MOLECULAR DIVERSITY OF 3 *Allium spp*. LOCAL RACES BASED ON RAPD DATA AND ASSESSMENT OF SSR MARKERS IN CROSS-SPECIES TRANSFERABILITY

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: June 30, 2018 **Accepted:** October 07, 2018

Abstract: To understand the extent of genetic diversity among local races of *Allium* species is an important prerequisite for proper plant conservation and genetic resource utilization. This study was conducted to assess the genetic relatedness and diversity in 10 onion (*A. cepa* L.), 4 garlic (*A. sativum* L.) and 3 bunching onion (*A. fistulosum* L.) local races representing various states in Nigeria using eight RAPD markers. In addition, the study included the evaluation of cross-species transferability in the 3 *Allium spp.* based on six expressed sequence tag (EST-SSR) bulb onion and three genic Welsh onion SSR markers. All the RAPD markers were polymorphic, the total of 66 alleles detected ranging from 4 to 10 (mean 8.25) alleles per locus. Across all the loci, the resolving power (Rp) for the RAPD markers varied from 2.11 to 5.05 (mean 4.18). The results showed that the 3 *Allium* species contained considerable moderate genetic diversity. Hierarchical neighbor-joining cluster analysis (NJ) based on Jaccard's dissimilarity matrix of the RAPD data clustered the 17 local races into three major groups (GI, II, III) corresponding to species types, two of which (GI and GII) were further divided into two sub-groups. Furthermore, the factorial analysis clearly showed three groupings, each group represented each type of species used in the study which were similar to the groupings in the NJ-based clustering with some minor differences. In total, the frequency of transferability of EST-SSR and genic SSR loci was from 65 to 100 % and 47 to 100 % respectively across all local races. A total of 13 alleles were detected using the EST and genic SSR loci in the 17 local races, ranging from 1 to 3 with an average of 2.3 alleles per polymorphic locus. Six of those 9 primer pairs were monomorphic in the 17 local races. This assessment demonstrated the potential of RAPD markers in elucidating clear genetic relationship and diversity among the studied species. This result suggests that these SSR markers could be used to analyse the genetic diversity and phylogenetic relationships in a large set of *Allium* species in the future.

Keywords: *A. cepa*, *A. fistulosum*, *A. sativum*, Molecular diversity, Nigeria

Introduction

Allium L. (Amaryllidaceae, Allioideae) is a large genus comprising about 750 species. Some species are well known including common onion (*A. cepa* L.; 2n = 2X = 16), garlic (*A. sativum* L.; 2n = 2X = 16), shallot (*A. cepa* L. *Aggregatum group*; 2n = 2X = 16), and bunching or Welsh onion (*A. fistulosum* L.; 2n = 2X = 16) (Gregory *et al*., 1998). They have been widely grown locally to the climatic conditions of almost all the states of the northern region and some states in the southern region of Nigeria (Adesoye *et al*., 2012). In Nigeria, some *Allium* species, e.g., bulb onion, garlic, shallots and bunching onion have socio-economic importance; they are commonly consumed and used as vegetables, flavourings, spices, and medicinal herbs. Garlic and bulb onion have been shown to contain sulfur-containing compounds such as allyl propyl disulfide that contribute to their pungent odor (Gurushidze *et al*., 2007).

The characterisation of genetic variability levels and interrelationship present within onion, garlic and bunching onion local races can be studied using molecular markers. This analysis will provide a basis of genetic information for making accurate decisions regarding the improvement and management of genetic resources for the purpose of conservation (Maxted *et al*., 2015). More importantly, the molecular diversity data can be employed for the establishment of genetic reserves like gene banks for the preservation of *Allium* local races. DNA-based molecular markers are highly esteemed tools in cultivar fingerprinting, phylogenetic analysis and genetic diversity assessment of *Allium* species (Gurushidze *et al*., 2007; Mukherjee *et al*., 2013). Furthermore, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR), Single Nucleotide Polymorphism (SNP), chloroplast, and mitochondrial DNA markers have been successfully applied to characterise genetic relationships, assess genetic diversity and cultivar identification among the species of the genus *Allium* (Bark and Havey, 1995; Song *et al*., 2004; Martin *et al*., 2005; McCallum *et al*., 2008; Mallor *et al*., 2014; Anandhan *et al*., 2014).

The RAPD and SSR markers have been used to assess the diversity and the genetic relationships within crops (Anwar *et al*., 2017). For *Allium* species, RAPD markers have been successfully used for their genetic characterisation because it is simple, quick and an easy method (Al-Zahim *et al*., 1997; Ipek *et al*., 2003; Kutty *et al*., 2006; Paredes *et al*., 2008). SSRs derived from the bulb and welsh onions have been widely used for characterising genetic diversity levels of other species of *Allium* taxon, thereby studying their cross-species transferability (McCallum *et al*., 2008; Khar *et al*., 2011; Khosa *et al*., 2013; Yang *et al*., 2015). SSRs are abundant within the genome of crop species, multi-allelic in nature, with high repeatability. Cross-species transferability of SSR loci can be considered as a cost-effective method for developing SSR markers for some plant species, making them useful in the *Allium* species genetic diversity analyses.

Although, there was a genetic diversity study carried out with RAPD analysis to assess the genetic variation in fifteen local cultivars of two *Allium* species grown in Nigeria (Adesoye *et al*., 2012). Until now, no study on the genetic relationship and diversity involving the local races of bulb onion (*A. cepa* L.), garlic (*A. sativum* L.) and bunching onion (*A. fistulosum* L.) cultivated in Nigeria is reported to the best of our knowledge. Therefore, additional study is imperative to understand the extent of molecular diversity of the three *Allium* species. In the present study, we genotyped a set of 17 of three *Allium* spp. local races representing different states in Nigeria using RAPD and SSR markers.

Materials and Methods

Plant materials

Seventeen (17) *Allium* species, comprising 10 bulb onions, 4 garlic and 3 bunching onion local races representing examples from the northern and southern regions of Nigeria were collected, are presented in Table 1.

DNA extraction

The bulb onion and garlic were cut and planted, while the whole bunching onion was planted in pots. Total genomic DNA from these local races was isolated from 2-week old young leaf tissue of individual plants following a modified cetyltrimethylammonium bromide procedure described earlier (Doyle and Doyle, 1987) and preserved at -20 °C. The DNA quality was checked on a 1% agarose and quantity was determined by Nanodrop ND 1000 spectrophotometer (Thermo Scientific, USA). Before polymerase chain reaction (PCR), the DNA was diluted to 20/100 ng with double distilled water and stored at -20 ºC.

RAPD genotyping analysis

Eight arbitrary 10-bp RAPD markers obtained from Operon Technologies, Alameda, California were selected for genotyping (Table 2). The amplification conditions were optimized for all the primers. RAPD PCR amplifications were performed in PCR mixture (10 µl) which contained 100 ng of genomic DNA as the template, 10X PCR buffer, 2.5 mM dNTPs, 50 mM $MgCl_2$, 5 units (U) of Taq DNA polymerase and 5 pMol of each primer. PCR conditions were as follow: initial temperature of 94 ºC for 5 min, followed by 40 amplification cycles at 94ºC for 1 min, 38ºC for I min, and 72ºC for 5 min. Amplification products were separated by electrophoresis on 1% agarose gels in TBE buffer. DNA marker was used to determine the sizes of the PCR products. All the gels were visualized and photographed under UV light after staining with ethidium bromide.

SSR genotyping analysis

In this study, six expressed sequence tag (EST)-SSR bulb onion markers which have been previously developed (McCallum *et al*., 2008), together with three genic-SSR Welsh onion published by Yang *et al*. (2015) were selected for genotyping across the three different *Allium* species (Table 3). All SSR primers were synthesized from INQABA (South Africa). Preliminary optimization for all the primers was carried out. Polymerase chain reaction (PCR) for SSR markers was performed under the following conditions: 15 µl containing 20 ng genomic DNA, 1X PCR buffer, 1.5mM $MgCl_2$, 0.2 mM dNTPs, 0.75 unit Taq polymerase and 1 µM each of forward and reverse primer. Two PCR programs were used. For the majority of markers the PCR conditions were as follow: initial DNA denaturation at 94 °C for 3 min followed by 30 amplification cycles of 94°C for 20 s, 38°C for 40 s, 72°C for 1 min and a final extension at 72°C for 4 min. The PCR product was stored at 10°C. Amplified products were separated by 2% SFR agarose gel electrophoresis. DNA marker was used to determine the sizes of the PCR amplicons. The gel was visualized with staining by ethidium bromide, and then ultraviolet-trans illuminated gels were photographed.

Statistical analysis

The RAPD and SSR amplified alleles were scored as 0 for their absence and 1 for their presence. The number of alleles per primer and mean number of alleles per locus were evaluated for the RAPD and SSR. For RAPD data, the number of polymorphic alleles (NP), percentage of polymorphic alleles (PPA) and resolving power (R_p) were estimated. R_p was determined to show the ability of the most informative primers to differentiate between the genotypes which was assessed according to Prevost and Wilkinson, (1999) using: $R_p = \sum I_b$, where I_b is the band informativeness with $I_b = 1 - [2 \times (0.5\text{-}p)]$ and where p is the proportion of lines containing the band. Polymorphism information content (PIC) was calculated for each locus using the formula $PIC = 1\text{-}\Sigma pi^2$, where pi is the frequency of the ith allele (Sehgal *et al*., 2009). Marker indexes (MI) were conducted according to Kumar *et al*. (2013). The MI was used for characterizing the capacity of each primer to detect polymorphic loci among the lines. The effective multiplex ratio (EMR) was applied to determine the number of polymorphic fragments detected per assay. The MI for each primer was calculated as a product of two functions - the PIC and EMR (Varshney *et al*., 2007) i.e., $MI = PIC \times EMR$. The RAPD allelic diversity data among the local races were used to compute the Jaccard's dissimilarity matrix using Darwin 6.0 software (Jaccard, 1908; Saitou and Nei, 1987). The dissimilarity matrix was further used to construct Hierarchical neighbour joining (NJ) cluster and factorial analyses using Darwin 6.0 software to assess the genetic clustering of the local races of the three species to reveal their molecular diversity. For SSR, the following statistical calculations were performed: a) polymorphic information content (PIC) was calculated for each polymorphic marker using the following formula: $PIC = 1\text{-}\Sigma P^2 ij$, where Pij is the frequency of the jth allele b) percentage of transferability of each SSR was calculated as the presence of target loci to the total number of loci amplified in different *Allium* species, as given below:

$$\text{Amplification (\%)} = \frac{\text{Number of amplified lines}}{\text{Total number of lines}} * 100$$

Table 1: Morphological descriptions of the 17 *Allium* species local races used in the study

Codes/Local races	Collection sites	Species	Common names	Flesh colour	Pungency
BcON01	Bauchi	*A. cepa*	Bulb onion	Light purple	Very pungent
GbON02	Gombe	*A. cepa*	Bulb onion	Light purple	Pungent
JjON03	Jigawa	*A. cepa*	Bulb onion	White	Slightly pungent
KbON04	Kebbi	*A. cepa*	Bulb onion	White	Slightly pungent
KnON05	Kano	*A. cepa*	Bulb onion	Very purple	Slightly pungent
KsON06	Katsina	*A. cepa*	Bulb onion	White	Pungent
SoON07	Sokoto	*A. cepa*	Bulb onion	White	Pungent
SoON08	Sokoto	*A. cepa*	Bulb onion	Purple	Pungent
YbON09	Yobe	*A. cepa*	Bulb onion	Light purple	Pungent
ZmON10	Zamfara	*A. cepa*	Bulb onion	Light purple	Slightly pungent
KdGC11	Kaduna	*A.sativum*	Garlic (C-10)	White	Slightly pungent
KbGC12	Kebbi	*A.sativum*	Garlic (C-72)	White	Slightly pungent
KnGC13	Kano	*A.sativum*	Garlic (C-15)	White	Slightly pungent
YbGC14	Yobe	*A.sativum*	Garlic (C-46)	White	Slightly pungent
LaSONT15	Lagos	*A. fistulosum*	Spring onion	White	Slightly pungent
LaSONM16	Lagos	*A. fistulosum*	Spring onion	White	Slightly pungent
LaSONI17	Lagos	*A. fistulosum*	Spring onion	White	Slightly pungent

C- the number of cloves

Table 2: RAPD primers used in this study and their amplification characteristics

Primer	Total of alleles	PA	MA	PA (%)	PIC	EMR	MI	R_p
H05	8	7	1	88	0.30	3.29	1.00	3.18
TO7	10	9	1	90	0.30	3.34	1.00	4.12
B12	10	10	0	100	0.33	3.12	1.04	4.94
H02	9	9	0	100	0.38	2.59	0.98	5.06
T01	9	9	0	100	0.36	3.18	1.15	4.82
T05	4	4	0	100	0.37	1.76	0.66	2.12
B10	9	9	0	100	0.37	3.88	1.43	4.94
TO4	7	7	0	100	0.39	2.41	0.95	4.24
Average	8.25	8	0.25	97	0.35	2.95	1.03	4.18

PA= Number of Polymorphic alleles, MA= Number of monomorphic alleles, Percentage of Polymorphic alleles, PIC= Polymorphic Information Content, EMR = effective multiplex ratio, MI = Marker index, Rp = resolving power

Table 3: Information of 9 primer pairs used for cross-species transferability in *Allium* species

SSR markers	Type	Forward primer	Reverse Primer	NA	PIC	AS (bp)	FT (%)
ACM004	EST-SSR	TCGTTCTTTAGAACACGTTAGG	GTCGGCGGATATAGTGACA	1	-	120	100
ACM065	EST-SSR	GCTCTGATGGAGGATGGTTC	CTTGCCATCTTTGTCGGT	3	0.79	150-180	65
ACM187	EST-SSR	GTACTCGGGCAGTGGAGGTA	GGAGCTGTCCAAATGCTAGG	1	-	250	100
ACM227	EST-SSR	AGCAGCTCATTCAGCAAAA	GAGGTCGGAGAAGGAGGAGT	1	-	240	100
ACM238	EST-SSR	TGATAGCCAGTTGATTGCGA	TTCCCCAGTACACACCTTCC	2	0.03	255-280	100
ACM326	EST-SSR	AAACCAGCAACAACCAATG	AAAATTGGAGAGCAGGCAAA	1	-	280	100
MCL42	gSSR	CGGGAACGAAGAGATGGATA	AACGACCAACAACGTCCTTC	1	-	380	100
MCL37	gSSR	AAGGAATGCTACGCCAGAGA	CTGAATTCTGCTGGGTCTCC	1	-	400	47
WC228	gSSR	CCACCACCACCTCAATATCC	CTAGTCGAGGTGCAGCATCA	2	0.03	350-400	100
Total alleles				13			
Mean					0.28		

NA= Number of alleles amplified, AS = Range of allele (bp), FT= Frequency of transferability of markers
EST-SSR and gSSR denote expressed sequence tag(EST) and genic sequence, respectively

Results and Discussion

Molecular diversity of 3 Allium species based on RAPD markers

Molecular markers have become a helpful genotyping tool for improving, managing and detecting genetic diversity. RAPD markers have been used in many studies for genotyping of various *Allium* species. This study evaluated the genetic diversity of some *Allium* local races that are grown in Nigeria using the RAPD marker analysis and also assessed the cross-species transferability of SSR primers among the local races. The generated RAPD data were found useful to detect genetic variability of *Allium* species which make them handy for molecular genotyping of plants. In RAPD analysis of local races of *Allium*, total number of alleles, percentage of polymorphic alleles (PA), polymorphic information content (PIC), EMR, MI and Rp values for overall genetic variability across all the 17 local races are shown (Table 2). A total of eight RAPD markers produced a total of distinct 66 alleles among the 17 local races of *Allium* species, yielding a minimum of 4 (T05) and a maximum of 10 (T07 and B122), with the number of alleles averaged 8.25 per primer (Table 2). Of the total alleles, 64 loci were polymorphic (97%) and only 2 alleles were monomorphic. Each *Allium* species exhibited a specific group of alleles among the 66 alleles based on the RAPD data (Fig. 1). However, the RAPD data generated for the identification of local races within each *Allium* sp. showed a few numbers of alleles. These results indicated low levels of diversity in the 17 local races under investigation.

The PIC value for each primer ranged from 0.30 to 0.40, with an average number of 0.35, indicating the allelic diversity in the *Allium* local races. The PIC values seem low suggesting low allelic diversity and very close genetic relationships among the *Allium* local races examined. Among the *Allium* species, the *A. cepa* analyzed showed a moderate allelic diversity than the two other species (*A. sativum* and *A. fistulosum*). Previous observations of low allelic diversity in onion based on RFLP (Bark and Havey, 1995) and EST-SSR markers (McCallum *et al*., 2008) have been reported. The genetic similarity observed in this study is nearly comparable to a previous study which reported a high genetic similarity among the two *Allium* species (14 of *A. cepa* and 1 *A. ascalonicum*) grown in Nigeria using 6 RAPD primers (Adesoye *et al.*, 2012).

The highest EMR value was observed with primer B10 (3.88) and the lowest to be T05 (1.76) with a mean of 2.94. The MI values ranged from 0.66 to 1.42 with an average of 1.03 (Table 2). The resolving power (R_p) of each RAPD primer ranged from 2.11 to 5.05 with an average of 4.18 per primer. The R_p of the markers revealed that they were highly informative and the capability of RAPD primers to detect moderate levels of genetic diversity among the *Allium* local races studied. The *A. sativum* local races studied possessed different numbers of cloves, however, the allelic diversity was

low. This study confirms earlier observations on low genetic diversity among 65 garlic clones (Paredes *et al*., 2008). Also, it has been reported that garlic genotypes have low genetic diversity since they undergo clonal reproduction by vegetative propagation and this could lead to low genetic diversity (Kamenetsky *et al*., 2015). The low genetic diversity observed in the other two species (*A. sativum* and *A. fistulosum*) may be due to the few numbers of lines analysed and further study of allele diversity with more number of local races of these species from other parts of Nigeria is needed for the clearer understanding of the intraspecific and genetic diversity of these species.

The genetic dissimilarity coefficient between the pair of local races was evaluated by calculating the Jaccard's dissimilarity coefficient based on the proportion of shared alleles. The Hierarchical neighbor-joining (NJ) cluster analysis based on Jaccard's dissimilarity matrix showed the genetic relationships and grouped by species (Fig. 2). It was observed that the 17 *Allium* local races were grouped into three clear groups (I, II and III). Group I contained 8 local races of *A. cepa* with moderate genetic distance among the local races. This group had two subgroups such as subgroup I containing 5 and subgroup II included 3 local races. Meanwhile, the group II was further split up into two sub-groups. Genetic distances were lowest among the *A. fistulosum* which grouped together in one sub-group and the other second sub-group comprised all the 4 *A. sativum* of the group II. The similarity based on RAPD data suggested that the *A. fistulosum* two local races (LASONM16 and LASONT15) were possibly the same in this study. The location of the two subgroups of *A. sativum* and *A. fistulosum* formed from the same node in the NJ tree between the two groups of *A. cepa* has shown an interspecific relationship among the three *Allium* species.

Two of the *A. cepa* local races (GbON02 and ZmON10) formed the third main group, a distinct one which did not form with those in the group I. Thus the *A. cepa* local races were represented by two distinct groups. Finally, the clustering of local race GbON02 in another group (group III) might be due to its possession of two unique alleles. The grouping of 17 local races (*A. cepa*, *A. sativum*, and *A. fistulosum*) suggested that there was a clear distinction between the three *Allium* species (Fig. 2). Furthermore, amongst the 3 *Allium* species studied, the grouping showed that *A. fistulosum* was closer to *A. sativum* than *A. cepa*. Hierarchical neighbour-joining (NJ) analysis revealed that the grouping of 17 *Allium* local races was related to their species types, thus clear intraspecific and interspecific genetic diversity among the three species were observed. Consequently, this might be indicative of similar genetic background and origin of the local races, meaning that they were from the same geographical region (Nigeria) in spite that they were collected from different locations where they are presently grown. The 17 *Allium* local races presented low genetic variability. This result is consistent with a previous study revealing a relatively low genetic diversity among tropical-adapted onion gemplasm based on RAPD data (Santos *et al*., 2012).

The factorial plot (Fig. 3) which was conducted using the Jaccard's dissimilarity matrix revealed that all the *A. cepa* were grouped together in one axis (within a single group I) which were divided into groups and subgroups in the NJ clustering output. Their genetic similarities were clearly observed within the *A. cepa*. The seventeen local races were clustered into three groups. G-I consisted of all the 10 *A. cepa* based on the plot, while G-II consisted of 4 *A. sativum* and G-III contained 3 *A. fistulosum*. Moreover, in the factorial based clustering, local races from the same *Allium* species tended to form a distinct group, implying more genetic similarities to each other than from the different species of *Allium*. Wherefore, the factorial analysis clearly delineated each of the three species and helped to complement the clustering of the NJ analysis. Here, in this study, it was also observed that the groupings of the *A. sativum* and *A. fistulosum* local races formed closely, which may be indicative of a degree of more genetic relatedness among these two species as the case in the NJ analysis. Genetic relatedness within the local races of the three *Allium* species could clearly be distinguished from each other.

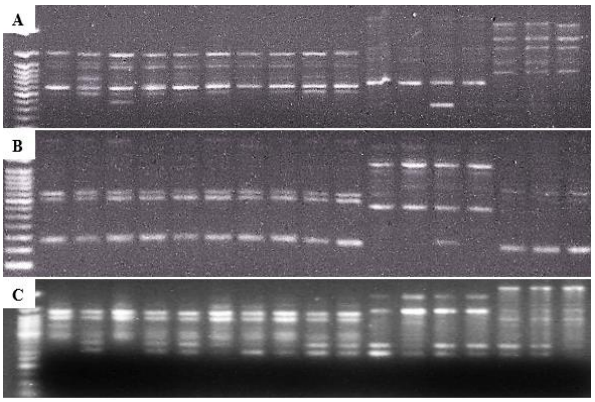

Fig. 1: The gel represents PCR amplification gels using RAPD primers T04 (A), B12 (B) and B10 (C) among 17 local races of Allium species .Lanes1: DNA marker Lanes 2-18: *A. cepa* L. (1 – 10) , *A. sativum* L. (11-14) and *A. fistulosum* L. (15-17)

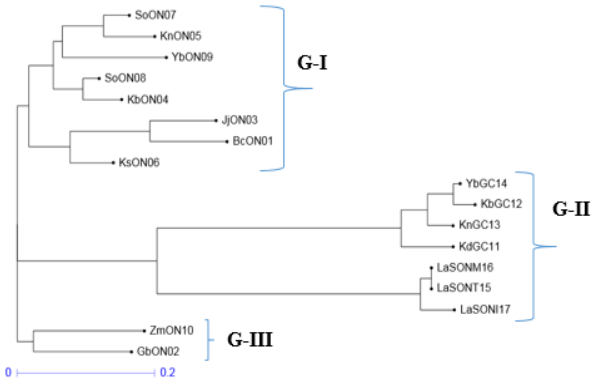

Fig. 2: Hierarchical neighbour joining (NJ) tree based on the Jaccard's dissimilarity matrix (66 RAPD alleles) of 17 local races of *Allium* species. All the local races clustered into three major groups (G-I, G-II with sub-groups and G-III)

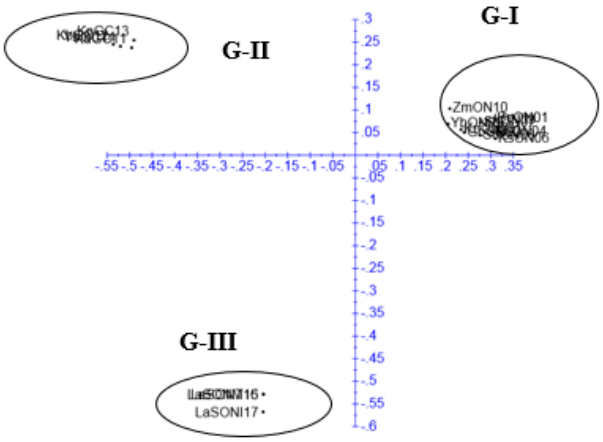

Fig. 3: The factorial analysis based on Jaccard's dissimilarity matrix generated from 8 RAPD markers in 17 local races of *Allium* species. G-I - (*A. cepa* L.), G-II - (*A. sativum* L.), G-III - (*A. fistulosum* L.)

Assessment of cross-species transferability of SSR markers

This study also assessed the transferability of a set of 6 EST-SSR bulb onion markers and 3 genic- SSR Welsh onion in three species belonging to the genus *Allium*, of which they produced successful amplification with an expected range of sizes (Table 3). The number of alleles, polymorphic information content (PIC), and the frequency of transferability for overall genetic variability across all the 17 local races are presented (Table 3). The 6 EST-SSR primers detected a total of 9 alleles in the study, 2 EST-SSR primers were polymorphic. The number of alleles varied from 1 (ACM004, ACM187, ACM227, ACM326) to 3 (ACM065). The primer ACM238 amplified two alleles among the evaluated lines and the only marker that detected the interspecific relationship between *A. fistulosum* L and the other two species. The 3 SSR Welsh onion markers generated a total of 4 alleles, one was polymorphic with only two alleles (WC228). The MCL42 locus produced no polymorphic alleles, and the MCL37 locus amplified monomorphic allele in some local races, while null alleles in the other local races. Overall, 3 of the 9 scored SSR loci were polymorphic, with the average allele per primer was 2.3. A low variation in the number of alleles per locus was observed across all the 17 local races. The PIC value revealed by these markers ranged between 0.03 and 0.78 with a mean of 0.28. This result is incomparable with a previous study where PIC varied from 0.29 to 0.90 with an average of 0.73 using 28 SSR markers among 24 *Allium* species (Khosa *et al*., 2013). In present study there appeared no clear molecular diversity of the three *Allium* species based on the evaluated SSR markers. This indicates that these SSR markers were not informative and could not detect polymorphism in the present set of *Allium* species nor differentiate the 17 local races from one another.

In addition, in a previous study that used a panel of 15 SSR primer pairs among 16 onion cultivars from the Czech Republic amplified the number of alleles per SSR locus ranging from 2 to 3, with an average of 2.2 alleles (Mitrová *et al*., 2015). However, these values were lower than the results of two previous studies (Yang *et al*., 2015; Baldwin *et al*., 2012) but comparable to the present study. Generally, higher polymorphism levels in genomic SSR is exhibited than in EST-SSR markers for different plants (Varshney *et al*., 2005; Liu *et al*., 2012) which has been reported in the *Allium* species (Hanci and Gökçe, 2016). The reason for the inability of EST-SSR and genic SSR to detect allelic diversity in the 17 local races might be attributed to the sample size, few SSR markers and method for separation of amplicons used in this study. Similarly, the SSR loci evaluated were developed from the coding regions which are highly conserved regions of the genome (Khosa *et al*., 2013). In addition, the lack of polymorphism is possibly due to a high degree of inbreeding in these local races.

The frequency of transferability of 6 EST-SSR bulb onion markers was ranged between 65 and 100% in all *Allium* species. Out of a total of six EST-SSR markers, 5 markers (ACM 004, ACM 187, ACM 227, ACM 238 and ACM 326) produced amplification in all the 17 local races of the different *Allium* species. ACM065 had 65% transferability in the studied *Allium* local races, with the lowest value observed in *A. sativum* L. (0%) and followed by *A. cepa* L. (10%). Meanwhile, the 3 genic Welsh onion derived SSR markers had a frequency of transferability varying from 47% (MCL37) to 100% (WC228 and MCL42) in all *Allium* species. MCL37 locus exhibited low transferability with *A. cepa* (47 %) and 0% in all the (*A. sativum* L.) local races. It is noteworthy that, only 7 out of 9 SSR primers had 100% transferability (ACM004, ACM187, ACM227, ACM238, ACM326, WC228, and MCL42) across all the local races of different *Allium* species in this study. Hence, demonstrating their usefulness as tools for future genetic diversity studies, phylogenetic relationships and cultivar identification in the *Allium* species. The bulb onion derived EST-SSR markers used for cross amplification in *A. fistulosum* has been reported to be high (75.10%) than the genomic SSR (43.30%) markers (Tsukazaki *et al*., 2008). This is similar to the present study. Furthermore, Mallor *et al*. (2014) assayed a wide collection of Spanish onions and related six *Allium* species using 12 SSR, had cross transferability rates ranging from 25 to 91.7%. Thus, this suggests that the transferability of SSR markers may vary from one *Allium* germplasm to another as it is in the present study.

Conclusion

These results clearly demonstrated the potential of RAPD markers in elucidating the genetic diversity and relationship in the *A. cepa*, *A. sativum* and *A. fistulosum* local races. Thus, and this study contributes to the knowledge about the level of genetic relatedness among the three *Allium* species. The results could be used for their genebanks and also *in situ* conservation for sustainable use of the plant genetic resources. Furthermore, the cross-species transferability of some genic Welsh onion SSR and bulb onion EST-SSR markers among the three *Allium* species was established. The present study suggests that the SSR markers could be judiciously used to unravel the genetic diversity and interspecific relatedness of a large set of the three *Allium* species.

Acknowledgement

We are grateful to Mrs. Adetutu Udofia, International Institute of Tropical Agriculture (IITA) for the genotyping of plant materials.

Conflicts of Interest

There is no conflict of interest.

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