Identification of groundnut (Arachis hypogaea) SSR markers suitable for multiple resistance traits QTL mapping in African germplasm

Busisiwe T. Ncube Kanyika a, Davies Lungu a, Alice M. Mweetwa b, Evans Kaimoyo c, Vincent M. Njung’e d, Emmanuel S. Monyo d, Moses Siambi d, Guohao He e, Channapata S. Prakash e, Yongli Zhao e, Santie M. de Villiers d, f, *

a Plant Science Department, School of Agricultural Sciences, University of Zambia, Box 32379, Lusaka, Zambia
b Soil Science Department, School of Agricultural Sciences, University of Zambia, Box 32379, Lusaka, Zambia
c Biological Sciences Department, School of Agricultural Sciences, University of Zambia, Box 32379, Lusaka, Zambia
d ICRISAT—Nairobi, PO Box 36993 00623, Nairobi, Kenya
e Department of Agricultural and Environmental Sciences, College of Agriculture, Environment and Nutrition Studies, Tuskegee University, AL, USA
f Department of Chemistry and Biochemistry, Pwani University, PO Box 195, 10801 Kilifi, Kenya

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Background: This study aimed to identify and select informative Simple Sequence Repeat (SSR) markers that may be linked to resistance to important groundnut diseases such as Early Leaf Spot, Groundnut Rosette Disease, rust and aflatoxin contamination. To this end, 799 markers were screened across 16 farmer preferred and other cultivated African groundnut varieties that are routinely used in groundnut improvement, some with known resistance traits.

Results: The SSR markers amplified 817 loci and were graded on a scale of 1 to 4 according to successful amplification and ease of scoring of amplified alleles. Of these, 376 markers exhibited Polymorphic Information Content (PIC) values ranging from 0.06 to 0.86, with 1476 alleles detected at an average of 3.7 alleles per locus. The remaining 423 markers were either monomorphic or did not work well. The best performing polymorphic markers were subsequently used to construct a dissimilarity matrix that indicated the relatedness of the varieties in order to aid selection of appropriately diverse parents for groundnut improvement. The closest related varieties were MGV5 and ICGV-SM 90704 and most distant were Chalimbana and 47–10. The mean dissimilarity value was 0.51, ranging from 0.34 to 0.66.

Discussion: Of the 376 informative markers identified in this study, 139 (37%) have previously been mapped to the Arachis genome and can now be employed in Quantitative Trait Loci (QTL) mapping and the additional 237 markers identified can be used to improve the efficiency of introgression of resistance to multiple important biotic constraints into farmer-preferred varieties of Sub-Saharan Africa.

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1. Introduction

Cultivated groundnut or peanut (Arachis hypogaea L.) is a cleistogamous allotetraploid leguminous annual crop with a genome of 2891 Mbp [1]. In Africa, where undernourishment from 2007–2008 increased by 10% with an increase in the price of nutritious foods, groundnut is an important cash crop, an affordable source of edible oil rich in omega-3 fatty acids, protein and vitamin E and its stover provides nutritious fodder for livestock [2,3,4]. Yield per hectare in Eastern and South Central Africa averages 1604 kg/ha, which is low compared to the 3393 kg/ha and 3801 kg/ha routinely harvested in China and the United States of America, respectively [4]. A major constraint to achieving the yield potential of groundnuts in Eastern and Southern Africa has been the prevalence of viral Groundnut Rosette disease (GRD), fungal rust and Early Leaf Spot (ELS) diseases [5]. Aspergillus flavus/parasiticus is also an important fungus that attacks groundnut post-harvest since consumption of aflatoxins can result in death [6] and its presence inevitably lowers yield quality.

The high cost of chemicals limits control of groundnut diseases in Africa and its use depends on ideal weather conditions, cultural practices and good application skills [7,8,9,10]. Biological control studies with mycoparasites [11] and Bacillus cereus [12] have been successful but limited to controlled environments.

Groundnuts exhibit low outcrossing rates ranging from 0 to 8% [13,14,15] and innate disease resistance is seldom attained through natural outcrossing. Historically, introgression of existing resistance
and other farmer preferred traits is accomplished only through artificial hybridization in targeted breeding from, for example, diploid wild relatives of groundnut with known abiotic and biotic stress resistance and/or tolerance [5]. In general, inheritance of disease resistance has been governed by quantitative recessive genes with low heritability that are controlled by epistatic effects and the environment [9]. The narrow genetic base of cultivated groundnut and variation in ploidy levels further limits introgression of resistance traits by interspecific hybridization [2].

Detection of polymorphic molecular markers associated with genes governing disease and insect resistance has progressed rapidly over the past two decades. This accelerated the development of cultivar resistance breeding programs for enhanced yield and grain quality [16,17,18]. SSR markers are preferred due to their co-dominance, simplicity, high polymorphism, repeatability, multi-allelic nature and transferability within the genus Arachis and significant polymorphism has been identified in novel Simple Sequence Repeat (SSRs) by He et al. [19]. These markers have enhanced phylogenetic studies of the Arachis species, for pre-breeding parent determination and integration of SSR based maps in both diploid and tetraploid species [20,21,22], comprehensive Quantitative Trait Loci (QTL) analysis for linkage to disease and pest resistance [23,24,25], comparative

### Table 1

| Category                  | Genotype             | Essential traits                          | Other agronomic traits | Country of cultivation                      |
|---------------------------|----------------------|-------------------------------------------|------------------------|---------------------------------------------|
| Disease resistance/     | ICGV-SM 95342        | LLS and rust resistant                     | –                      | Malawi                                      |
| tolerance                | ICGV 94114           | Rust resistant (Good parent for            | –                      | Malawi                                      |
|                          | ICG 12991            | resistance breeding)                      | –                      |                                              |
|                          | ICGV-SM 90704        | GRV resistant, *Aphis* sp. susceptible     | Virunga bunch type, high-yielding, medium-duration, difficult to shell | Malawi, Uganda, Mozambique, Zambia |
|                          | ICG 7878             | LLS resistant, ELS tolerant               | Virginia bunch type, amenable to technology, large seeds | – |
|                          | ICGV 95714           | ELS resistant (Good parent for resistance breeding) | Short duration | – |
| High yield and other     | 55–437              | Atafloxin tolerant                        | Drought resistant, high oil content | West Africa |
| quality traits           | FLEUR II             | ELS and atafloxin susceptible             | Non-dormant            |                                             |
|                          | CG 7 (MGV 4)         | GRD, ELS, rust susceptible                | Drought tolerant, good taste, short cooking time, uniform kernels, high oil content | Malawi, Zambia |
|                          | MGV 5                | Virginia runner type, confectionery, high oil content, roasts well, attractive tan-colored kernels | – | Zambia |
|                          | Chalimbana           | GRD, ELS and rust susceptible             | Virginia runner type, large seeds, high oil content, easy shelling, good taste, pre-harvest dormancy | Malawi, Zambia |
| Farmer preferred traits  | ICGV-SM 99557        | High-yielding                            | High-yielding, large seeds | Malawi                                      |
|                          | Pendo                | High-yielding                            | High-yielding, large seeds | Tanzania                                    |
|                          | ICGV 86124           | Spanish, early-maturing, high-yielding    | Spanish, early-maturing, high-yielding | Senegal, Mali. |
|                          | 47–10                | Resistance to *Phytium* sp.               | –                      |                                             |
|                          | JL 24 (Luena)        | GRD, ELS, rust susceptible                | Spanish, early-maturing, high-yielding, drought tolerant, non-dormant | India, Malawi, Mali, Philippines, DR Congo, Zambia, South Africa, Zimbabwe |

**Fig. 1.** SSR fragment analysis images showing examples of the different allele grades allocated according to ease of scoring.
mapping studies [26,27] and as a basis for identification of candidate genome regions controlling rust and LLS resistance [28,29]. Wang et al. [30] constructed a genetic linkage map from SSR derived bacterial artificial chromosome end sequences, facilitating the identification of markers linked to resistance gene homologs and map-based cloning. Even markers with low polymorphism enhanced the total available SSRs in wild species for transfer of target traits and should not be disregarded [31].

This study was undertaken to identify and select informative SSR markers that may be linked to resistance to ELS, GRD, rust and aflatoxin contamination across 16 varieties of farmer-preferred and other cultivated African groundnut varieties that are routinely used in groundnut improvement in order to aid the identification of suitable parents for mapping populations or marker-assisted introgression and to select a subset of SSR markers that are evenly spread across the groundnut genome for future resistance QTL mapping.

Table 2
Dissimilarity matrix of 16 Arachis sp. Genotypes. Appropriate disease resistance/tolerance pair wise comparisons between varieties (> 0.532) are highlighted for ELS (orange), GRD (red), GRD-aphid (green), rust (blue) and aflatoxin (pink).

| Genotype | ICG 7878 | ICG 12991 | 55–437 | ICGV 90704 | ICGV 94114 | ICGV 95342 | ICGV–SM 95714 | ICGV–SM 99557 | 47–10 | CG7 | Chalimbana | FLEUR–II | JL24 | MGV 5 |
|----------|----------|----------|--------|------------|------------|------------|----------------|----------------|-------|----|-----------|----------|------|-------|
| ICG 12991 | 0.458    |          |        |            |            |            |                |                |       |    |          |          |      |       |
| 55–437   | 0.508    | 0.407    |        |            |            |            |                |                |       |    |          |          |      |       |
| ICGV 86124 | 0.582    | 0.506    | 0.407  |            |            |            |                |                |       |    |          |          |      |       |
| ICGV-SM 90704 | 0.479    | 0.468    | 0.546  | 0.547     |            |            |                |                |       |    |          |          |      |       |
| ICGV 94114 | 0.538    | 0.458    | 0.441  | 0.496     | 0.542      |            |                |                |       |    |          |          |      |       |
| ICGV-SM 95342 | 0.507    | 0.572    | 0.552  | 0.520     | 0.550      | 0.544      |                |                |       |    |          |          |      |       |
| ICGV-SM 95714 | 0.567    | 0.532    | 0.491  | 0.514     | 0.519      | 0.543      | 0.548          |                |       |    |          |          |      |       |
| ICGV-SM 99557 | 0.532    | 0.452    | 0.422  | 0.488     | 0.499      | 0.427      | 0.549          | 0.499          |       |    |          |          |      |       |
| 47–10    | 0.607    | 0.504    | 0.383  | 0.468     | 0.501      | 0.509      | 0.571          | 0.566          | 0.511 |    |          |          |      |       |
| CG7      | 0.513    | 0.479    | 0.579  | 0.551     | 0.404      | 0.499      | 0.537          | 0.522          | 0.446 | 0.611 |          |          |      |       |
| Chalimbana | 0.409    | 0.483    | 0.577  | 0.594     | 0.400      | 0.566      | 0.534          | 0.512          | 0.527 | 0.662 | 0.439   |          |      |       |
| FLEUR–II | 0.570    | 0.526    | 0.394  | 0.454     | 0.536      | 0.522      | 0.560          | 0.503          | 0.487 | 0.493 | 0.593 | 0.543 |      |       |
| JL24     | 0.597    | 0.532    | 0.419  | 0.412     | 0.503      | 0.542      | 0.567          | 0.567          | 0.525 | 0.419 | 0.615 | 0.580 | 0.425 |       |
| MGV 5    | 0.471    | 0.485    | 0.547  | 0.567     | 0.534      | 0.549      | 0.535          | 0.523          | 0.518 | 0.651 | 0.438 | 0.310 | 0.533 | 0.589 |
| PENDO    | 0.532    | 0.471    | 0.475  | 0.419     | 0.540      | 0.509      | 0.594          | 0.528          | 0.452 | 0.526 | 0.511 | 0.517 | 0.447 | 0.370 | 0.563 |

Fig. 2. Neighbor-joining tree illustrating the sub-clusters representing the 16 Arachis genotype, represented according to its predominant characteristic of disease resistance (green), yield and quality (pink) and farmer preferred traits (blue).
2. Materials and methods

2.1. DNA extraction

A total of 799 SSRs (supplementary data), comprising of di-and tri-nucleotide motifs from both genomic and expressed sequence tag (EST) SSRs, as compiled by Zhao et al. [32], were screened across 16 cultivated groundnut varieties indigenous to Africa. These varieties are listed in Table 1 and varied in yield and quality traits and tolerance to biotic stresses such as rust resistance (ICGV-SM 95342 and ICGV 94114), aphid resistance of GRD (ICG 12991) and virus resistance of GRD (ICGV-SM 90704), ELS resistance (ICGV-SM 95714 and ICG 7878), aflatoxin tolerance (53–47) high yield and quality traits (Fleur II, CG7/MGV4, MGV5 and Chalimbana), and other farmer preferred varieties (FPVs) (ICGV-SM 90704, Pendo, ICGV 86124, 47 II, CG7/MGV4, MGV5 and Chalimbana), and other farmer preferred varieties (FPVs) (ICGV-SM 90704, Pendo, ICGV 86124, 47 II, CG7/MGV4, MGV5 and Chalimbana).

Genomic DNA was extracted from 14-day old seedlings with one leaf from three individual plants combined into a single sample for each genotype. The genomic DNA was extracted according to the CTAB method of Mace et al. [33] with the exclusion of the phenol-chloroform extraction step.

2.2. SSR analysis

DNA from each variety were analyzed by PCR at the 799 selected SSR loci [32]. All forward primers contained an M13-tag (S) and each reaction comprised of 1× PCR Buffer (20 mM Tris–HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% Triton X-100; 50% glycerol), 2 mM MgCl2, 0.16 mM dNTPs, 0.04 μμM forward primer, 0.2 μμM reverse primer, 0.16 μμM fluorescent labeled M-13 tagged forward primer (FAM, VIC, NED PET), 0.2 U Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30 ng DNA. PCR conditions were 94°C for 5 min, 35 cycles of 0.5 min at 94°C, 1 min at 59°C and 2 min 72°C and final extension at 72°C for 20 min using a GeneAmp® 9700 (Applied Biosystems). Amplification was confirmed by electrophoresis of PCR products (4 μL) on a 2% agarose gel against a 3500 Genetic Analyzer of successful PCR products. These (1.5–3.5 μL each) were co-loaded in sets of 4 markers together with the internal size standard, GeneScan™-500 LIZ® (Applied Biosystems). Gene Mapper Software (Version 4.0, Applied Biosystems) was used for allele scoring, followed by data analysis using PowerMarker Version 3.25 [35]. A dissimilarity matrix was compiled with DARwin software v5 [36].

3. Results and discussion

3.1. SSR marker properties and performance

A total of 799 markers (Supplementary data) were screened to identify the most informative markers for QTL mapping and pre/post-breeding applications.

Marker allele profiles obtained after capillary electrophoresis using GeneMapper 4.0, were graded on a scale of 1 to 4 for ease of scoring as illustrated in Fig. 1 (1 = clear single peaks, 2 = clear peaks with multiple stutter peaks, 3 = peaks not well defined but could be scored and, 4 = difficult to score due to noise, multiple loci binding or low availability). For grades 1, 2 and 3 the numbers of polymorphic markers obtained were 182, 61 and 133, respectively. In total, 423 markers were excluded from the final data set. These included 93 that were scored as grade 4, 169 that failed to amplify PCR products in the majority of the 16 varieties (i.e. availability <0.38) and 161 monomorphic markers. This screening provided 376 high quality polymorphic markers that worked well (average success rate of 94.2%) across the 16 varieties.

PowerMarker results were compiled for allele number, major allele frequency, how well each marker worked (availability), heterozygosity and PIC (Supplementary data).

Markers that were highly heterozygous confounded data interpretation and were carefully considered to determine if they had amplified two loci and if so, were split into two sets of alleles denoted with (_1/2) to the marker name. If both sets of alleles were heterozygous and polymorphic, these markers were retained. If one set of alleles was homoygous, this allele was discarded. Markers that would have resulted in two homozygous loci were not split. The total number of retained split markers was 18 and thus resulted in 394 polymorphic loci from a total of 376 markers.

The PIC range observed (0.06 for Ah-671 to 0.86 for Ah1TC4F12) in this study was similar to that reported by Pandey et al. [37] (PIC range of 0.10 to 0.89). The mean PIC value obtained in the current study was

| LG | Markers |
|----|---------|
| a04 (LG9) | GM1062 Ap40 GM890 GM2246 TC11B04 GM1720 IPAHM105 GM2589 GM1919 GM1311 |
| a09 (LG18) | GM2450 GM849 GM2359 GM1291 GM1911 PM675 AHC50695 IPAHM868 AHC50993 |
| a06 (LG5,10) | IPAHM659 GM1489 GM1490 GM2337 IPAHM245 TC11A04 GM1573 IPAHM689 AHC50916 AHC50706 |
| a03 (LG7) | GM1717 GM2402 GM2215 GM2528 GM2206 GM1954 Ah1TC0A01 pPGSeq19C7 AHC50132 AHC50382 |
| a05 (LG19) | GM1049 GA34 GM1577 GM2078 R16F05 GM1702 pPGSeq10D4 GM2557 pPGSeq0D5 AHC50990 |
| b07 (LG2) | GM1953 GM2156 GM2067 GM2073 GA24 GM2557 pPGSeq0D5 AHC50990 |
| a07 (LG4) | GM1494 GM1937 GM1076 GM1880 GM1986 GM1922 GM1990 |
| a08 (LG12) | GM2289 GM1628 GM2089 Ah1TC3804 Ah2TC7A02 GM1713 GM2571 |
| b03 (LG14) | GM1854 GM1618 GM1996 GM2388 GM2009 Ah1TC3812 GM2574 |
| b05 (LG21) | GM2137 GM1555 IPAHM136 GM1843 Ah1TC5001 AHC50702 |
| b01 (LG6) | GM1501 GM1331 Ah3 GM2607 pPGSeq13A7 AHC50138 |
| b10 (LG5) | TC10E05 GM1742 GM2165 GM2032 Ah1TC1002 Ah1TC1A02 |
| a10 (LG1) | GM2531 GM1788 GM2411 GA161 GM799 |
| b02 (LG16) | GM2196 Ah26 GA161 Ah1TC4F12 |
| b04 (LG13) | GM2584 GM1445 GM2033 AHC50230 |
| b08 (LG4) | GM1961 IPAHM123 IPAHM606 GM1798 |
| (LG3) | CM2063 AHC50369 AHC50798 AHC50278 |
| (LG17) | CM1821 pPGSeq2F5 GM1985 |
| (LG20) | AHC50147 Ah1TC9H08 AHC50151 |
| (LG11) | AHC50357 pPGSeq1B9 GM1598 |
| b09 | GM1483 Le1 |
| (LG15) | CA166 RI1F06 Ah1TC4F12 |

Table 3
Polyomorphs SSRs loci identified in this study that were previously mapped to Arachis linkage groups (LG) (Gautami et al. [45], Wang et al. [30]).
Table 3 (continued)

| Marker          | Value   |
|-----------------|---------|
| IPAHM108 Ah-671 | GM2313  |
|                 | AHGS0347|
|                 | AHGS0134|
|                 | pPGSeq18C5|
|                 | GM2480  |
|                 | Ah1TC5A07|
|                 | Ah2TC7G10|

0.49, with values above 0.5 observed in 174 (44%) of the loci analyzed, which was high compared to the findings of Cuc et al. [39], where only 15.7% of SSR markers showed PIC values > 0.5. A study by He et al. [19] gave a lower percentage (34%) of polymorphic markers than that shown in our study. The number of polymorphic markers identified in this study (376 or 47% of the total number screened) was also high compared to other studies in groundnuts, which ranged from 3 to 33% of the markers analyzed [19,20,38]. However, the values were comparable to those reported by Cuc et al. [39] (44% with mean PIC 0.46; PIC range 0.12 to 0.75) and Mace et al. [40] (PIC range 0.29 to 0.60; mean PIC 0.47) with variations ascribed to genotype differences. The polymorphic markers identified in this study are therefore highly informative.

Marker GM2009 had a PIC value of 0.67 and has been shown to be closely associated with the major QTL for Late Leaf Spot (LLS) [23]. The genetic similarity of LLS and ELS disease resistance mechanisms [9] further supports the significance of this marker for QTL analysis for ELS resistance. Markers IPAHM108.1/2 and IPAHM123.2 had PIC values of 0.69/0.72 and 0.73 across 5 and 6 alleles, respectively. These were similar to that from a previous study by Cuc et al. [39] in which IPAHM108/123 had PIC values of 0.62/0.75 across 3 and 4 alleles, respectively. Other polymorphic IPAHMx markers varied from those of Cuc et al. [39] in terms of both low (IPAHM659_2/105/136/177) and high polymorphisms (IPAHM689) whilst allele numbers were fairly consistent in comparison. These variations in marker characteristics could be due to the inherent genotypic constitution of the cultivars used but cannot be confirmed as there were no common genotypes between this study and that of Cuc et al. [39]. Other markers that had high PIC values in this study as well as in that of Varshney et al. [41] were Ah1TC1E01, Ah1TC4F12 and Ah1TC6E01 with PIC values of 0.60–0.90. These similarities across different studies further highlight their usefulness in the present study across globally cultivated Arachis spp. The polymorphic markers identified in this study may also be useful across other legume species in comparative genomics studies as was ascertained with polymorphic soybean derived EST-SSRs in the Arachis genome [28]. These markers produced an average of 3.7 alleles per locus, for a total of 1476 alleles. The number of alleles per marker ranged from 2 to 11 with a mean of 3.74. Both higher numbers of alleles ranging from 2 to 14 [2,19] and lower numbers ranging from 2 to 8 [39] have been reported by previous studies. The most polymorphic markers with PIC values > 0.70, reported by Hildebrand et al. [42] had allele values ranging from 5 to 11. The most informative SSR markers in this study were Ah2TC7H11, Ah1TC3E02, Ah1TC4F12, GNB70, Ah2TC11H06, AHGS0798, pPGSeq385, Ah2TC9H09, Lec1, Ah1TC2G05, AHGS0965, GA161, TC04G02, Ah1TC3B04, TC11A04, TC3E05, TC05A06 and GNB18 and had allele numbers ranging from 8 to 11 and PIC values of 0.78 to 0.86 and were considered important to distinguish all the varieties for use in MAS and other diversity studies.

Major Allele Frequency (MAF) ranged from 0.18 to 0.97 with a mean of 0.58 and heterozygosity ranged from 0 to 0.38 with a mean of 0.20. Markers with MAF between 0.5 and 0.8 (181 polymorphic markers in this study) have been reported to contribute approximately equally to information in linkage disequilibrium studies and should be useful in QTL mapping [43].

3.2. Genetic relationships and marker map locations

3.2.1. Dissimilarity matrix pair wise comparisons across the sixteen Arachis sp. varieties

A dissimilarity matrix was calculated from the allelic data of the 376 polymorphic markers (Table 2) and values ranged from 0.34 for the closest related varieties MGV5 and ICGV-SM 90704 to 0.66 for the most distant varieties Chalimbana and 47–10, with a mean value of 0.51. The dissimilarity values obtained were high in comparison to genetic distance values of previous studies in Arachis sp. [27,44] and ranged from 0.091 to 0.288 and 0.083 to 0.117, respectively. Subsequently, the most appropriate combinations for the development of bi-parental mapping populations for disease tolerance/resistance QTL mapping were identified, selecting the most distantly related varieties with contrasting expression of the trait and dissimilarity values above 0.5. As such, for ELS and LLS QTL mapping, ICG 7878 can be combined with FPVs 47–10, JL 24 and ICGV 86124 (dissimilarity values of 0.607, 0.597, 0.567 respectively). JL24 may be further improved by crossing with other resistant varieties such as ICG 12991 (GRD sp. resistant), ICGV-SM 95714 (ELS resistant) and ICGV-SM 95432 (LLS and rust resistant) and ICGV-SM 95714 (ELS resistant) with dissimilarity values of 0.532, 0.563, 0.567 and 0.567 respectively. The matrix also indicated good varieties to combine in order to pyramid ideal abiotic
and resistance traits. In this regard, ICGV-SM 95714 (ELS resistant) will combine well with rust resistant ICGV 94114 and ICGV-SM 95432 (dissimilarity values 0.543 and 0.548 respectively) and drought tolerant *Aphis* sp. resistant ICGV 12991 with rust resistant ICG 95432 (0.572) and ELS resistant ICGV-SM 95714 (0.532) varieties. Other varieties may also be considered for pair wise introgression of disease resistance, such as rust resistant genotype ICGV-SM 95432 with *A. flava* resistant 55–437 or ICGV 12991 and ICGV-SM 90704 for GRD resistance.

Sixty-three percent of the dissimilarity values calculated ranged from 0.50–0.66 and resulted from 237 polymorphic markers that could differentiate all varieties for the various traits of yield, quality and disease resistance. Nineteen percent of these values were associated with recommended crosses for introgression of ELS resistance. The high number of markers used in this study therefore enhanced the potential for targeted introgression of multiple disease resistance, yield and quality traits into farmer preferred and commercial groundnut varieties.

3.2.2. Genetic tree analysis

A neighbor-joining tree, illustrating the relatedness among the varieties, is presented in Fig. 2. The 16 varieties were grouped into three large clusters and a single outlier, ICGV-SM 95714. The majority of FPVs (47–10, ICGV 86124, JL 24 and Pendo) were grouped together in cluster 1 with ICGV 86124, 47–10, JL 24 and Pendo forming a more closely related sub-group (sub-cluster 1a). This may be attributed to low levels of out crossing [13,14,15]. Seed exchange among small holder farmers, planting proximity of preferred varieties, farmer preference for specific varieties and collection of seed for this study from a common geographic location may also have influenced the overall composition and relatedness of the varieties over the years. ELS resistant varieties ICG 7878 and ICGV-SM 95714 were noticeably distant from the majority of the varieties and hence more useful for trait QTL mapping and introgression into the other 14 varieties. ICGV-SM 95714 showed the lowest score for PCR performance across the varieties (90.5%), which may have contributed to its independent clustering.

3.3. Marker map distribution

A total of 139 (37%) of the 376 markers that were found to be polymorphic in this study have been previously mapped [30,45] (Table 3) and the number of markers per linkage groups (LG) and chromosomes (aa and bb) ranged from 0 for LG b06 to 18 for LG9 of chromosome a04. On average, the mapped markers were distributed evenly across all LGs with the exception of LG b06 of chromosome bb. The markers polymorphic in this study have been previously mapped [30,45] and quality trait QTLs and their locations on the genome. The 139 is evenly across all LGs with the exception of LG b06 of chromosome bb. On average, the mapped markers were distributed (aa and bb) ranged from 0 for LG b06 to 18 for LG9 of chromosomes (aa and bb) ranged from 0 for LG b06 to 18 for LG9 of chromosome a04. Sixty-three percent of the dissimilarity values calculated ranged from 0.50–0.66 and resulted from 237 polymorphic markers that could differentiate all varieties for the various traits of yield, quality and disease resistance. Nineteen percent of these values were associated with recommended crosses for introgression of ELS resistance. The high number of markers used in this study therefore enhanced the potential for targeted introgression of multiple disease resistance, yield and quality traits into farmer preferred and commercial groundnut varieties. In this regard, ICGV-SM 95714 (ELS resistant) will combine well with rust resistant ICGV 94114 and ICGV-SM 95432 (dissimilarity values 0.543 and 0.548 respectively) and drought tolerant *Aphis* sp. resistant ICGV 12991 with rust resistant ICG 95432 (0.572) and ELS resistant ICGV-SM 95714 (0.532) varieties. Other varieties may also be considered for pair wise introgression of disease resistance, such as rust resistant genotype ICGV-SM 95432 with *A. flava* resistant 55–437 or ICGV 12991 and ICGV-SM 90704 for GRD resistance.

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