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ARTICLE

Genetic Similarity from Collections of Seed for Two Peanut (*Arachis hypogaea* L.) Cultivars in Ghana

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ABSTRACT

Maintenance of genetic purity of crop cultivars is critical to meet the needs of farmers, processors, and consumers. In Ghana where the informal seed sector is popular (e. g., farmer-saved seed), purity of improved cultivars in the farming community can be compromised. The objective of the study was to assess the genetic purity of farmer-saved seed of two peanut (*Arachis hypogaea* L.) cultivars (Shitaochi and Yenyawoso) using molecular marker techniques. Twenty samples of Shitaochi and eight samples of Yenyawoso were collected from farmers across five regions of the country and were compared with seed provided by the research institutes of the Council for Scientific and Industrial Research (CSIR). Genetic similarity ranged from 43% to 100% when comparing seed collections from farmers to those from research institutes. Only five of the 20 seed samples of Shitaochi (25%) and two of the 8 samples of Yenyawoso (25%) collected from farmers were found to be completely similar to the reference samples provided by CSIR. These results demonstrate the lack of purity among described cultivars in the informal seed sector in Ghana. While diversity between samples of Shitaochi have been reported and would be expected in the informal seed system, the pace at which Yenyawoso has lost genetic purity in a relatively short period of time indicates that a more effective formal seed system is needed to maintain cultivar purity.

INTRODUCTION

In Ghana, there has been an increase in the development and release of peanut cultivars in recent years. Ghana's seed sector is characterized by two dominant seed systems from which peanut farmers obtain seeds for production: the formal seed system and

the informal/farmers' seed system. The formal seed system involves the production and purchase of commercial certified seed. Major activities under this system include development of crop varieties, approval and registration of varieties, production of breeder and foundation seed, production of commercial seeds, as well as quality control and certification of seeds (Etwire et al., 2013; Tripp and Mensah-Bonsu, 2013). Crops Research Institute (CRI) and Savanna Agricultural Research Institute

(SARI) are responsible for developing improved varieties of peanut, while the National Variety Release and Registration Committee (NVRRC) releases the varieties. Typically, the Ministry of Food and Agriculture through the extension department are formally responsible for dissemination of the released improved varieties (Asiedu-Darko, 2014). For improved peanut cultivars, it is important to maintain genetic purity of cultivars so that yield and quality attributes are predictable throughout the peanut value chain. However, it is estimated that less than 5% of peanut cultivated in Ghana are certified seed sourced from the formal seed system (Puozaa et al., 2021) while the vast majority of seed is supplied by the informal seed system (e. g., farmer saved seeds, exchanges with other farmers and the grain markets).

Genetic quality entails the underlying genetic potential of the cultivar and determines yield potential (Biemond, 2013). A high level of genetic purity in crop cultivars or hybrids is required to ensure that the advances in productivity and quality imparted by breeders are delivered to the farmer and ultimately to the consumer (Smith and Register, 2008). Knowing the overall quality status of a seed helps the farmer with important crop input decisions during production. Farmers expect high-quality, genetically pure seeds. To ensure this, seed testing is required so that farmers receive the quality of seed they desire. Seed testing ensures that seeds meet minimum quality standards and minimizes the risk of crop failure and mitigate the negative effects of using seed contaminated with weed seeds, pathogens and inert matter (FAO, 2018). However, in Ghana, testing the genetic purity of seeds by farmers before planting is seldom implemented and thus genetic purity of peanut cultivars is unknown once received by farmers and incorporated in the informal seed system. According to Ibrahim and Florkowski (2015), most farmers involved in on-field trials with improved varieties share the seeds with other farmers even before the variety is released, which leads to the contamination of the seed prior to release. For the farmers who are able to purchase seeds of the improved peanut varieties from the formal seed system, it often takes about 10 years to replace initial seeds (Anonymous, 2016). Additionally, through seed diffusion between farmers and cultural practices including selection and seed storage conditions, diversity among seeds produced by individual plants within a cultivar can shift (Thomas et al., 2012). Ibrahim and Florkowski (2015) reported that both peanut farmers and traders in northern part of Ghana are not able to distinguish among varieties and often give descriptive names to varieties other than the original, official name. For instance, 'Simbaligu', which literally means 'small kernels' is the name given to Shitoachi because of its small kernel size.

Goals of cultivar testing includes identifying the cultivar, discriminating between cultivars, and documenting genetic purity of the cultivar (Powell, 2009). Depending on the objective, approaches to cultivar testing can include grow-out tests, biochemical methods, and DNA-based methods. Historically, genetic purity testing was achieved through plant grow-outs in the field that relies on the visual identification of plant morphological characteristics (Ballester and de Vincente, 1998; Della Vecchia et al., 1998). However, this method is time consuming, labor intensive, and space demanding (Liwang et al., 2004). Recently, genetic identity and purity testing have shifted to DNA-based molecular marker methods (Lai et al., 2015).

Molecular markers, which are based on variation in DNA sequence, provide an unbiased and objective way of identifying cultivars (Bora et al., 2016). While numerous molecular markers are available for variety identification and genetic purity studies, simple sequence repeats (SSR) have been widely employed. This is largely based on characterization by co-dominance, multiallelic variation, relative abundance, reproducibility, and good genome coverage (Lu et al., 2018; Stachel et al., 2000).

Simple sequence repeats are short tandem repeat motifs that may vary in the number of repeats at a given locus (Tautz, 1989). The use of SSR as markers is based on the differences in repetition of usually two or three nucleotide bases (Ghosh et al., 2014). SSR markers have been used to differentiate accessions of the peanut species (Kottapalli et al., 2007), genetic diversity in peanut (Oteng-Frimpong et al., 2015; Patel et al., 2015; Tang et al., 2007), QTL analysis (Khedikar et al., 2010; Zhao et al., 2016), and construction of genetic linkage maps (Li et al., 2019; Qin et al., 2012). Also, SSR markers have been successfully used to assess the genetic purity of farmer-saved seeds in soybean [*Glycine max* (L.) Merr.] (Meesang et al., 2001), commercial hybrids of soybean (Li et al., 2019; Zhang et al., 2014), corn (*Zea mays* L.) (Chaudhary et al., 2018; Daniel et al., 2012; Elçi and Hançer, 2015), and rice (*Oryza sativa* L.) (Kumar et al., 2012; Yashitola et al., 2002; Ye-Yun et al., 2005).

The cultivar Shitoachi was released in Ghana more than 40 years ago (NVRRC, 2019) and is currently estimated to be grown on about 80 to 90% of land area devoted to peanut cultivation in Ghana (Owusu-Akyaw et al., 2019). More recently, the cultivar Yenyawoso was released in Ghana in 2012 and is estimated to be planted on a limited amount of ha in the country (Owusu-Akyaw et al., 2019). Using these two cultivars as references, the objective of this research was to estimate genetic purity of farmer-saved seeds using SSR markers for the cultivar Yenyawoso in a relatively short period of time after release. Should contamination of seed be observed for this cultivar, the cultivar Shitoachi would be the likely source of adulteration in the informal seed system in Ghana.

MATERIALS AND METHOD

Seeds of two commercially available peanut cultivars in Ghana were collected from farmers and research institutes to determine genetic similarity using SSR. Seeds from 28 farmers were collected with 20 being the cultivar Shitoachi and eight being Yenyawoso. One seed sample of Shitoachi and two samples of Yenyawoso were collected from the Council of Scientific and Industrial Research's Savanna Agricultural Research Institute (CSIR-SARI) and Crops Research Institute (CSIR-CRI) as standards against the farmer-saved samples.

For each accession, fifteen seeds were randomly selected and sown in plastic containers in the greenhouse. DNA was extracted from three recently expanded leaflets from 10 individual plants for each sample. Total genomic DNA was isolated following the Cetyl-trimethyl ammonium bromide (CTAB) procedure (Cuc et al., 2008).

Quality and quantity of the extracted DNA were assessed using a UV-Vis Spectrophotometer Nanodrop 2000 (Thermo Scientific) and diluted to final concentration of 100 ng/µl.

DNA from 10 plants representing one sample were bulked prior to Polymerase Chain Reaction (PCR) analysis.

A total of twenty-five SSR primer pairs were randomly selected from the existing literature on the basis of their

polymorphic nature. Following screening for their ability to discriminate between Shitaochi and Yenyawoso cultivars, four polymorphic primers were selected for genetic analysis of the 28 farmer-saved seed samples (Table 1).

Table 1. List of SSR primers used in the genetic analysis.

Primer	Sequence	A.T.°C	Reference
PM3	F - GAAAGAAATTATACACTCCAATTATGC R - CGGCATGACAGCTCTATGTT	55	He et al., 2003
PM36	F - ACTCGCCATAGCCAACAAAC R - CATTCCCACAACCTCCACAT	50	He et al., 2003
PM50	F - CAATTCATGATAGTATTTTATTGGACA R - CTTTCTCCTCCCAATTTGA	50	He et al., 2003
PM204	F - TGGGCCTAAACCAACCTAT R - CCACAAACAGTGCAGCAATC	55	He et al., 2003
PM210	F - CCGCAGATCTTCTCCTGTGT R - CCTCCTCATCCTCTAAACTCTGC	55	He et al., 2003
PM375	F - CGGCAACAGTTTGTATGGTT R - GAAAAATATGCCGCCGTTG	55	He et al., 2003
Seq2B10	F - AATGCATGAGCTTCCATCAA R - AACCCCATCTTAAAATCTTACCAA	51	Ferguson et al., 2004
Seq2C11	F - TGACCTCAATTTGGGGAAG R - GCCACTATTCATCGCGGTA	52	Ferguson et al., 2004
Seq5D5	F - AAAAGAAAGACCTTCCCCGA R - GCAGGTAATCTGCCGTGATT	52	Ferguson et al., 2004
Seq2F5	F - TGACCAAAGTGATGAAGGGA R - AAGTTGTTTGTACATCTGTACATCG	51	Ferguson et al., 2004
Seq15F12	F - AAAGTCAACCGCTCACACTG R - AGGGTTAGGATTTTGGGTGG	60	Ferguson et al., 2004
Seq17F6	F - CGTCGGATTTATCTGCCAGT R - AGTAGGGGCAAGGGTTGATG	52	Ferguson et al., 2004
IPAHM105	F - CAGAGTTTGGGAATTGATGCT R - GCCAGATCTGAGCAAGAACC	60	Cuc et al., 2008
IPAHM123	F - CGGAGACAGAACACAAACCA R - TACCCTGAGCCTCTCTCTCG	60	Cuc et al., 2008
IPAHM406	F - TGAAAGGGATTGGACCAAAA R - TGTGGACAGGATTCACACA	60	Cuc et al., 2008
IPAHM689	F - GATGACAATAGCGACGAGCA R - GTAAGCCTGCAGCAACAACA	60	Cuc et al., 2008
IPAHM716	F - CACTATGCCACGAGCTTCAA R - ACACACCACAACCACAGAGC	60	Cuc et al., 2008
GM1911	F - CAGCTTTCTTTCAATTCATCCA R - CACTTCGTGTTCTTCCCTGCTC	59	Guo et al., 2012
GM1577	F - GCGGTGTTGAAGTTGAAGAAG R - TAACGCATTAACCACACACCA	59	Guo et al., 2012
GM1991	F - GAAAATGATGCCGAGAAATGT R - GGGGAGAGATGCAGAAAGAGA	59	Guo et al., 2012
S021	F - AGTCCTACTTGTGGGGGTTG R - TCCCTTTTGCAGTGAATCC	59	Wang et al., 2007
S080	F - GCGGTCCCATGCTTAC R - AGAATGCGTTGATGTTATGAA	59	Wang et al., 2007
TC7A02	F - CGAAAACGACACTATGAACTGC R - CCTTGGCTTACACGACTTCTCT	59	Moretzshon et al., 2005
TC4F12	F - GATCTTTCCGCCATTTTCTC R - GGTGAATGACAGATGCTCCA	59	Moretzshon et al., 2005
PMc588	F - CCATTTTGGACCCCTCAAAT R - TGAGCAATAGTGACCTTGCAAT	60	He et al., 2003

Polymerase Chain Reaction (PCR) amplification was performed using 20 µl of PCR mixture solution containing 10 µl of 1X PCR master mix (SBS), 1 µl of each primer (20 µM) (forward and reverse), and 100 ng of genomic DNA templates. The PCR program was set at initial denaturation temperature of 94 °C for 5 minutes, followed by denaturation at temperature of 95 °C for 1 minute, 51-60 °C for annealing for 45 seconds, based on the primers, 72 °C for 45 seconds for extension, all run for 35 cycles and final extension cycle at 72 °C for 8 minutes, and then an indefinite hold at 4 °C.

Amplified products from 5 µl of the PCR reaction were separated by electrophoresis in a 6 % polyacrylamide gel together with a Quick-Load Purple 100 bp DNA ladder (New England Biolabs), for 75 minutes at 80 V and 300 amh in 1x TBE buffer. Gels were visualized using an Alpha Imager TM 2200 (AlphaInnotech Inc., San Leandro, CA) gel documentation system.

Gel images were scored for the presence (1) and absence (0) of SSR bands manually. The band scores were used to generate a similarity data matrix using Simple Matching (SM) coefficient. A dendrogram was constructed from the similarity coefficient values following the unweighted pair-group method with arithmetic average (UPGMA) technique in NTSYS-pc version 2.20v (Rohlf, 2009). The Sequential Agglomerative Hierarchical and Nested (SAHN) method was adopted for clustering.

RESULTS AND DISCUSSION

The UPGMA dendrogram based on simple matching coefficients of binary scores of polymorphic bands obtained from the 21 seed lots of Shitaochi and 10 seed lots of Yenyawoso is provided in Figure 1. Genetic similarity values ranged from 0.43 to 1.0. Overall, cluster analysis put the seed lots into one group at 43% similarity but subsequently separated them into two clusters at 0.6 coefficient of similarity. Two Yenyawoso seed

lots from CSIR-SARI and CSIR-CRI (e. g., Yen-S and Yen-C, respectively) were grouped in Cluster I along with eight farmer-saved Yenyawoso seed lots (ASF-2, NRF-8, NRF-15, NRF-9, ASF-5, ASF-4 and UWF-10) and one farmer-saved Shitaochi seed lot (UEF-2). Cluster II similarly contained the Shitaochi seed lot obtained from CSIR-SARI (Sh), nineteen farmer-saved Shitaochi seed lots (NRF-14, UEF-6, ASF-1, UEF-5, UEF-1, UEF-10, NRF-5, UWF-11, UWF-4, UWF-2, UWF-5, NRF-11, UWF-7, NRF-13, NRF-4, UWF-6, ASF-7, ASF-6 and NRF-12), and one farmer-saved Yenyawoso seed lot (UWF-1).

In the screening of twenty-five SSR markers for polymorphic markers, only four (16%) discriminated between Shitaochi and Yenyawoso. These include Seq17F6, Seq5D5, PM50, and Seq2B10. This suggests there is a low level of polymorphism between these two peanut cultivars. The results, however, corroborate the findings of Gaikpa *et al.* (2015) who reported a low level of polymorphism between Shitaochi and Yenyawoso using the SSR primer Seq17F6.

Farmers select varieties on the basis of their agricultural characteristics such as resistance to biotic and abiotic stresses, or their productivity and the recognized value of their products (Powell, 2009). As such, it is pertinent that farmers obtain seeds of good genetic quality pertaining to their chosen variety. Out of the twenty seed lots reported by the farmers to be the Shitaochi peanut cultivar in this study, only five were completely genetically similar (NRF-14, UEF-6, ASF-1, UEF-5 and UEF-1) to the reference Shitaochi seed in cluster I. With regards to Yenyawoso farmer-saved seeds, only two samples were 100% genetically similar (ASF-2 and NRF-8) to the reference Yenyawoso seed samples. This is indicative that these seed samples are very similar to the original breeder seeds and thus have not changed in the hands of the farmers during seed production. This also means that the owners of these seed samples have accurately identified the cultivar. The other farmer-saved seed samples that were grouped in the two clusters at a coefficient of similarity less than 1.0 suggests that they are

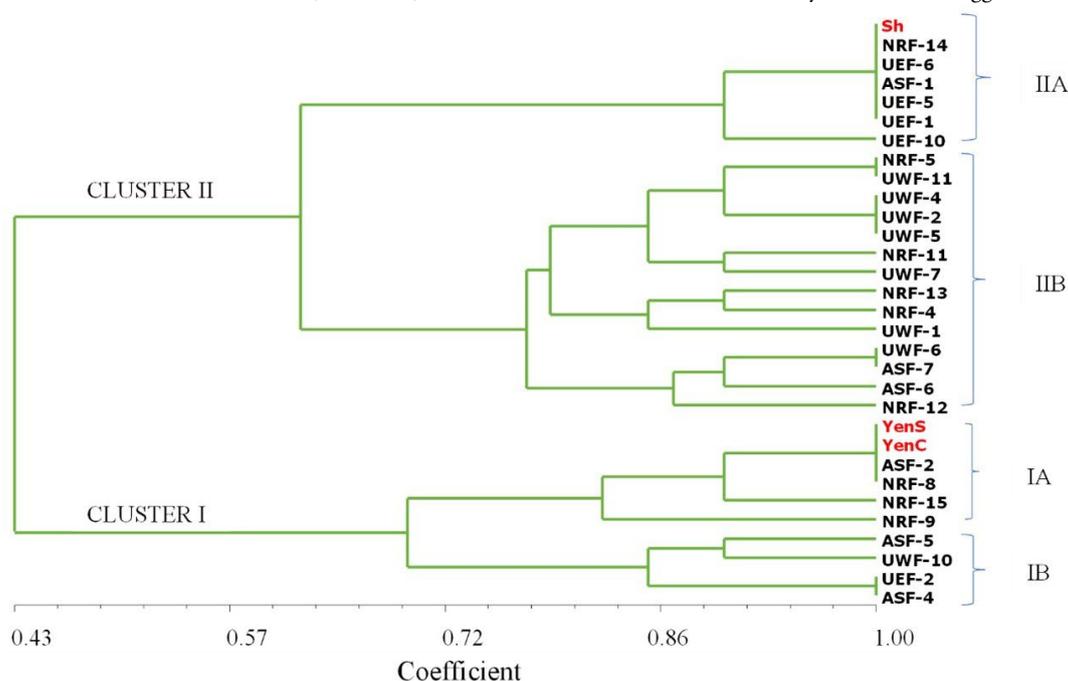


Figure 1. Dendrogram showing genetic relationship among 31 peanut seed lots generated using UPGMA based on Simple Matching (SM) coefficient. Reference samples: Sh-'Chitaochi', YenS-YenyawosoSARI, YenC-YenyawosoCRI; Farmer samples: UWF-Upper West Farmer, UEF- Upper East Farmer, NRF-Northern Region Farmer, ASF-Ashanti Region Farmer.

different from the seeds from SARI and CRI, and that their genetic purity has been lost in the informal seed chain. This perhaps is due to inadvertent errors in the identification of cultivars in the informal seed exchange system among farmers. Additionally, different selection methods used by farmers during seed saving processes could be responsible for the deterioration in genetic purity of some of the farmer-saved seeds. Samples UEF-2 and UWF-1 were identified by the farmers in this study to be Shitaochi and Yenyawoso, respectively. However, UEF-2 was grouped in the same cluster as the Yenyawoso reference samples while UWF-1 was grouped in the same cluster as the Shitaochi reference sample. This could be a result of misidentification of the samples by the farmers. Sometimes, farmers name cultivars apart the original name designated by the breeder on different basis: specific characteristics of the cultivar or the person/organization who introduced the cultivar to the community

In conclusion, these results indicate that Yenyawoso and Shitaochi cultivars in the hands of farmers are different genetically when compared to those from the research institutes. Results from this research have limitations due to several factors. First, specific practices for each farmer relative to how seeds were handled previously was not determined. The cause of impurity of seed noted through SSR markers may have been elucidated more clearly with greater information from farmers on their seed sourcing. Secondly, determining if phenotypic differences were present for plants grown from seed used for SSR markers would have been informative and is a common practice when determining genetic differences among cultivars. Additionally, future research should be conducted on the genetic purity of cultivars by employing a greater number of markers due to the low level of polymorphism detected by primer pairs used in this research. The low level of polymorphism was not unexpected based on the origin of *Arachis hypogaea* (Seijo et al. 2007). There is also a need to screen more peanut cultivars that have been in cultivation for many years. None-the-less, results from this research provide information on genetic purity of cultivars in the informal seed system in Ghana.

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