Assessment of genetic diversity of *Musa* species accessions with variable genomes using ISSR and SCoT markers

David Okeh Igwe · Onyinye Constance Ihearahu · Anne Adhiambo Osano · George Acquaah · George Nkem Ude

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**Abstract** Assessing the effectiveness of different molecular markers is essential for identification of appropriate ones for crop improvement and conservation, hence, inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) markers were used for this study. Sixty-six accessions with different genomes obtained from International Transit Center, Belgium, were used for DNA extraction, amplification with ISSR and SCoT markers and agarose gel electrophoresis. The reproducible bands were scored for analyses. We identified high allelic richness of 299 (ISSR) and 326 (SCoT). Polymorphic information contents (ISSR: 0.9225; SCoT: 0.9421) were high but SCoT exhibited higher level of informativeness. The two markers demonstrated high percentage polymorphic loci (ISSR: 91.21–100%; SCoT: 96.97–100%). Other genetic indicators including effective number of alleles, Nei’s genetic diversity, and Shannon information index were higher in SCoT and further elucidated the usefulness of the markers. Intraspecific genetic diversity, interspecific genetic diversity, coefficient of gene differentiation and level of gene flow revealed extensive gene flow and larger variability within the accessions. Both ISSR and SCoT grouped the accessions via dendrogram, biplot and structure analyses. Though the two marker systems varied in their informativeness, they demonstrated high effectiveness in resolving genetic diversity (GD) of the different accessions, with higher efficiency in SCoT markers. Due to higher GD indices exhibited by SCoT, AS is the most genetically endowed one. Our study showed that SCoT markers are more informative than ISSR for GD exploration, assessment and cluster resolution of *Musa* species, thereby revealing the potential of SCoT markers for improved breeding and conservation.

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D. O. Igwe · O. C. Ihearahu · A. A. Osano · G. Acquaah · G. N. Ude (✉)
Department of Natural Sciences, Bowie State University, 14000 Jericho Park Road, Bowie, MD 20715, USA
e-mail: gude@bowiestate.edu

D. O. Igwe
e-mail: digwe@bowiestate.edu

O. C. Ihearahu
e-mail: oihearahu@bowiestate.edu

A. A. Osano
e-mail: aosano@bowiestate.edu

G. Acquaah
e-mail: gacquaah@bowiestate.edu

D. O. Igwe
Department of Biotechnology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

D. O. Igwe
Plant Pathology and Plant Microbe Biology, School of Integrated Plant Sciences, Cornell University, Ithaca, NY 14853, USA
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Introduction

Bananas and plantains (Musa spp.), perennial crops, are the favorite fruit crops of the world. They are globally distributed in more than 120 countries where production is estimated at 116 million tonnes (FAOStat 2014, 2019; Chab et al. 2018). Musa spp., originating from South-East/West Oceania zone, possess edible ones including either Musa acuminata Colla (genome A), or a mixture of A and B called M. balbisiana Colla (genome B), or A and S (M. schizcarpa N. W. Simmonds), or A and T (M. textilis Nee) (Perrier et al. 2011; Christelova et al. 2017; Sardos et al. 2018). Bananas and plantains are the highest export fruit crops (Kumar et al. 2014a, b) and rated fourth most important in sub-Saharan Africa (SSA) after cassava, maize and yam (FAOStat 2014). They are good sources of carbohydrates, vitamin C, potassium and sodium (IBA 2007). Many genomic constitutions exist based on their genomic origins with respect to A and B genomes (Simmonds 1966). Different genotypes were derived from M. acuminata (AA) and M. balbisiana (BB) and classified into different genomic constitutions including diploids (AA, AB and BB), triploids (AAA, AAB and ABB) and tetraploids (AAAA, AAAB, AABB and ABBB) (INIBAP 2008; Pollefeys et al. 2004). The East African bananas, mainly desert ones, (AA, AAB, AAA, ABB and AB), the African plantain bananas (AAB) grown mainly in Central, and the West African and the East African Highland Bananas (EAHB: AAA), among others are used for cooking and beer brewing (Karamura et al. 1998). Other subgroups including ‘Mutika’ (formerly known as ‘Mutika-Lujugira’ and currently a well-known EAHB of AAA) and ‘Mchare’ (AA) are grown as major crops in East African countries (Perrier et al. 2011). Six of these genomes (AA, AAA, AB, AAB, ABB and ABBB) are classified as naturally occurring ones based on taxonomic features and ploidy constitutions (Simmonds and Shepherd 1995).

However, these vital crops have become vulnerable to both abiotic and biotic factors depending on their genomic constitutions. Interestingly, it has been reported that genotypes with “B” genome (in particular ABB type) are more tolerant to abiotic and biotic stresses than those solely possessing “A” genome (Tripathi et al. 2007; Vanhove et al. 2012; Davey 2013; Hu et al. 2015). The world population is fast growing, and it is expected to attain over 9 billion by the year 2050 (Ehrlicha and Harte 2015; FAO 2017). Feeding this overwhelming population level is generating much pressure on agricultural crop production (Kastner et al. 2012; Dempewolf et al. 2014; Khoury et al. 2014). To increase productivity of Musa species, it is essential to investigate the degree of relatedness of different genomes and their GD strength for increased tolerance/resistance to different stressors and to meet present and future challenges.

Harnessing genetic diversities and relationships can reveal accessions capable of withstanding the changing environmental factors. This is because crops with narrower range of GD may fail to survive under climatic extremes. Breeders need Musa species with high GD endowment for resistance to abiotic and biotic stressors, but the accurate identification of such unique ones cannot easily be achieved through conventional breeding, due to the complicated genetic system of Musa species. It is possible by complementing it with informative and functional gene-based molecular markers that are not influenced by changes in climatic factors (Martínez et al. 2006). Different molecular markers (with emphasis on gel-based molecular marker techniques) including random amplified polymorphic DNA (RAPD) marker (Bhat et al. 1995; Ude et al. 2003a, b; Lamare and Rao 2015), restriction fragment length polymorphism (RFLP) (Bhat et al. 1995), simple sequence repeat (SSR) (Perrier et al. 2011; Hippolyte et al. 2012; Christelova et al. 2017), sequence-related amplified polymorphism (SRAP) (Youssef et al. 2011), inter-simple sequence repeats (ISSR) (Godwin et al. 1997; Lamare and Rao 2015), directed amplified minisatellite DNA (DAMD) (Lamare and Rao 2015) and amplified fragment length polymorphism (AFLP) (Ude et al. 2003a, b; Bhat et al. 2004) have been used in Musa species. Considering the “indirect random markers” such as RAPDs, ISSRs, or SSRs (Andersen and Lübberstedt 2003) only without regards to other gene-based markers, limits the expected progress in germplasm improvement, utilization and conservation. Informative molecular markers can effectively exploit GD and...
relatedness inherent in *Musa* species and their wild relative species for genetic improvement as earlier reported in other crops (Hajjar and Hodgkin 2007; Ford-Lloyd et al. 2011). In addition, conserving GD of domesticated and wild crop relative species via the use of functional gene-based markers can provide a strong basis for the sustainable use of these species. Knowledge of the GD within different accessions of *Musa* species possessing diverse genomic constitutions can facilitate the efficiency of banana and plantain breeding programs. Comparative utilization of ISSR and Start codon targeted (SCoT) markers has become very important to identify more informative and better resolving markers for *Musa* species. Inter-simple sequence repeat markers which are arbitrary in nature target variable genomic loci for amplification of DNA segments present between two similar microsatellite regions that are opposite with each other (Zietkiewicz et al. 1994), while SCoT markers were developed from the conserved regions of the genomes across different plant species and they possess longer primer lengths and high annealing temperatures (Collard and Mackill 2009). Although, ISSR and SCoT markers have been found useful in various crops for GD studies because of their high reproducibility and efficiency (Godwin et al. 1997; Galvan et al. 2003; Cao et al. 2006; Collard and Mackill 2009; Guo et al. 2012; Hamidi et al. 2014; Etminan et al. 2016; Igwe et al. 2017), SCoT is yet to be utilized in bananas and plantains. To the best of our knowledge, comparative assessment of effectiveness of ISSR and SCoT markers has not been utilized in bananas and plantains with different ploidy or genomic constitutions to harness their usefulness in assessing GD, cluster resolution and population structure. Therefore, the objectives of this study were to: (1) study the GD and cluster resolution among the 66 accessions of *Musa* species using ISSR and SCoT markers; (2) compare the effectiveness of ISSR and SCoT markers in assessing GD and population structure of 66 accessions of *Musa* species with different genomes.

**Materials and methods**

Sample collection and DNA extraction

Sixty six (66) accessions of bananas and plantains from different genomes, namely, AA, AAA, AAAAA, AAB, BB, AB, ABB, AAAB and AS, as well as other three diploid accessions (*Musa beccarii* N. W. Simmonds, *M. coccinea* Andrews, and *M. textilis*) were obtained from the International Transit Center (ITC), Kasteelpark Arenberg 13 bus 2455, 3001 Leuven, Belgium (Supplementary file 1 Table S1). Thirty-two (32) out of 66 were obtained as tissue cultured plantlet materials, each in five replicates, and were grown and maintained at the screenhouse of the Department of Natural Sciences, Bowie State University, while the remaining 34 were obtained in lyophilized form. Approximately 100 mg and 120 mg were, respectively weighed from young fresh and lyophilized leaves of *Musa* species for DNA extraction using Cetyltrimethylammonium bromide (CTAB) method (Abarshi et al. 2010) with a little modification by using ratio of 241:1 of chloroform and isoamyl alcohol, respectively without phenol.

Polymerase chain reaction and agarose gel electrophoresis

Polymerase chain reaction (PCR) amplification was performed in volume of 25 μL which consisted of 2.0 μL 100 ng DNA, 5.0 μL of 5 × Green GoTaq Buffer (Promega Corporation, Madison, USA), 2.0 μL of 2.5 mM dNTPs (Bioline, Massachusetts, USA), and 0.2 μL GoTaq DNA polymerase (5 U/μL) (Promega Corporation, Madison, USA), 1.0 μL of 10 μM each of ISSR or SCoT primer and 14.80 μL of 500 mL diethyl pyrocarbonate (DEPC)-treated water (Invitrogen, Carlsbad, CA, USA). The list of ISSR and SCoT primers, their sequences, Guanine-Cytosine (GC) content, annealing temperatures and sources were presented (Table 1). The PCR amplification profile was run for 35 cycles, each consisting of a denaturation step at 94 °C for 5 min, followed by annealing at 35–40 °C for 35 s, an extension step at 72 °C for 1 min, and a 10 min final extension at 72 °C using a Bio-Rad T100 Thermal cycler (Bio-Rad Laboratories Inc. Singapore). Ten (10) μL of the PCR reaction products were resolved with 1.5% agarose gel containing 0.5 mg/mL ethidium bromide and photographed using Aplegen Omega Lum G gel documentation system (Minnesota 55303, USA).
Data analyses

Data matrices of ISSR and SCoT marker profiles were generated by scoring (1) for presence and (0) for absence of individual allele. The generated data matrices were used for computing GD, allele frequency and polymorphic information content (PIC) using PowerMarker (Version 3.25). Analyses of number of polymorphic loci (NPL), percentage polymorphic loci (PPL), effective number of alleles (Ne), Nei’s gene diversity, Shannon’s information index (I), and population structure (total gene diversity or intraspecific GD, Ht; gene diversity within accessions or interspecific GD, Hs; coefficient of gene differentiation, G_{ST}; and level of gene flow, Nm) of the accessions were analyzed using POPGENE software version 1.32 (Yeh and Boyle 1997). Brown’s analysis for multilocus associations among and within populations was also performed with the POPGENE program. The multilocus association variances were

Table 1  List of primers, their sequences, percentage guanine-cytosine (GC) contents and annealing temperatures (Tms)

| Primer name | Sequence | GC | Tm | Source |
|-------------|----------|----|----|--------|
| ISSR        |          |    |    |        |
| ISSR825     | ACACACACACACT | 46.7 | 47.3 | Lamare and Rao (2015) |
| UBC835      | AGAGAGAGAGAGAGYCYC | 52.8 | 50.2 | Lamare and Rao (2015) |
| UBC814      | CTCTCTCTCTCTCTCAT | 47.1 | 45.7 | Lamare and Rao (2015) |
| UBC826      | ACACACACACACACC | 52.9 | 52.8 | Lamare and Rao (2015) |
| UBC827      | ACACACACACACACAG | 52.9 | 53  | Lamare and Rao (2015) |
| UBC840      | GAGAGAGAGAGAGAGATT | 44.4 | 46.3 | Lamare and Rao (2015) |
| UBC840B     | GRAGAGAGAGAGAGATTT | 44.1 | 44.8 | This study |
| UBC808      | AGAGAGAGAGAGAGAGGC | 52.9 | 48.8 | Lamare and Rao (2015) |
| UBC811      | GAGAGAGAGAGAGAGAGGC | 56.3 | 47  | Lamare and Rao (2015) |
| UBC868      | GAGAGAGAGAGAGAGAGAAGAA | 33.3 | 43.2 | Lamare and Rao (2015) |
| UBC901      | ACACACACACACACACARY | 50 | 52.5 | Lamare and Rao (2015) |
| UBC834      | AGA GAG AGA GAG AGY T | 46.9 | 45.6 | Lamare and Rao (2015) |
| UBC812      | GAGAGAGAGAGAGAGAGAA | 47.1 | 45.7 | Lamare and Rao (2015) |
| SCoT        |          |    |    |        |
| SCoT2       | CAACAATGGCTACACCACC | 55.6 | 53.6 | Collard and Mackill (2009) |
| SCoT2Musa   | CAACAATGGSTACCCACC | 52.9 | 50.6 | This study |
| SCoT28      | CCATGCGTACACCGCCA | 61.1 | 57.7 | Collard and Mackill (2009) |
| SCoT16B     | ACCATGGCTACACGTGCA | 55.6 | 54.4 | This study |
| SCoT16BmusA | ACTATGTCTATGTGCAAC | 38.9 | 45.3 | This study |
| SCoT16BmusA | ACTATGTCTATGTGTMAC | 38.2 | 42.8 | This study |
| SCoT36B     | GCAACATGGCTACTACACC | 50 | 50.7 | This study |
| SCoT36BmusA | GCAACATGGCTAYACC | 50 | 49.6 | This study |
| SCoT20B     | ACCATGGYACCACCGCG | 67.6 | 60  | This study |
| SCoT20BmusA | TAAACATGGCTACCMCG | 55.9 | 52.8 | This study |
| SCoT13      | ACCATGGCTACACCGGAC | 61.1 | 58  | Collard and Mackill (2009) |
| SCoT16      | ACCATGGCTACACCGGAC | 61.1 | 57.3 | Collard and Mackill (2009) |
| SCoT20      | ACCATGGCTACACCGGAC | 66.7 | 60.8 | Collard and Mackill (2009) |
| SCoT22      | ACCATGGCTACACCGGAC | 55.6 | 55  | Collard and Mackill (2009) |
| SCoT24      | ACCATGGCTACACCGGAC | 55.6 | 54.8 | Collard and Mackill (2009) |
| SCoT28      | CCAATGGCTACACCGC | 66.7 | 60.7 | Collard and Mackill (2009) |
| SCoT33      | CCAATGGCTACACCGC | 66.7 | 58.9 | Collard and Mackill (2009) |
| SCoT35      | CAACTGGCTACACCGGCCC | 72.2 | 61.7 | Collard and Mackill (2009) |
| SCoT36      | GCAACATGGCTACCCACC | 55.6 | 54.2 | Collard and Mackill (2009) |

GC guanine and cytosine, Tm melting temperature
partitioned into single-locus and two-locus components (Brown and Feldman 1981). Dendrogram reconstruction using Unweighted Pair Group Method with Arithmetic Mean (UPGMA Pakseresht et al. 2013), SAHN clustering and dissimilarity index in Jaccard’s option was conducted using NTSYSpc software version 2.02 (Rohlf 2000). Principal component analysis (PCA) and population structure of ISSR and SCOT datasets were computed as Discriminant analysis of principal component (DAPC) using Bayesian information criterion (BIC) as described by Jombart (2008), Jombart et al. (2010), Jombart and Collins (2015) in the Adegenet package v.2.1.2 in R (R Development Core Team 2015). Biplot function in R (R Development Core Team 2015) was used to plot the first two PCAs with individual points coloured and shaped according to cluster membership identified through DAPC.

**Results**

Allelic variation, gene diversity and polymorphic information content obtained using inter-simple sequence repeat and start codon targeted markers

Nine out of the 13 primers of ISSR tested were replicated and found reproducible and scorable, while eight out of 19 were selected from SCOT as in some of the representatives of the gel images (Fig. 1a, supplementary file 2 Figs. S1a and S1b; Fig. 1b, supplementary file 2 Figs. S1c and S1d). Each of the representatives of the gel images had unique banding patterns across some of the accessions. For the ISSR, the reproducible ones were UBC808, UBC825, UBC827, UBC835, UBC840, UBC901, UBC811, UBC812, and UBC840B, while SCOT consisted of SCOT16, SCOT2, SCOT20BM, SCOT33, SCOT36, SCOT20, SCOT22, and SCOT26. A total of 299 numbers of alleles were generated from the reproducible ISSR markers, while SCOT yielded 326 (Table 2). The major allele frequency in ISSR was 0.1424, while SCOT had 0.1717. The gene diversities obtained with ISSR and SCOT were 0.9282 and 0.9432, respectively. Polymorphic information content values of 0.9225 (ISSR) and 0.9421 (SCOT) were obtained. The ISSR primers UBC808, UBC825, UBC827, UBC835, UBC840, UBC901, UBC811 and UBC812 were polymorphic, while UBC840B was monomorphic. The PIC values from the ISSR primers were ranked in a descending order as UBC808 > UBC811 > UBC827 > UBC825 > UBC835 > UBC901 > UBC840 > UBC812 > UBC840B. The identified polymorphic SCOT primers were SCOT16, SCOT2, SCOT20BM, SCOT33, SCOT36, SCOT20 and SCOT22, while SCOT26 was monomorphic. The PIC values from the SCOT were SCOT20BM > SCOT2 > SCOT20 > SCOT36 > SCOT33 > SCOT16 > SCOT22 > SCOT26. Allelic scores, counts and frequencies obtained using ISSR and SCOT marker systems were informative with variable allelic count ranges (ISSR: 1–21; SCOT: 1–24) and frequencies (ISSR: 0.0152–0.3182; SCOT: 0.0152–0.3636) (supplementary file 3 Table S2; supplementary file 4 Table S3).

Genetic diversity within inter-simple sequence repeat and start codon targeted markers

The identified NPL and PPL from the nine reproducible set of primers of ISSR markers were 47–66 and 91.21–100, respectively, while the eight reproducible set of primers of SCOT markers yielded 65–66 (NPL) and 96.97–100 (PPL) (Table 3). Based on the GD richness, 8 of the ISSR primers exhibited 91.21–100% polymorphisms, while seven of the SCOT primers showed 96.97–100% polymorphism. Within the nine primers of ISSR markers, Ne, H and I values and their standard deviations ranged from 1.3590 ± 0.2986–1.7802 ± 0.2219, 0.2269 ± 0.1739–0.4272 ± 0.0925 and 0.3491 ± 0.2531–0.6132 ± 0.1149, respectively, while the Ne, H and I values and their standard deviations from SCOT markers were 1.4447 ± 0.1775–1.8249 ± 0.2231, 0.2848 ± 0.1209–0.4411 ± 0.0917 and 0.4505 ± 0.1504–0.6288 ± 0.1064, respectively.

Genetic diversity of different genomic constitutions of bananas and plantains as revealed by Inter-simple sequence repeat and Start codon targeted markers

Within the *Musa* species of diverse genomic constitutions assessed with ISSR and SCOT marker systems, variable GD indices were identified in both ISSR and SCOT (Table 4). Assessing the genomes of AA, AAA, AAAA, AAAB, AAB, BB, and AB, ISSR produced genetic parameters that were different from those obtained with SCOT markers. For the wild and other
diploid (M. textilis, M. coccinea, and M. beccarii) accessions studied with ISSR, different GDs (Ne = 1.2941, H = 0.2273, I = 0.3878) were detected in relation to those from SCoT (Ne = 1.8312, H = 0.4539, I = 0.6463).

The GD endowment in an ABB genome was found to be the highest, with the values of Ne, H and I as 1.6310, 0.3801 and 0.5657, respectively, using ISSR, while SCoT identified AS to be the highest (Ne = 1.8312, Ne = 0.44539, I = 0.6463). On the other hand, the GD in the AS accession was lowest (Ne = 1.2941, H = 0.2273, I = 0.3878) with ISSR, while SCoT determined textilis as the lowest judging from the values of GD (Ne = 1.4382, H = 0.3047, I = 0.4826). The GD indicators identified in these variable genomic (ploidy) groups were ranked: ABB > AAB > AAAB > BB > AAA > AA > coccinea > AAAA > textilis > AB > beccarii > AS
from high to low based on polymorphic loci of the selected ISSR, while SCoT ranked them as AS > AAA > coccinea > AAB > AAA > AA > beccarii > ABB > AB > BB > AAAB > textilis from high to low. From the ISSR, the overall mean values of Ne, H and I and their respective standard deviations across the diverse genomic constitutions were 1.5474 ± 0.1509, 0.3476 ± 0.0649 and 0.5296 ± 0.07460, respectively, while SCoT yielded 1.6440 ± 0.1749 (Ne), 0.3841 ± 0.0737 (H) and 0.5694 ± 0.0858 (I).

The assessment of genetic variations within and among the different accessions revealed that the values of Ht, Hs, G ST and Nm were distinctly different based on the two markers (Table 5). For the ISSR, there were different values of Ht (0.2273–0.3837), Hs (0.2076–0.3516), G ST (0.0537–0.1180) and Nm (5.2826–12.4521) across the entire accessions relative to those (Ht = 0.3047–0.4539; Hs = 0.0323–0.4222; GST=0.0630–0.1699; and Nm = 2.4421–14.7687) that were generated with SCoT. With ISSR, the overall mean values of Ht, Hs, GST and Nm were 0.3476 ± 0.0042, 0.3229 ± 0.0036, 0.0709 and 0.5694 ± 0.0858 (I).

Table 2 Major allele frequency, number of alleles, gene diversity and polymorphic information content obtained from Musa species using Inter-simple sequence repeat (ISSR) and Start codon targeted (SCoT) markers

| Marker | Major allele frequency | No. of obs | No of allele | Gene diversity | PIC |
|--------|------------------------|------------|-------------|----------------|-----|
| ISSR   |                        |            |             |                |     |
| UBC808 | 0.0455                 | 66         | 57          | 0.9803         | 0.9799 |
| UBC825 | 0.0909                 | 66         | 39          | 0.9596         | 0.9581 |
| UBC827 | 0.0758                 | 66         | 48          | 0.9706         | 0.9698 |
| UBC835 | 0.1364                 | 66         | 32          | 0.95           | 0.9479 |
| UBC840 | 0.2576                 | 66         | 19          | 0.8747         | 0.8643 |
| UBC840B| 0.3182                 | 66         | 14          | 0.7961         | 0.7698 |
| UBC901 | 0.1364                 | 66         | 25          | 0.9293         | 0.925  |
| UBC811 | 0.0606                 | 66         | 48          | 0.9715         | 0.9708 |
| UBC812 | 0.2879                 | 66         | 17          | 0.8653         | 0.8549 |
| Mean   | 0.1424                 | 66         | 36.5        | 0.9282         | 0.9225 |
| SCoT   |                        |            |             |                |     |
| SCoT16 | 0.1667                 | 66         | 26          | 0.9449         | 0.9427 |
| SCoT2  | 0.0606                 | 66         | 53          | 0.9829         | 0.9822 |
| SCoT20BM| 0.0455                | 66         | 60          | 0.9789         | 0.9986 |
| SCoT3  | 0.0909                 | 66         | 34          | 0.9541         | 0.9522 |
| SCoT36 | 0.1364                 | 66         | 44          | 0.9605         | 0.9592 |
| SCoT26 | 0.3636                 | 66         | 29          | 0.832          | 0.8214 |
| SCoT20 | 0.1364                 | 66         | 45          | 0.9605         | 0.9593 |
| SCoT22 | 0.3736                 | 66         | 35          | 0.932          | 0.9214 |
| Mean   | 0.1717                 | 66         | 41          | 0.9432         | 0.9421 |

PIC polymorphic information content
6.5531, respectively, while 0.3841 ± 0.0054, 0.3452 ± 0.0044, 0.1013 and 4.4377 were obtained with SCoT. Using ISSR markers, the $G_{ST}$ of 7.09% was recorded as the total genetic divergence among the accessions and 92.91% was found within the accessions, while SCoT identified 10.13% as the total genetic divergence among the accessions, and 89.87%, within the accessions.

### Table 3
Genetic diversity within inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) markers used in accessing genetic diversity of different genomic groups of banana and plantain crops

| Marker | NPL | PPL | Ne      | H        | I        |
|--------|-----|-----|---------|----------|----------|
| ISSR   |     |     |         |          |          |
| UBC808 | 65  | 98.48 | 1.7802 (0.2219) | 0.4272 (0.0925) | 0.6132 (0.1149) |
| UBC825 | 65  | 98.48 | 1.5556 (0.2459) | 0.3393 (0.1164) | 0.5132 (0.1456) |
| UBC827 | 61  | 92.42 | 1.4723 (0.2653) | 0.2980 (0.1315) | 0.4592 (0.1769) |
| UBC835 | 66  | 100  | 1.5379 (0.1691) | 0.3418 (0.0743) | 0.5222 (0.0869) |
| UBC840 | 66  | 100  | 1.4457 (0.1607) | 0.2997 (0.0792) | 0.4726 (0.0899) |
| UBC840B| 65  | 98.48 | 1.3608 (0.1601) | 0.255 (0.0887) | 0.4158 (0.1174) |
| UBC901 | 66  | 100  | 1.5331 (0.2329) | 0.3316 (0.1091) | 0.5063 (0.1318) |
| UBC811 | 66  | 100  | 1.6657 (0.2295) | 0.3869 (0.0952) | 0.5706 (0.1104) |
| UBC812 | 47  | 91.21 | 1.3590 (0.2986) | 0.2269 (0.1739) | 0.3491 (0.2531) |

| SCoT   |     |     |         |          |          |
|--------|-----|-----|---------|----------|----------|
| SCoT16 | 66  | 100  | 1.7301 (0.2445) | 0.4082 (0.1007) | 0.593 (0.1170) |
| SCoT2  | 66  | 100  | 1.8249 (0.2231) | 0.4411 (0.0917) | 0.6288 (0.1064) |
| SCoT20BM| 64  | 96.97 | 1.6380 (0.3119) | 0.3638 (0.1394) | 0.537 (0.1739) |
| SCoT33 | 66  | 100  | 1.5796 (0.2188) | 0.3539 (0.0969) | 0.5335 (0.1150) |
| SCoT36 | 65  | 98.48 | 1.4604 (0.2543) | 0.2848 (0.1209) | 0.4505 (0.1504) |
| SCoT26 | 66  | 100  | 1.4447 (0.1775) | 0.3026 (0.0842) | 0.4756 (0.1009) |
| SCoT20 | 65  | 98.48 | 1.4604 (0.2543) | 0.2948 (0.1209) | 0.4605 (0.1504) |
| SCoT22 | 66  | 100  | 1.4547 (0.1775) | 0.3026 (0.0842) | 0.4756 (0.1009) |

Standard deviations are in parentheses, NPL = number of polymorphic loci, PPL = percentage polymorphic loci, mean Ne = Effective number of alleles, mean H = Nei’s gene diversity, mean I = Shannon’s information index

### Table 4
Genetic diversity indices obtained from 66 accessions of *Musa* species using Inter-simple sequence repeat (ISSR) and Start codon targeted (SCoT) markers

| Genome group | ISSR marker | SCoT marker |
|--------------|-------------|-------------|
|              | Ne          | H           | I         | Ne          | H           | I         |
| AA           | 1.4952      | 0.3276      | 0.5078    | 1.6327      | 0.3809      | 0.5664    |
| AAA          | 1.5380      | 0.3413      | 0.5210    | 1.6984      | 0.3969      | 0.5801    |
| AAAA         | 1.4662      | 0.3169      | 0.4965    | 1.6435      | 0.3902      | 0.5786    |
| AAB          | 1.6297      | 0.3837      | 0.5710    | 1.6707      | 0.3981      | 0.5866    |
| BB           | 1.5390      | 0.3443      | 0.5261    | 1.5697      | 0.3586      | 0.5428    |
| AB           | 1.393       | 0.2821      | 0.4558    | 1.6000      | 0.3750      | 0.5623    |
| ABB          | 1.6310      | 0.3801      | 0.5657    | 1.6033      | 0.3669      | 0.5494    |
| AAAB         | 1.5453      | 0.3529      | 0.5378    | 1.554       | 0.3565      | 0.5419    |
| AS           | 1.2941      | 0.2273      | 0.3878    | 1.8312      | 0.4539      | 0.6463    |
| *textilis*   | 1.4604      | 0.3153      | 0.4949    | 1.4382      | 0.3047      | 0.4826    |
| *coccinea*   | 1.4774      | 0.3231      | 0.5040    | 1.6897      | 0.4082      | 0.5983    |
| *beccarii*   | 1.3596      | 0.2645      | 0.4344    | 1.6228      | 0.3838      | 0.5719    |
| Overall mean | 1.5474      | 0.3476      | 0.5296    | 1.644       | 0.3841      | 0.5694    |
| Overall st. dev | 0.1509 | 0.0649     | 0.0746    | 0.1749      | 0.0737      | 0.0858    |
Dendrogram analysis of different genomic constitutions of *Musa* species as revealed by inter-simple sequence repeat and Start codon targeted markers

A dendrogram analysis generated five major groups at a similarity index of approximately 0.80 using ISSR markers (Fig. 2). Group I consisted of ‘Fougamou 1’, ‘Pisang Palembang’, ‘Improved Lady Finger’ and ‘Silk’, with AAB-genome accessions dominating the group. Group II contained ‘Pisang Rajah’ (South Johnstone) that had AAB genome. In group III, five subclades, SCI, SCII, SCIII, SCIV and SCV were respectively identified. For SCI, accessions such as ‘Obino I’Ewa’, ‘Higa’, ‘Garbon 2’, ‘Plantain n°17’, ‘Calcutta 4’, ‘Cardaba’, ‘Lidi’, ‘Pisang Lilin’, ‘Lai’ and ‘Green Red’ were found with AA/AAA genome as the most abundant ones compared to AAB. ‘Garbon 2’ (AAB), ‘Plantain n°17’ (AAB) got closely clustered. Similar clustering was also observed between ‘Lidi’ (AA) and ‘Pisang Lilin’ (AA); ‘Lai’ (AAA) and ‘Green Red’ (AAA) within SCI. Exceptions were found between ‘Obino I’Ewa’ and ‘Higa’ that had different genome groups but still tightly clustered together and this similar grouping was also detected between ‘Calcutta 4’ and ‘Cardaba’. In SCII, only ‘Truncata’ (AA) was found even though it had the same genome group possessed by some accessions in SCI, while SCIII had ‘Gros Michel’, ‘Chinese Cavendish’, ‘Honduras’, ‘Umalag’, ‘Hsein Jen Chiao’, ‘Dwarf Parfitt’, ‘Mons Mari’ (Pedwell) and ‘Lady Finger Nelson’ that were mostly triploid A (AAA), except ‘Lady Finger Nelson’ (AAB) and ‘Honduras’ (BB).

In SCIV, accessions of different ploidy groups including ‘Pisang Keling’, ‘Figure Pome Geante’, ‘Williams’ (Bell, South Johnstone), ‘Highgate’, ‘Selangor’, ‘Long Tavoy’, ‘Malaccenesis’, ‘J.D. Yangambi’ and ‘Pome’ were identified, while ‘Khae’ (Phrae), ‘Wompa’, ‘Tjau Lagada’, ‘Tomolo’, ‘Pisang Berlin’, ‘No.110’, ‘SH-3436-6’, ‘Borneo’ and ‘FHIA-23’, which were dominated by A genome, were detected in SCV subclade. In group IV that got descended from *M. coccinea* accession, mixed triploids (AAA and AAB) and A diploid were revealed in SCI, while both diploid and tetraploid A genome dominated SCII. Triploid accessions ‘Pisang Lawadin’, ‘Plantain n°3’, ‘Pata’, ‘Poteau Geante’ and ‘Balanakowe’ with mostly AAB genome clustered in SCI. Furthermore, accessions ‘Zebrina-G.F’, ‘Niyarma Yik’, ‘Honduras’, ‘Kunnan’, *M. beccarii* (diploid), ‘Dwarf Cavendish’, ‘Lal Velchi’, *M. textilis* (diploid) and ‘Dole’ were contained in SCII and this sub-group contained AA, BB,AB,ABB and AAA. In group V, accessions of different ploidy groups including ‘Blue Torres Strait Island’, ‘Pelipita’, ‘Pelipita Manjoncho’, ‘Tani’, ‘Pisang Klutuk Wulung’, ‘Pisang Awak’, ‘Khrae Tiparot’, ‘Madang’

| Genome group | ISSR marker | SCoT marker |
|--------------|-------------|-------------|
|              | Ht          | Hs          | G_st        | Nm           | Ht          | Hs          | G_st        | Nm           |
| AA           | 0.3276      | 0.3102      | 0.0538      | 11.9913      | 0.3809      | 0.3478      | 0.0848      | 8.9499       |
| AAA          | 0.3413      | 0.3213      | 0.0618      | 9.9344       | 0.3969      | 0.3529      | 0.1025      | 9.1105       |
| AAAA         | 0.3169      | 0.2943      | 0.0701      | 9.4502       | 0.3902      | 0.3023      | 0.0630      | 8.8145       |
| AAB          | 0.3837      | 0.3516      | 0.1043      | 7.7087       | 0.3981      | 0.3534      | 0.1117      | 7.5796       |
| BB           | 0.3443      | 0.3015      | 0.1180      | 6.1982       | 0.3586      | 0.3065      | 0.1420      | 4.4524       |
| AB           | 0.2821      | 0.2630      | 0.0678      | 6.8705       | 0.3750      | 0.3418      | 0.0884      | 5.1538       |
| ABB          | 0.3801      | 0.3520      | 0.0721      | 8.7957       | 0.3669      | 0.3365      | 0.0743      | 14.7687      |
| AS           | 0.2273      | 0.2076      | 0.0865      | 5.2826       | 0.4539      | 0.4222      | 0.0699      | 6.6533       |
| AABB         | 0.3529      | 0.3260      | 0.0760      | 6.0764       | 0.3565      | 0.2959      | 0.1699      | 2.4421       |
| AAAA         | 0.3153      | 0.2983      | 0.0537      | 8.8182       | 0.3047      | 0.2768      | 0.0916      | 4.96         |
| textilis     | 0.3231      | 0.3106      | 0.0386      | 12.4521      | 0.4082      | 0.3495      | 0.1437      | 2.9783       |
| coccinea     | 0.2645      | 0.2368      | 0.1047      | 4.2778       | 0.3838      | 0.3278      | 0.1458      | 2.9288       |
| beccarii     | 0.3476      | 0.3229      | 0.0709      | 6.5531       | 0.3841      | 0.3452      | 0.101       | 4.4377       |
| Overall mean | 0.0042      | 0.0036      | 0.0054      | 0.0044       |

Ht = total gene diversity, Hs = gene diversity within *Musa* species accessions, G_st = coefficient of gene differentiation and Nm = estimate of gene flow from G_st or Gc's. E.g., Nm = 0.5(1 - GST)/GST.
and FHIA-21 (#68) clustered together but ‘Madang’ and FHIA-21 (#68) were more distantly isolated.

A dendrogram analysis with SCoT markers identified five major groups at similarity index of 0.75 (Fig. 3). For group I, three sub-clades of SCI, SCII and SCIII were detected. In SCI, ‘Fougamou1’, ‘Pelipita Manjoncho’, ‘Plantain n°17’, ‘Pome’, ‘Zebrina G.F’, ‘Wompa’, ‘Williams’ (Bell, South Johnstone), ‘Silk’, ‘Pisang Palembang’ and ‘Figure Pome Geante’ with mixed diploids (AA, AS) and triploids (AAA, ABB, AAB) as their respective genomic groups were revealed. The ‘Silk’, ‘Pisang Palembang’ and ‘Figure Pome Geante’ were more isolated in this group. For SCII, accessions ‘Calcutta 4’, ‘Pisang Lawadin’, ‘FHIA-23’, ‘No.110’, ‘JD-Yangambi’, ‘Plantain n°3’, ‘Lady Finger Nelson’, ‘Gros Michel’, ‘Highgate’, ‘Mons Mari’ (Pedwell), ‘Pisang Awak’, ‘Selangor’, ‘Long Tavoy’, ‘Tjau Lagada’, ‘Tomolo’, and ‘Pisang Berlin’. In this subclade, ‘Calcutta 4’ and ‘Pisang Berlin’ were genetically isolated from other accessions. In SCIII, diverse genomic compositions (AA, AAA, AAAB) associated with different

Fig. 2 Dendrogram of 66 accessions of *Musa* species generated using inter-simple sequence repeat markers
accessions clustered together. The identified accessions included ‘Improved Lady Finger’, ‘Pelipita’, ‘Poteau Geante’, ‘Garbon 2’, ‘Chinese Cavendish’, ‘Hsein Jen Chiao’, ‘Hochuchu’, ‘Lidi’, ‘Borneo’, and ‘Pisang-Lilin’. ‘Chinese Cavendish’, ‘Hsein Jen Chiao’ and ‘Hochuchu’ each had AAA and got closely grouped together and the same clustering was applied to ‘Lidi’, ‘Borneo’, and ‘Pisang-Lilin’ that AA.
In group II, two subclades, SCI and SCII originating from ‘Cardaba’ (ABB) and ‘SH-3436-6’ (AAAA) were formed. Within the SCI, ‘Obino I’Ewa’, ‘Blue Torres Strait Island’, ‘Green Red’, ‘Balonkawe’, ‘Pata’, ‘Dwarf Parfitt’, ‘Dole’, ‘Niyarma Yik’, ‘Dwarf Cavendish’, ‘Malaccenensis’, ‘Truncata’, ‘M. textilis (textilis)’, ‘Lal Velchi’ and M. beccarii (beccarii) grouped together, with ‘Truncata’, ‘M. textilis (textilis)’, ‘Lal Velchi’ and M. beccarii (beccarii) demonstrating much distant separation from other accessions. Subgroup SCII consisted accessions with B genome as the most dominant, but ‘Madang’ (AA) and FHIA-21 (#68) (AAAB) were distantly related. In group III, only ‘Khae (Phrae)’, ‘Honduras’ and ‘Kunnan’ were identified. Groups XIV and V had ‘Lai’ and ‘Umalag’ each with AAA genome.

Principal component analysis, biplot and population structure of different genomic constitutions of Musa species as revealed by inter-simple sequence repeat and start codon targeted markers

PCA was computed through DAPC to identify association among the accessions with variable genomic groups, with the first three PCs showing 28.5% and 27.8% variance in ISSR and SCoT marker datasets, respectively. Biplots revealed clustering pattern with PCA 1 and PCA 2 explaining about 12.3% and 9.3% variance in ISSR and 12.5% and 8.5% in SCOT, respectively (Fig. 4). Cluster 1 of the population structure obtained with ISSR markers, contained accessions with three variable genomes (BB, ABB, AAB), while cluster 1 in SCOT had four (AA, AAA, AAB, AAAA) (Fig. 5). For cluster 2 in ISSR, nine genomic groups including wild diploids (AA, BB, AB, ABB, AAA, AAAA, AAAB, AAB, AS, wild diploids), were detected and seven with wild diploids (AA, BB, ABB, AAA, AAAA, AAAB, AAB, AAAA, wild diploids) in SCOT. In cluster 3, four genomic groups (AA, AAA, ABB, AAB) and seven with wild diploids (AA, AB, AAB, ABB, AAA, AAAA, AS, wild diploids), were respectively identified with ISSR and SCoT marker systems.

Comparison of data generated from inter-simple sequence repeat and start codon targeted markers across 66 different genomes of banana and plantain accessions

A total number of 299 alleles (14–57 per primer) were obtained with ISSR markers, while 326 alleles (26–60 per primer) were identified with SCoT (Table 6). Other genetic indices including mean major allele frequency, allelic count, gene diversity, PIC, NPL, PPL, Ne, H, and I were distinctly and differently revealed by the two marker systems. The overall mean values of Ne, H and I in ISSR were 1.5474 ± 0.1509, 0.3476 ± 0.0649, and 0.5296 ± 0.07460, while SCoT had 1.6440 ± 0.1749, 0.3841 ± 0.0737, and 0.5694 ± 0.0858. Also, the values of Ht, Hs, GST and Nm parameters varied between ISSR and SCoT markers. Overall average values of Ht, Hs, GST and Nm in ISSR were 0.3476 ± 0.0042, 0.3229 ± 0.0036, 0.0709 and 6.5531, whereas 0.3841 ± 0.0054, 0.3452 ± 0.0044, 0.1013 and 4.4377 were, respectively obtained with SCoT marker systems. Nine and 15 major groups with various subclades at similarity indices of 0.81 and 0.77 were, respectively obtained from the dendrogram analysis of the 66 accessions using ISSR and SCoT data.

To further evaluate the genetic structure associated with the accessions, analysis of multilocus associations was applied to both the ISSR and SCoT data (Table 7). For the entire population of the Musa accessions, the mean gene diversity (MH) of single-locus components obtained with ISSR markers accounted for 13.4% (with variance of diversity, VH = 0.7882), while SCoT accounted for 14% (with VH = 0.6357). For the populations, the obtained Wahlund’s effect (WH) indices were 0.4951 with ISSR and 0.6275 with SCoT markers. Considering the two-locus effect, the mean disequilibria (MD) obtained with ISSR markers (289.0036) varied with those of SCoT (194.3081). The Wahlund’s effect (WC) values in two-locus effect in the populations were 11.8337 (ISSR) and 14.8983 (SCoT). Other parameters including interactions between MD and WC (abbreviated as AI), variance of disequilibrium (VD), covariance of interactions (CI), total variance (σ²t) and average variance (σ²a) in the populations varied between the two markers.
Fig. 4 Principal component analysis of 66 *Musa* species accessions assessed with Inter-simple sequence repeat (a) and Start codon targeted (b) marker systems.
Discussion

Genetic diversity of banana and plantain is important to the development of sound *Musa* breeding programs, especially now that the issue of climate change is globally threatening crop productivity and yields (Ortiz 2000; Khayat and Ortiz 2011). Assessing the GD of these vital crops using informative and gene-based molecular markers can readily facilitate the exploration of *Musa* accessions for selection and integration into breeding programs for crop improvement and conservation. Comparative application of different molecular markers to explore GD or allelic richness of crops has provided a detailed and more
informative characterization as well as the choice of the best approach of classification available to researchers than a single marker system (Souframanien and Gopalakrishna 2004; Scariot et al. 2007; Lamare and Rao 2015; Igwe et al. 2017). To the best of our knowledge, this is the first report of utilizing and comparing the effectiveness of ISSR and SCoT markers in accessing GD and population structure of accessions of Musa species with different genomic compositions. Our results demonstrate high potential in assessing GD of the accessions as exhibited by the unique banding patterns or allelic variations, high GD and population parameters including NPL, PPL, PIC, Ne, H, I, Ht, Hs, GST, among other essential indices generated with these markers. Reports show that accessions with the high GD of neutral markers and alleles might be suitable candidates for high adaptive variation, fitness, and conservation (Van Zonneveld et al. 2012; Ilves et al. 2013). In addition, it has been reported that allelic richness is an indicator of GD and it is mostly utilized to assess molecular markers and to identify crops for conservation and breeding purpose (Vinceti et al. 2013).

Both marker systems in this study showed high values for the total number of alleles, mean numbers of alleles, major allele frequency, allelic counts, GD, PIC, and other genetic indicators; but these crucial parameters were higher in SCoT than those obtained with ISSR markers. This demonstrates that SCoT markers may be more effective and efficient in dissecting GD, and relationships among the accessions. The total number of alleles or bands and their

Table 6 Comparative assessment of data obtained 66 accessions of different genomes of bananas and plantains using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) markers

| Parameters                        | Molecular marker data matrix compared |
|-----------------------------------|---------------------------------------|
| Total alleles/range               | ISSR 329 (14–57) SCoT 326 (26–60)     |
| Mean alleles                      | ISSR 36.5 SCoT 41                   |
| Major allele frequency            | ISSR 0.1424 (0.0455–0.3182) SCoT 0.1717 (0.0455–0.3736) |
| Allelic count range               | ISSR 1–21 SCoT 1–24                |
| Allelic frequency range           | ISSR 0.0152–0.3182 SCoT 0.0152–0.3636|
| Gene diversity                    | ISSR 0.9282 (0.7961–0.9803) SCoT 0.9432 (0.8320–0.9986) |
| Polymorphic information content (PIC) | ISSR 0.9225 (0.7698–0.9799) SCoT 0.9421 (0.8214–0.9986) |
| Total number of polymorphic loci  | ISSR 524 SCoT 567                  |
| Number of polymorphic loci        | ISSR 47–66 SCoT 65–66              |
| Percentage polymorphic loci       | ISSR 91.21–100% SCoT 96.97–100%    |
| Effective number of alleles (Ne)   | ISSR 1.2941–1.6310 SCoT 1.5540–1.8312|
| Net’s gene diversity (H)          | ISSR 0.2273–0.3801 SCoT 0.3565–0.4539|
| Shannon information index (I)     | ISSR 0.3878–0.5710 SCoT 0.5419–0.6463|
| Overall mean of Ne                | ISSR 1.5474 ± 0.1509 SCoT 1.6440 ± 0.1749|
| Overall mean of H                 | ISSR 0.3476 ± 0.0649 SCoT 0.3841 ± 0.0737|
| Overall mean of I                 | ISSR 0.5296 ± 0.0746 SCoT 0.5694 ± 0.0858|
| Total gene diversity (Ht)         | ISSR 0.2273–0.3837 SCoT 0.3565–0.4539|
| Gene diversity within population (Hs) | ISSR 0.2076–0.3516 SCoT 0.0323–0.4222|
| Coefficient of gene differentiation (GST) | ISSR 0.0538–0.1180 SCoT 0.0630–0.1699|
| Estimate of gene flow (Nm)        | ISSR 5.2826–11.9913 SCoT 2.4421–14.7687|
| Overall mean Ht                   | ISSR 0.3476 ± 0.0042 SCoT 0.3841 ± 0.0054|
| Overall mean Hs                   | ISSR 0.3229 ± 0.0036 SCoT 0.3452 ± 0.0044|
| Overall mean G_ST                 | ISSR 0.0709 SCoT 0.1013               |
| Overall mean Nm                   | ISSR 6.5531 SCoT 4.4377              |
| No of clusters from dendrograms   | ISSR 9 SCoT 15                      |
| Similarity index from dendrograms | ISSR 0.8140 SCoT 0.7700             |
| Principal component analysis (PCA) | ISSR 5 SCoT 7                     |

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averages (no of alleles/bands, n = 299; average, av = 36.5) obtained from ISSR are higher than the ones earlier reported in 21 and 25 accessions of Musa species with mixed genomic constitutions (Lamare and Rao 2015; Silva et al. 2016), but lower than the ones (n = 326; av = 41) from SCoT. The differences could be due to the nature and number of accessions assessed as well as the loci of the markers. The major allele frequency of 0.1424 from ISSR was lower than 0.1717 from SCoT and this is expected since SCoT markers are derived from the functional section of genomes and more informative (Collard and Mackill 2009). Polymorphic information contents from the two maker techniques are quite high, thereby demonstrating the informative nature of these marker systems but SCoT displayed the higher value of PIC. Different but lower values of PIC were shown in 21 Musa species that consists of triploid and tetraploid accessions (Silva et al. 2016) and 25 variable accessions (Lamare and Rao 2015). Efficiencies of the two marker systems have been exploited in another plant in which SCoT was considered more efficacious than ISSR, but the identified PIC of SCoT is slightly higher (Igwe et al. 2017). The noted discrepancy could be associated with the mixed ploidy nature and number of the accessions used in this study.

The mean major allele frequencies produced by the two markers, ISSR and SCoT from the 66 accessions are high especially the one from SCoT, but lower than the one obtained using SSR markers across 695 accessions, including 327 diploids, 363 triploids and 5 tetraploids (Hippolyte et al. 2012). This could be due to the large difference in the number of accessions analyzed with the SSR markers.

The NPL is used to evaluate the efficiency of primers in dissecting the GD among individuals of accessions (Luz et al. 2015) and this is consistent with our study that generated high NPL with ISST (524) and SCoT (567). Interestingly, both markers produced high PPL varying from 91.21 to 100%, with SCoT demonstrating higher efficiency by possessing a higher range of PPL (96.97–100%) than ISSR (91.21–100%). In a study involving polyploid plant, durum wheat (Triticum turgidum var. durum) that is similar to Musa species, high polymorphisms were recorded using ISSR (98.70%) and SCoT (100%) markers (Etminan et al. 2016). Other researchers identified varying rates of polymorphisms of 85.1% (Ying et al. 2011), but slightly in agreement with 97.5% (Silva et al. 2016).

The overall mean values of Ne, H and I from ISSR (Ne = 1.5474 ± 0.1509, H = 0.3476 ± 0.0649, I = 0.5296 ± 0.07460) are lower than those from SCoT

### Table 7 Brown’s multilocus analysis of inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) diversities and linkage disequilibria of the populations of 66 accessions of Musa species

| Source of variance                  | Abbreviated as | Values obtained with the two markers compared |
|-------------------------------------|----------------|---------------------------------------------|
|                                     |                | ISSR     | SCoT     |
| Single-locus effect                |                |          |          |
| Mean gene diversity                | MH             | 13.4093  | 13.9975  |
| Variance of diversity              | VH             | 0.7882   | 0.6357   |
| Wahlund’s effect                   | WH             | 0.4951   | 0.6275   |
| Two-locus effect                   |                |          |          |
| Mean disequilibrium                | MD             | 289.0036 | 194.3081 |
| Wahlund’s effect                   | WC             | 11.8337  | 14.8983  |
| Interaction between MD and WC      | AI             | 14.1258  | 19.5816  |
| Variance of disequilibrium         | VD             | 40.9389  | 55.0301  |
| Covariance of interactions         | CI             | −21.0491 | −3.8697  |
| Total variance                     | σ²t            | 329.6558 | 244.0488 |
| Average variance                   | σ²a            | 336.4287 | 279.0477 |

σ²t = MH + VH + WH + MD + WC + AI; σ²a = MH + MD + AI + VD + CI

ISSR inter-simple sequence repeat, SCoT start codon targeted
ness (Na and Ne), Nm and G ST assess the genome reduction process (Voisin et al. 2005; Doebley et al. 2006). It has also been reported in various crops that inbreeding and extensive selection decreases effective population size and increases genetic drift in the course of domestication (Doebley et al. 2006; Tang et al. 2010). It has also been reported in Durum wheat (Etminan et al. 2016). The minor polymorphism was among the accessions as within the populations of the accessions whereas with SCoT, indicating that majority of the variation is with the populations of the accessions whereas minor polymorphism was among the accessions as reported in Durum wheat (Etminan et al. 2016). The identified G ST in this study is lower than the one obtained by Lamare and Rao (2015) using combined G ST values from three markers (RAPD, ISSR and DAMD); Ge et al. (2005) using wild M. balbisiana with cpDNA PCR–RFLP; and Mukunthakumar et al. (2013) that analyzed wild banana populations with RAPD markers. This finding could be linked to the effect of summation of G ST values from three marker systems, and the use of wild populations that have proved to be more genetically diverse than the cultivated ones (Zawko et al. 2001). The higher proportion of the genetic polymorphisms within the accessions and lower degree of genetic differentiation among the accessions and lower degree of genetic differentiation among the populations using the two markers (ISSR: 7.09% for among and 92.91 within accessions; SCoT: 10.13% for among and 89.87 within the accessions) corroborate with other studies (Lamare and Rao 2015). It has been reported that most of the out-breeding accessions or species usually have higher GD within populations and low diversity among populations (Hamrick and Godt 1996; Hogbin and Peakall 1999; Zawko et al. 2001). Estimate of gene flow has been categorized as low (Nm << 1), moderate (Nm > 1) and extensive (Nm > 4) in nature (Kumar et al. 2014a, b). The values of Nm from the ISSR and SCoT data in the present study are high since Nm > 4 in both markers, indicating a high and extensive profile but a higher value was obtained with ISSR. This high level of gene flow demonstrates lack of habitat fragmentation, which could be natural or human disturbance that determine the rate of gene flow as earlier reported (Padmesh et al. 2012).

Dendrogram analysis revealed five major groups from ISSR and SCoT data, respectively with different subclades in 66 sets of accessions. This variation in the number of sub-clusters obtained from the both markers could be due to the more effectiveness of the SCoT markers in targeting different loci, which in turn resulted to existence of some accessions in separate groups instead of aligning with their respective larger groups. Most of the genomic groups were well resolved by the markers. For instance, in group I of ISSR, the AAB triploids dominated the group. In group III with several subclades (SCI-SCV), each sub-cluster resolved the accessions based on genomic constitutions as obtained in SCI that had most A genome (AA/AAA) compared to those that had AAB. However, the ‘Gros Michel’ and ‘Chinese Cavendish’ with triploid AAA clustered closely in SCI of group III with AAA, AA and AAB and this relatedness is not unusual since they share AA genomic group as earlier reported (Perrier et al. 2011; Rotchanapreeda et al. 2015). In group IV, mixed triploids (AAA, AAB) and diploid A were found in SCI, while diploid and tetraploid A genomes were more predominant in SCI. SCIII had accessions with triploid AAA clustered closely in SCI of group III with AAA, AA and AAB and this relatedness is not unusual since they share AA genomic group as earlier reported (Perrier et al. 2011; Rotchanapreeda et al. 2015). In group IV, mixed triploids (AAA, AAB) and diploid A were found in SCI, while diploid and tetraploid A genomes were more predominant in SCI. SCIII had accessions with triploid A (AAA) genome as the most frequently occurring, except AAB (‘Lady Finger Nelson’), and BB (‘Honduras’) that were also detected in the same sub-group. Furthermore, AAA and AAB dominated the group and this over-
representation of these two genomes has been reported using SSR markers (Hippolyte et al. 2012). Within SCIV of group III of the ISSR dataset, ‘Figure Pome Geante’ (AAB), a donor of the balbisiana genome, clustered closely to ‘Pisang Keling’ (AAB) but distantly isolated from ‘Pome’ (AAB), while ‘Williams’ (Bell South Johnstone) and ‘Highgate’ with AAA genome are tightly grouped together compared to others (‘Selangor’ and ‘JD-Yangambi’) with the similar genome. This similar heterogenic grouping was reported in Musa accessions analyzed with AFLP markers (Ude et al. 2002) and SSR markers (Sardos et al. 2018). It has also been reported that the triploid AAB had vital contribution of the genome from M. acuminata Colla (Simmonds 1966). In SCI of group I of SCoT marker dataset, genomes of AA, AS, and AAA were found with AA dominating the group. Group II contained all the plantain genomes (AAB and ABB) but ‘Balonkawe’ is more distantly related to others (‘Selangor’ and ‘JD-Yangambi’) with the similar genome. This similar heterogenic grouping was also reported by de Jesus et al. (2013), where three clusters. Existence of homo and hetero accessions identified within the clusters. de Jesus et al. (2013) reported this similar grouping of accession with variable genomes (diploids, triploids, tetraploids) errors as reported (Hippolyte et al. 2012). It could also be linked to inefficiency of the markers to resolve them well based on homo, hetero, diploid, triploid or tetraploid. Population structure of the accessions obtained with ISSR distinctly resolved them into three clusters without any admixture occurring among the accessions and this contradicted the output from SCoT marker system that resulted to admixtures among the accessions identified within the clusters. de Jesus et al. (2013) reported this similar grouping of accession with variable genomes (diploids, triploids, tetraploids) in one cluster or different clusters.

The multilocus structure indices obtained with ISSR and SCoT markers varied across the populations,
as suggested by the large portion of the disequilibrium variance (VD) in the total variance from ISSR (VD = 40.94%) and SCoT (VD = 55.03%) in all the accessions. It was also found that Wahlund’s effect (WH) was small in all the populations based on the values from the two marker systems (ISSR, WH = 0.4951; and SCoT, WH = 0.6275), indicating that selection, rather than population admixture or founder effect, was largely responsible for the observed multilocus structure.

**Conclusion**

Knowledge of distribution of genetic variability, and relatedness among populations or accessions contributes significantly toward crop improvement. The two marker systems demonstrated high effectiveness in resolving GD among the different accessions of *Musa* species with diverse genomic constitutions. Due to apparent display of higher GD indices (PIC, gene diversity, alleles, Ne, H, I, NPL, PPL, Ht, Hs, GST and Nm) by SCoT, AS genomic group, was considered the most genetically endowed accession. The markers identified higher genetic variability within the populations, with SCoT markers exhibiting higher marker efficiency indicators (PIC, NPL and PPL) in comparison with ISSR. Although the cluster analysis from ISSR and SCoT markers separated them into different groups, the population structure obtained with SCoT provided a more detailed clustering pattern with admixtures in some of the accessions of variable ploidy compositions. Interestingly, SCoT markers, the functional gene-based DNA markers targeting the conserved regions of genome across various plant species, could be more advantageous, informative and efficient in exploration of GD for selection of potential breeding populations of *Musa* species for improvement and germplasm conservation.

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**Availability of data and materials**  All data generated during this study are included in this published article [and its supplementary information files].

**Compliance with ethical standards**

**Conflict of interest**  The authors declare that they have no conflict of interest.

**Ethics approval and consent to participate**  This work does not involve living animals and no consent is needed.

**Consent to participate**  Not applicable.

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