cluster IIA had four minor clusters at 80% similarity with one cluster containing a single genotype (ICGV-IS 08837), which was medium-maturing and exhibited high tolerance to foliar diseases. Sub-cluster IIB had two minor clusters with similarity coefficients ranging from 84 to 100%.

Although the groundnut genotypes were grouped based on their phenotypic attributes, the clustering from DNA analysis did not match these phenotypic groupings. Genotypes from different groups were found to cluster together irrespective of their phenotypic characters, and this observation is consistent with the findings of other studies which also found low genetic diversity within cultivated groundnut (Jiang et al., 2007; Janila et al., 2013). Based on these results, there is a strong possibility that the majority of genotypes tested in this study shared a similar pedigree since few parents have historically been used in groundnut breeding programs (Nigam, 2000; Janila et al., 2013). The limited number of parents used in groundnut breeding programs stems from the fact that the hybridization of two possible diploid ancestors (*Arachis duranensis* and *Arachis ipaensis*), followed by chromosome doubling, resulted in the allotetraploid genome of cultivated groundnut (Nagy et al., 2012; Shirasawa et al., 2013; Janila et al., 2013), which introduced a crossing barrier with its wild diploid ancestors and therefore limited the sources of allelic variability needed for groundnut improvement (Nagy et al., 2012; Janila et al., 2013).

A number of reports on the use of SSR markers to characterise groundnut have produced results similar to those obtained in this study. For example, Mace et al. (2006), who used 23 SSR primers to study 22 groundnut genotypes with varying levels of resistance to rust and early leaf spot, recorded 52% polymorphism with a PIC value ≥ 0.5. In a study with 31 groundnut genotypes that exhibited different levels of resistance to bacterial wilt, Jiang et al. (2007) also found that 29 of the 78 SSR primers were polymorphic, and amplified a total of 91 polymorphic loci with an average of 2.25 alleles per primer. Similarly, Tang et al. (2007) employed 34 SSR markers to determine the genetic diversity in four sets of 24 accessions from the four botanical varieties of cultivated groundnut, and found that 16 primers were polymorphic. This led to the conclusion that abundant inter-variety SSR polymorphism exists in groundnut.

A recent study which assessed the diversity of 11 groundnut genotypes using 17 SSR markers, also recorded 24% polymorphism (Shoba et al.,

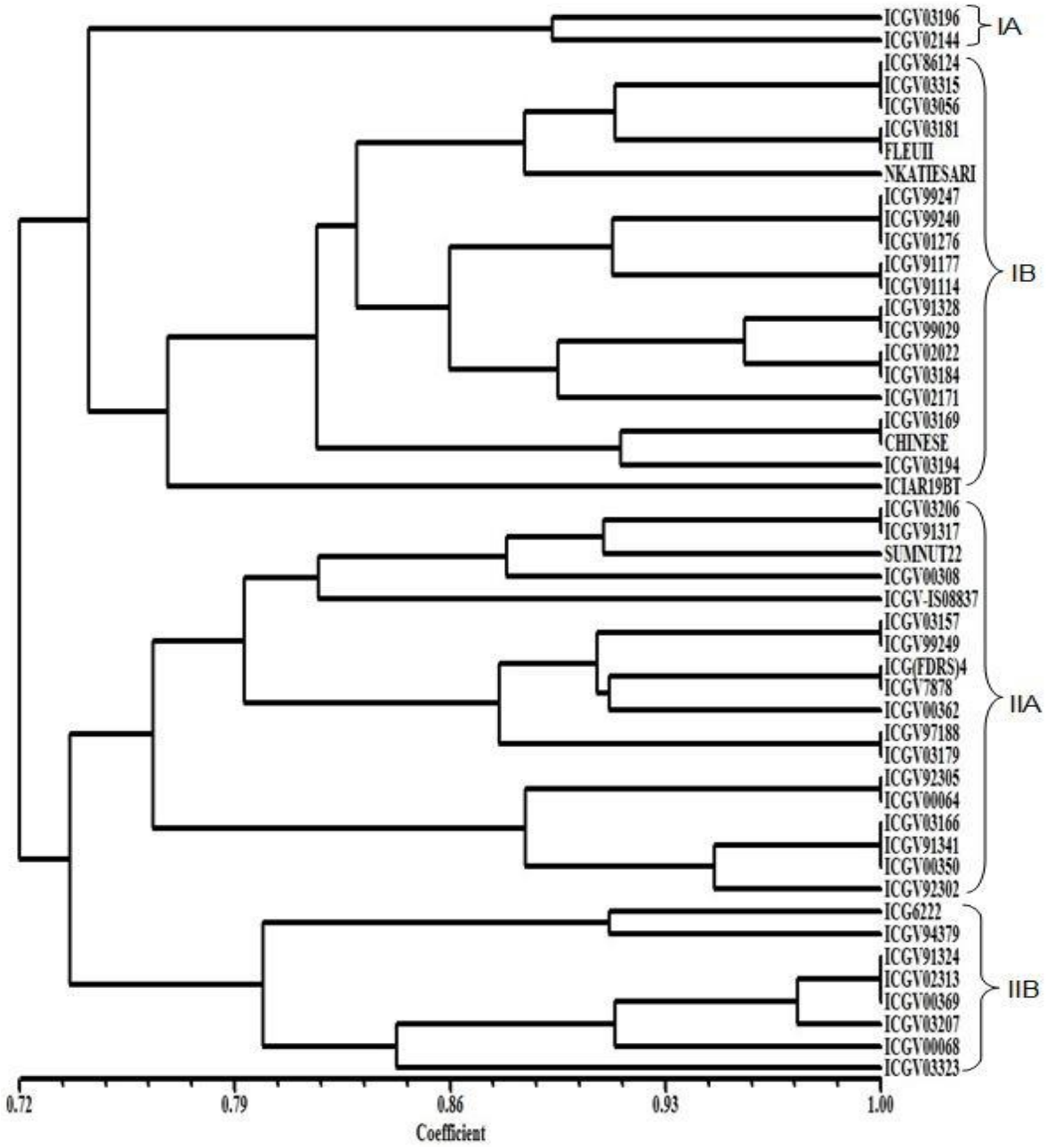

Figure 1. Genetic diversity among 48 groundnut genotypes generated using the unweighted pair group method with arithmetic mean (UPGMA) procedure based on the Jaccard's similarity coefficient created with NTSYSpc v. 2.1.

2010). Mondal and Badigannavar (2010) similarly used 26 SSR primers to amplify 136 bands and showed that 76.5% were polymorphic in 20 cultivated groundnut genotypes that differed in resistance to rust and late leaf spot disease. It is therefore interesting that, in this study, a total of 164 bands were amplified using 54 SSR markers on 48 groundnut genotypes, and 25 were found to be polymorphic. But more importantly, the map position and distance of some of the polymorphic markers used in this study (Table 3) have recently been identified on the consensus genetic map of the *Arachis* genome (Shirasawa et al., 2013). Furthermore, they have also been mapped on different linkage groups from the consensus groundnut maps created to identify QTLs associated with foliar disease resistance and drought tolerance (Table 4). Marker GM1911 in this study is, for example, linked to a drought tolerance QTL (Ravi et al., 2011; Gautami et al., 2012), while markers GM1577 and GM1991 are linked to QTLs associated with tolerance to late leaf spot disease (Sujay et al., 2012). No doubt, the

Table 4. Polymorphic markers associated with traits of interest in cultivated groundnut.

Marker	Linkage group	Position (cM)	Traits of interest	Reference
GM1483	AhIV	293.4	SCMR	Gautami et al., 2012
GM1577	AhV	187.4, 10.6	TE, LLS	Gautami et al., 2012; Sujay et al., 2012
GM1911	AhXIII	11.3, 53.0	SCMR, SLA, LLS, Rust	Ravi et al., 2011; Gautami et al., 2012; Sujay et al., 2012
GM1937	AhXVI	32.1, 6.9	HI, TDW, VW, SCMR	Gautami et al., 2012; Ravi et al., 2011
GM1959	AhXVII	24.6	SCMR, HI	Gautami et al., 2012
GM1991	AhX	21.8	SCMR	Ravi et al., 2011
GM1996	AhXIV	64.1	SLA	Gautami et al., 2012
GM2637	AhVII	55.4	TW	Gautami et al., 2012

SCMR - SPAD chlorophyll meter reading, TE - transpiration efficiency, LLS - late leaf spot resistance, SLA - specific leaf area, rust - Rust tolerance, HI - harvest index, TDW - total dry weight, VW - vegetative weight.

identification of polymorphisms associated with these important agronomic traits has potential for advancing groundnut improvement in Ghana, as they can be used in QTL mapping, and/or marker-assisted breeding activities (for example, marker-assisted backcrossing and marker-assisted recurrent selection).

Taken together, the results of this study demonstrate that SSR markers can be very effective in discerning variations among the 48 different groundnut genotypes despite their close relatedness, a finding consistent with other studies (Hopkins et al., 1999; Cuc et al., 2008; Carvalho et al., 2010). The mean PIC value of 0.57 suggests that the primers were highly polymorphic (Pandey et al., 2012b) and can be applied to different groundnut populations in breeding programs. The clustering of groundnut genotypes in this study was independent of their phenotypic attributes, and thus confirmed the low level of genetic variability in cultivated groundnut (Pandey et al., 2012a; Janila et al., 2013). The relatively low genetic diversity within the genotypes used in this study was likely due to the fact that they were crosses generated from a breeding program. The SSR markers could discern variations and differentiate between the closely related groundnut genotypes, makes this technology a powerful tool for genomic characterisation of groundnut. The relatively diverse genotypes identified in this study are potential candidates for use as parental material in future studies to advance groundnut breeding in Ghana.

Conflict of interests

The authors did not declare any conflict of interest.

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