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Transferability of SSR Markers used for Assessment of Genetic Relationship in Five Species/Genera in Cucurbitaceae

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THIS STUDY assessed the genetic diversity and relationships among 24 local accessions across five genera of the Cucurbitaceae family including *Lagenaria siceraria* (gourd), *Citrullus lanatus* (''Egusi''melon), *Cucurbita pepo* (pumpkin), *Telfairia occidentalis* (fluted pumpkin) and *Trichosanthes cucumerina* (snake tomato) based on the transferability of six *L. siceraria* (bottle gourd) and six *C. pepo* (pumpkin) derived SSR markers. The study revealed a high level of transferability of *C. pepo* markers, ranged from 83-100% across the genera/species. The transferability rate of the *L. siceraria* SSR markers was 66% within *T. occidentalis* and 83% in *T. cucumerina.* An average of 33% transferability of *L. siceraria* SSR markers amplified in *C. pepo*. The 12 SSR markers were polymorphic and a total of 109 alleles were obtained, ranging from 3 to 19 with an average of 9.08 per marker. The polymorphism information content (PIC) values ranged from 0.45 to 0.89 with an average of 0.79, indicating a high level of informativeness. Using un-weighted pair group method with arithmetic average cluster analysis (UPGMA), two groups of 15 and 9 accessions were identified, which comprised *C. lanatus*, *Lagenaria siceraria* and *C. pepo*, *T. occidentalis* and *T. cucumerina* accessions, respectively. The present study has shown the transferability of *L. siceraria* and *C. pepo* SSR markers across other genera in the Cucurbitaceae. The results also indicated the existence of a considerable level of inter/intraspecific genetic diversity and relationship among cucurbit species of the five genera under study.

Keywords: Cucurbitaceae, *C. pepo* (pumpkin), Genetic diversity, *L. siceraria* (gourd), Transferability of SSR markers.

Introduction

The Cucurbitaceae family is genetically diverse, it contains about 130 genera and approximately 900 species, mainly distributed in the tropical and subtropical regions. There are some important economic crops in this family, encompassing cultivated genera such as *Cucurbita*, *Citrullus*, *Lagenaria*, *Momordica* and *Trichosanthes* (Whitaker & Davis, 1962; Dhiman et al., 2012; Ajuru & Nmom, 2017). Recently, interest in the Cucurbitaceae plant family has been popularly known for medicinal and nutritional properties of the edible fruits. They are consumed as whole immature fruits or mature fruits flesh or seeds (Bates & Robinson, 1995; Paris et al., 2012). Cucurbitacins belonging to the phytochemical class of terpenoids have been suggested to be responsible for the cucurbits' anti-inflammatory, hepatoprotective, cardiovascular, anthelmintic, anti-cancer and immunoregulatory activities (Bisognin, 2002; Rajasree et al., 2016).

Previously, some were only considered as sources of edible seeds and seed oil and ornamental bottles/ utensils/ instruments (Jacks et al., 1972). Eight species of the Cucurbitaceae are some of the main vegetable crops for the edible fruits in Nigeria are bottle gourd (*Lagenaria siceraria), melon (Citrullus lanatus),* squash and pumpkin *(Cucurbita pepo),* fluted pumpkin (*Telferairia occidentalis), snake gourd (Trichosanthes cucumerina),*bitter

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gourds/ bitter melon *(Momordica charantia),* white seed melon *(Cucumeropsis mannii)* and *Cucurbita moschata.* These cucurbits are widely cultivated globally. Phylogenetically, cucurbit species such as cucumber and melon as well as watermelon are close to bottle gourds (Xu et al., 2011).

Various molecular markers have proven to be powerful tools in the assessment of genetic variation and the elucidation of genetic relationships within and among species (Chakravarthi & Naravaneni, 2006). Among the existing molecular approaches, Simple sequences repeats (SSRs), also known as microsatellites is one of the widely preferred methods in genetic studies due to its many known advantages like their high reproducibility, multi-allelic nature, high polymorphism and extensive genome coverage (Varshney et al., 2000). Development of SSR markers from an enriched genomic library and appropriate SSR primers designed have been currently carried out in some cucurbits. They are employed in the genetic analysis such as genetic relationships within the closely related species and cross-species transferability among the species in other genera of the family of Cucurbitaceae (Gong et al., 2008, Ji et al., 2012; Inan et al., 2012; Gong et al., 2012; Saxena et al., 2014; Zhu et al., 2016).

Several studies have demonstrated the efficacy of SSR markers from one species to closely related species or genera in the genetic diversity assessment and cross-species transferability (Kalia et al., 2011; Wang et al., 2012). Yildiz et al. (2015) reported the transferability of 27 *Cucurbita* SSR markers in bottle gourd and also compared the genetic relationship between bottle gourd and 31 other cucurbit accessions (11 *Cucurbita maxima*, 3 *C. moschata*, *5 C. pepo* subsp. ovifera, 10 *C. pepo* and 2 *Luffa cylindrica*). SSR markers developed in bottle gourd were investigated for their transferability in other four cucurbits (watermelon, pumpkin, loofah and bittergourd). This set of SSR markers showed high transferability in watermelon (41%) followed by loofah (20%), while relatively low cross-species SSR transferability was observed in pumpkin and bittergourd (Xu et al., 2011). In another study, Kazmi´nska et al. (2017) tested a set of 23 highly polymorphic SSR markers selected from 500 SSRs originally developed by Gong et al. (2008) in *C. moschata* and *C. pepo* and were successfully used to assess the genetic diversity of 88 *C. maxima.*

Therefore, the cross-specific amplification of some of these SSR loci in other poorly studied cucurbit genera/species can be evaluated. This may have potential use as molecular tools for the analysis of intra-specific/inter-generic crosses. However, to date, SSR markers for the *Telfairia* and *Trichosanthes* have not been identified nor available like other cucurbits. This is limiting the conservation, genetic characterization and germplasm utilization for the breeding of the species. Knowledge of their genetic relatedness will contribute to the effective conservation, genetic assessment and facilitate the development of strategies for crop improvement of cucurbit species.

Many cucurbit species belonging to the family Cucurbitaceae are typically diverse in fruit morphology (shape, size, colour), phytochemicals and widely distributed or adapted to the agro-climatic conditions in Nigeria. To our knowledge, the characterisation of genetic variability in the cucurbits grown in Nigeria has not been studied. Therefore, the objective of this research is to assess the genetic relationships among 24 local accessions across five genera of the Cucurbitaceae family (*Lagenaria siceraria*, *Citrullus lanatus*, *Cucurbita pepo*, *Telfairia occidentalis* and *Trichosanthes cucumerina* based on the cross-transferability of six bottle gourd and six pumpkin derived SSR markers.

Materials and Methods

Plant materials

In the Cucurbitaceae family, a total of twenty-four local cucurbit populations (accessions) including *L.siceraria* (11), *C. lanatus* (4), *C. pepo* (3), *T. occidentalis* (5) and *T. cucumerina* (1) collections were used to examine transferability and assess genetic relationships in this study; these cultivars were chosen largely based on their high medicinal importance (Table 1). The accessions were collected as fruits (Fig. 1) from the local farmers from the main regions of production and some were purchased from the local markets. For the proper documentation of the collection, the morphological characterisation of the fruit, seed and leaf was carried out in the laboratory (Table 2, Fig. 2). The following qualitative traits were recorded for each fruit, shape, presence or absence of neck and lines; the width (mm) and length (mm) (measured with a Digimizer image analysis software), (www.digimizer.com), number,

colour and texture of the seeds were scored. In June 2018, seeds from the 24 accessions were germinated in the greenhouse during the rainy season for the evaluation of the morphological traitsof leaf (shape and margin) evaluation. For the morphological characterisation, the standard descriptor for Cucurbitaceae by the International Board For Plant Genetic Resources (IBPGR) (Esquinas-Alca´zar & Gulick, 1983) was used.

TABLE 1. List of Cucurbitaceae genera/species used in the Study.

Codes	Places of collection	Local names	Common names	Scientific names
GL01	Ilorin, Kwara	Seere	Bottle gourd	*Lagenaria siceraria*
GL02	Ilorin, Kwara	Akeregbe	Giant bottle gourd	*Lagenaria siceraria*
GL03	Ilorin, Kwara	Ato	Penguin gourd	*Lagenaria siceraria*
GL04	Ilorin, Kwara	Ado	Bottle gourd	*Lagenaria siceraria*
GL05	Ibadan, Oyo	Ado	Bottle gourd	*Lagenaria siceraria*
GL06	Ibadan, Oyo	Seere	Bottle gourd /big kettle gourd	*Lagenaria siceraria*
GL07	Ibadan, Oyo	Gongo	Bottle gourd	*Lagenaria siceraria*
GL08	Ibadan, Oyo	Sooro	Bottle gourd	*Lagenaria siceraria*
GL09	Ibadan, Oyo	Akeregbe	Bottle gourd	*Lagenaria siceraria*
GL10	Lagos	Bara	Speckled gourd	*Lagenaria siceraria*
GL11	Lagos	Bara	Speckled gourd	*Lagenaria siceraria*
ML12	Lagos	Egusi	Melon	*Citrullus lanatus*
ML13	Lagos	Egusi	Melon	*Citrullus lanatus*
ML14	Abeokuta	Egusi	Melon	*Citrullus lanatus*
ML15	Ile-Igbo, Oyo	Egusi	Melon	*Citrullus lanatus*
RL16	Lagos	Pumpkin	Pumpkin/squash	*Cucurbita pepo*
RL17	Lagos	Pumpkin	Pumpkin/squash	*Cucurbita pepo*
RL18	Lagos	Pumpkin	Pumpkin/squash	*Cucurbita pepo*
FL19	Lagos	Ugu	Fluted gourd	*Telfairia occidentalis*
FL20	Lagos	Ugu	Fluted gourd	*Telfairia occidentalis*
FL21	Delta	Ugu	Fluted gourd	*Telfairia occidentalis*
FL22	Anambra	Ugu	Fluted gourd	*Telfairia occidentalis*
FL23	Calabar	Ugu	Fluted gourd	*Telfairia occidentalis*
ST24	Lagos	Tomato elejo	Snake gourd	*Trichosanthes cucumerina*

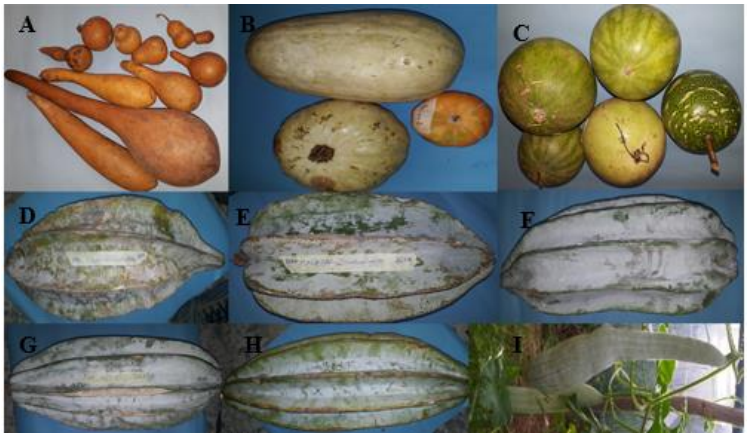

Fig. 1. Diversity of fruit size, shape and colour of the cucurbit accessions used in the study. Left to the right, *L. siceraria* (bottle gourds) [A)], *C. pepo* (pumpkins) [B], *C. lanatus* (melons) and *L. siceraria* (speckled gourd) [C], *T. occidentalis* (fluted pumpkins) [D-H] and *T. cucumerina* (snake tomato) [I].

TABLE 2. Morphological description of cucurbits used in the study.

Codes	Fruit			Seeds number	Leaf					
	Shape	Neck	Lines		Colour	Width (mm)	Length (mm)	Texture	Shape	Margin
GL01	Cavate	Yes	No	218	Brownish black	8.54	18.82	SR	Reniform	Finely serrate
GL02	Cavate	Yes	No	80	White/cream	5.34	10.42	SR	Reniform	Finely serrate
GL03	Elongated pyriform	Yes	No	2217	Brownish black	7.68	11.09	SR	Reniform	Finely serrate
GL04	Cavate	Yes	No	108	Cream	4.54	8.24	SR	Reniform	Finely serrate
GL05	Cavate	Yes	No	78	Brownish black	4.68	10.18	SR	Reniform	Finely serrate
GL06	Cavate	Yes	No	97	Brownish black	5.97	12.45	SR	Reniform	Finely serrate
GL07	Cavate	Yes	No	145	Brownish black	5.73	11.91	SR	Reniform	Finely serrate
GL08	Cavate	Yes	No	28	Brownish black	5.71	11.3	SR	Reniform	Finely serrate
GL09	Elliptical	Yes	No	106	Brownish black	5.28	11.4	SR	Reniform	Finely serrate
GL10	Cavate	Yes	No	519	White	4.76	10.33	S	Reniform	Finely serrate
GL11	Elliptical	No	No	204	White	3.50	7.96	S	Reniform	Finely serrate
ML12	Oblate	No	Yes	271	White	8.03	14.33	S	Lobed	Cleft
ML13	Oblate	No	Yes	97	White	8.51	15.01	S	Lobed	Cleft
ML14	Oblate	No	No	113	White	8.30	13.75	S	Lobed	Cleft
ML15	Oblate	No	Yes	93	White	9.32	15.56	S	Lobed	Cleft
RL16	Circular	No	Yes	63	White	7.88	11.94	S	Reniform	Finely serrate
RL17	Circular	No	Yes	317	Tan	7.96	11.94	SR	Reniform	Finely serrate
RL18	Cylindrical	No	Yes	265	Tan	8.49	11.54	SR	Reniform	Finely serrate
FL19	Pyriform	No	Yes	87	Brownish black	237.47	59.61	SR	Ovate	Dentate
FL20	Elongate slim	No	Yes	90	Brownish black	204.34	54.74	SR	Ovate	Dentate
FL21	Pyriform	No	Yes	97	Brownish black	214.32	57.15	SR	Ovate	Dentate
FL22	Pyriform	No	Yes	49	Brownish black	251.27	63.3	SR	Ovate	Dentate
FL23	Pyriform	No	Yes	63	Black	237.12	63.92	SR	Ovate	Dentate
ST24	Cylindrical	No	No	42	Grey	9.09	14.6	SR	Reniform	Finely serrate

SR-Slightly rough; S-Smooth

Fig. 2. Diversity of seed size, shape and colour of the cucurbit accessions used in the study. Left to the right, *L. siceraria* (bottle gourds) [A-J], *L. siceraria* (speckled gourd) [K-L], *C. lanatus* (M, Q-S), *C. pepo* (N-P), *T. occidentalis* (T-S) and *T. cucumerina* (Y).

SSR analyses

DNA extraction

Tissue samples (80-100mg fresh weight) were collected from young leaves, freeze-dried and ground to a fine powder in an extraction tube using stainless steel balls in a Genogrinder-2000. DNA was isolated using the method described by Dellaporta et al. (1983). The quality of genomic DNA was performed for each sample of the 24 samples by 2% agarose electrophoresis. The DNA concentration of all samples was determined using Nano Drop spectrophotometer (Nano Drop Technologies, Wilmington, Delaware, USA) and stored in a refrigerator until use.

Transferability of bottle gourd and pumpkin genomic SSR markers, amplification by PCR and genotyping

Genotyping of the cucurbit accessions in this study was performed using a set of 12 SSR markers for the Cucurbitaceae (Table 3). These markers were arbitrarily selected, 6 were derived from bottle gourd developed by Xu et al. (2011) and used in a recent study (Mashilo et al., 2015), 2 were derived from *C. pepo* (Gong et al., 2008) and the others also from *C. pepo* (Blanca et al., 2011). The primers were synthesized by Inqaba Biotec West Africa Ltd., South Africa. For PCR reactions and conditions, three sets were conducted with the appropriate annealing temperature using a PCR thermocycler as previously described by those of Xu et al. (2011), Gong et al. (2008) and Blanca et al. (2011). Of the 24 cucurbit accessions, three were initially used to optimize the primers. PCR products were first to run on a 2% agarose gel and visualization by ethidium bromide for successful amplification and was thereafter resolved on 6% non-denaturing polyacrylamide gel (29:1, acrylamide:bis), visualized under UV lights in the presence of the Safe View TM DNA stains (Applied Biological Materials Inc., Richmond BC, Canada) and images were captured. Allele sizes were estimated by comparison to the DNA ladder marker. The amplified products in the gel images were scored manually in a binary format, '1' and '0' indicating the presence and absence of the amplicons, respectively for all accessions and used for data analysis.

TABLE 3. The primer sequences (5'-3', forward/reverse) of the 12 SSR markers used in PCR reactions with genomic DNA of each of the cucurbit accessions.

Marker name	Forward primer	Reverse primer	Reference
LSR015	CTTACCTTCACAAAACCCCATC	ACTCTGTTTCGACTCTGCTTCC	Xu et al. (2011)
LSR040	TTCCATCCAGACCAAACCTATC	CAAAGGCCATAGACAAACACAA	Xu et al. (2011)
LSR056	TAATAATGCCACTGCACATGGT	AGATGAATCCCAATATCCCAGA	Xu et al. (2011)
LSR063	AAGAGAGGGGCAGGAAGTAAAT	AGAAAACACACAGTACGCCTCC	Xu et al. (2011)
LSR077	GACAGATCCTTCTGGGACTTTT	TTCTGCAATAGAGTACGTTGGC	Xu et al. (2011)
LSR112	CTCTCTATATGTCTAATTCCTCGCC	CAAATTCACAGTTGTTGTCACG	Xu et al. (2011)
CUTC017708	TGTGATTTGTTGGGCTCTCTGT	ACATGGAAATCCACCTCTTCGT	Gong et al. (2008)
CUTC002749	GTGGGCTAAGTTCAAATCGTTC	GAACCCAATCTTCTCATTTCCA	Gong et al. (2008)
CUTC046645	CCCTCGGTAGCAATTTTGTAGT	TTTGGTGGGAGTGATACTGATG	Gong et al. (2008)
CUTC009607	CAAAACTGCCCATGACCACTAC	CTTTCTACCCCAACCCCACAT	Gong et al. (2008)
CMTp46	TTCCCTTCTGCAGAGATGCT	CCATGCGCATAATTGTATCG	Blanca et al. (2011)
CMTp142	TCAACCAAGTGCCAATCTCA	ACTGATCCACCGACTGATACG	Blanca et al. (2011)

Data analysis

SSR marker was taken to be transferable if the amplification of a PCR product of the expected size range was observed (Gong et al*.,* 2008; Xu et al*.*, 2011; Blanca et al*.,* 2011). The percentage of transferability of the markers was calculated by determining by the presence of target SSR loci to the total number of the SSR loci analysed. The observed number of alleles (Na) per marker, an average of amplicons (alleles) per primer pairs, gene diversity, heterozygosity and Polymorphic Information Content (PIC) value for each locus were estimated using Power Marker version 3.25 (Liu & Muse, 2005). The generated binary data for all the SSR loci were used to compute the distance matrix using the Jaccard's similarity coefficient in the numerical taxonomy and multivariate analysis system (NSTYS-Pc) statistical package (Jaccard, 1908; Rohlf, 1997). The distance matrix was further used to generate dendrogram based on un-weighted pair group method with arithmetic average (UPGMA) cluster analysis and a principal component analysis (PCA) was also constructed. These analyses were done to group the cucurbit accession/species based on genetic diversity.

Results

Morphological variation of cucurbits

The diversity in fruit shape, size and colour of the cucurbits studied is presented in Fig. 1. The morphological variation in fruit, seed and leaf is shown in Table 2 and variation in seed morphology is depicted in Fig. 2. The studied cucurbits showed substantial variation in fruit shapes, size and seed number, colour and shape morphology, while leaf morphology is distinct for each genus.

Amplification of bottle gourd and pumpkin genomic SSR markers and transferability within species/genera

The 12 selected SSR primer pairs from *L. siceraria* and *C. pepo* genomes for cross-transferability, amplified genomic DNA from 24 accessions belonging to five genera in Cucurbitaceae (*L. siceraria*, *C. lanatus*, *C. pepo*, *T. occidentalis* and *T. cucumerina*). All the 12 SSRs studied successfully produced fragments within the expected range in at least in more than two of the Cucurbitaceae genera screened (Fig. 3, Table 4). These SSR markers also showed moderate to high transferability to other Cucurbitaceae species, ranged from amplification of 33-100% of donor species markers. The highest transferability, 83-100% was observed with the SSR markers developed in *Cucurbita pepo*. From them, 6 (100%) produced amplification in the *L. siceraria*, *C. lanatus*, *C. pepo* and 5 (83%) for other genera (*T. occidentalis* and *T. cucumerina*). The transferability rate of the *L. siceraria* markers was 66% within *T. occidentalis* and 83% in *T. cucumerina.* Two (33%) *L. siceraria* SSR markers amplified in *C. pepo,* while 100% transferability was observed in *C. lanatus.*

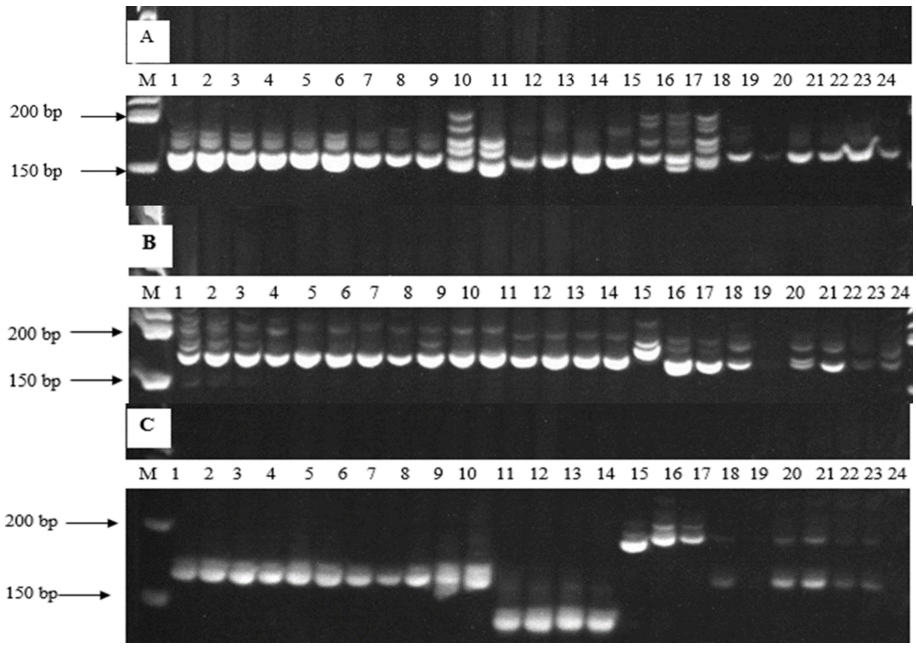

Fig. 3. Polyacrylamide gel picture showing examples of PCR amplification in 24 accessions of Cucurbitaceae family [Lanes 1-11 (*L. siceraria*); lanes 12-15 (*C. lanatus)*; lanes 16-18 (*C. pepo*); lanes 19-23 (*T. occidentalis*) and lane 24 (*T. cucumerina*)] using three pairs of SSR markers (A: LSR056, B: CUTC046645, C: CMTp142 and M: DNA ladder marker).

TABLE 4. Transferability of *L. siceraria* and *C. pepo* SSR markers in genus/species of the Cucurbitaceae family.

Donor species of markers	Number of markers	Number of amplified markers (percentage of transferability) in:				
		L. siceraria (11)	*C. lanatus* (4)	*C. pepo* (3)	*T. occidentalis* (5)	*T. cucumerina* (1)
L. siceraria	6	6	6	2	4	5
		100%	100%	33%	66%	83%
C. pepo	6	6	6	6	5	5
		100%	100%	100%	83%	83%

Polymorphism of bottle gourd and pumpkin genomic SSR markers in other species

The number of alleles amplified by each SSR marker varied with genera and species used in the study. A summary of the polymorphism for the 6 bottle gourd and 6 pumpkin SSR makers is shown in Table 5. The amplified 12 SSR markers were polymorphic in the five genera groups (Table 5). A total allele of 109 was amplified, with an average of 9.08 per marker. The gene or locus diversity varied greatly from 0.54 to 0.90 and the average gene diversity is 0.75. Heterozygosity values ranged from 0.00 to 0.96 with an average of 0.41, indicating moderate to high heterogeneity. The average polymorphic information content (PIC) value, which shows the extent of allele diversity among the species, was 0.72 and ranged from 0.45 to 0.89 for each locus. Among the SSRs, the highest allele number and the maximum PIC value was found with primer LSR077.

Genetic relationship assessment

The UPGMA analysis of the 24 cucurbit accessions depicted in a dendrogram, placed the accessions into two major groups (Fig. 4), Group I and Group II in this study. Group I had the largest number of accessions, contained 15 accessions from different genera, subdivided into two subgroups (Subgroup I-1 and Subgroup I-2). Subgroup I-1 included 4 *C. lanatus* (ML12 - ML15) and 2 *L. siceraria* (speckled gourd) accessions (GL10 and GL11). For the 9 accessions belonging to the *L. siceraria* (gourd type), were mainly assigned to sub-group I-2. Based on grouping, the most closely related gourd accessions identified were GL02 and GL04; GL03

and GL06; and GL07 and GL08 in subgroup I-2. In comparison, the 5 *T. occidentalis* (FL19-FL23), the only *T. cucumerina* (ST24) accessions and 3 *C. pepo* (RL16-RL18) were grouped under Group II. The genetic relationship of the 24 cucurbit accessions was also examined using principal component analysis (PCA). The PCA analysis showed a clear differentiation between the species of the five cucurbit genera in the two axes (Fig. 5). The *L. siceraria* and *C. lanatus* species were plotted in the first axis, which explains 28.04% of the variation. The *C. pepo*, *T. occidentalis* and *T. cucumerina* accessions displayed a distinct separation in the second axis in Fig. 5. The PCA plot obtained with the SSR data was consistent with the result of dendrogram analysis. The Jaccard's-based similarity coefficient among the 24 cucurbit collections ranged from 0.56 to 0.95 with an average of 0.76. The maximum dissimilarity was observed between GL01 (*L. siceraria*) and RL17 (*C. pepo*).

TABLE 5. Number of alleles, gene diversity, heterozygosity and PIC of 12 SSR markers used for the 24 accessions.

Marker	Observed number of alleles	Gene diversity	Heterozygosity	PIC
LSR015	9	0.83	0.59	0.81
LSR40	8	0.70	0.14	0.65
LSR056	12	0.78	0.63	0.75
LSR063	7	0.83	0.41	0.80
LSR077	19	0.90	0.59	0.89
LSR112	3	0.54	0.00	0.45
CUTC017708	4	0.73	0.24	0.68
CUTC002749	7	0.73	0.43	0.69
CUTC046645	9	0.83	0.96	0.80
CUTC009607	17	0.62	0.33	0.60
CMTp46	8	0.84	0.50	0.82
CMTp142	6	0.71	0.13	0.67
Mean	9.08	0.75	0.41	0.72

PIC - Polymorphic information content

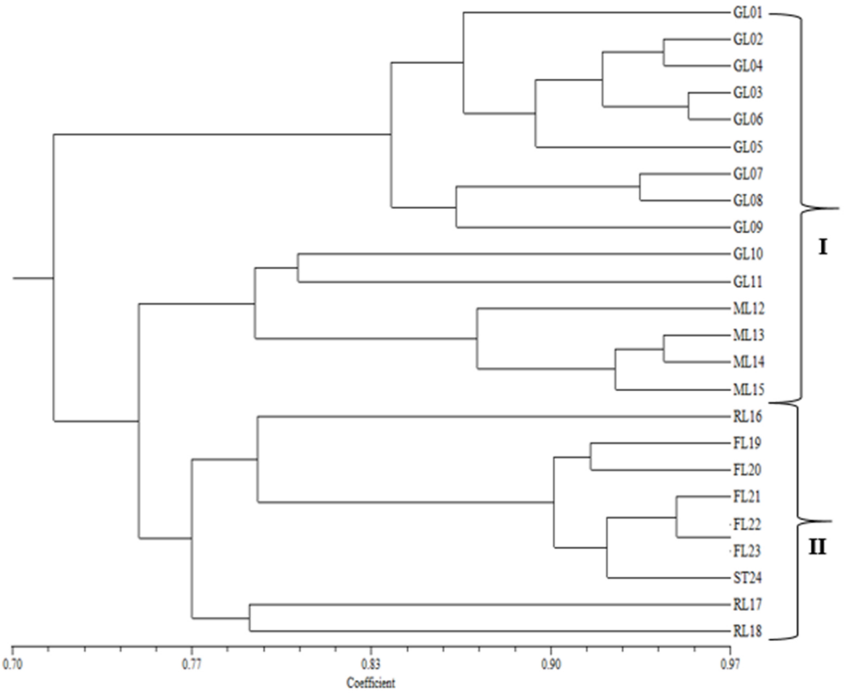

Fig. 4. Dendrogram from UPGMA cluster analysis among 24 accessions of the Cucurbitaceae family (The code of the accessions follow the designations listed in Table 1).

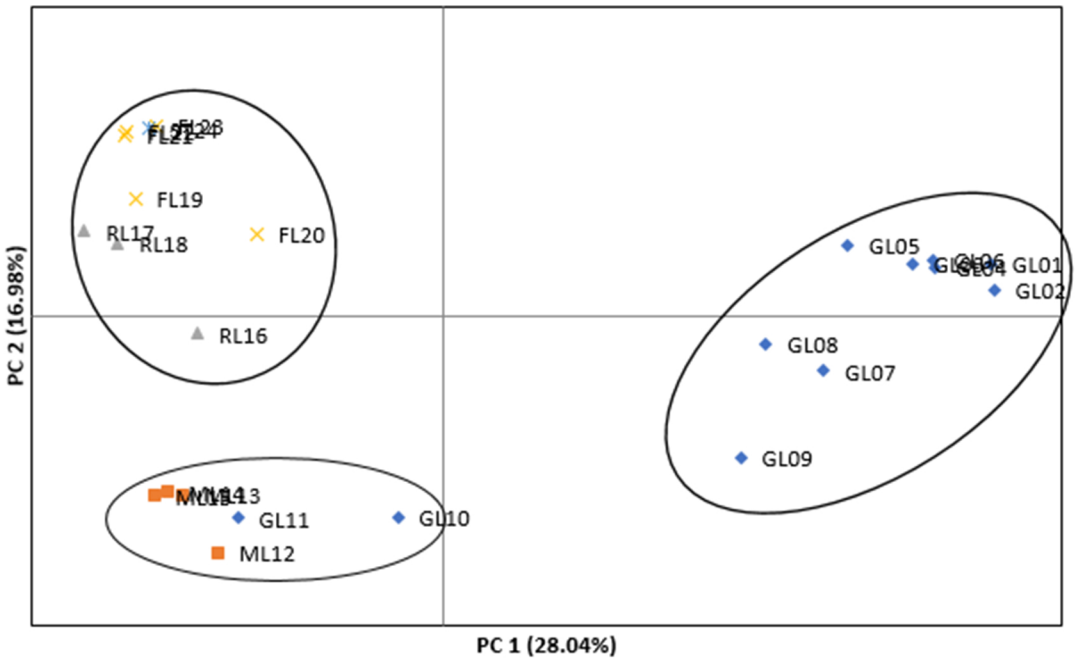

Fig. 5. PCA plot clustering of the 24 accessions of Cucurbitaceae family (The code of the accessions follow the designations listed in Table 1).

Discussion

PCR-based SSRs interspecific/generic transferability are widely used for genetic relationship analysis and identification of accessions (Soller & Beckmann, 1983). Transferability of SSR markers has been used to assess the genetic relationship in some species in various genera of the Cucurbitaceae family (Xu et al., 2011; Bhawna et al., 2014; Yildiz et al., 2015). In this study, 12 previously developed SSR markers (6 *L. siceraria* and 6 *C. pepo*) were evaluated for the cross-species transferability across 24 accessions of *Lagenaria*, *Citrullus*, *Cucurbita*, *Telfairia* and *Trichosanthes* cucurbit species/genera and thereafter used for characterisation of genetic diversity of the cucurbits. In this study, 100% of the gourd SSRs showed a successful cross-transferability in *C. lanatus* and a moderately high transfer rate (33, 66 and 83%) was obtained in *C. pepo*, *T. occidentalis* and *T. cucumerina,* respectively, which is higher than the rates observed in *C. lanatus* (watermelon) (41%) and in the genus *Luffa* (loofah) (20%) (Xu et al., 2011). Additionally, Saxena et al. (2014) reported about (78.4%) bitter gourd (*Momordica charantia* L.) SSR markers were transferable to six other *Momordica* species, ranged from 32.5% to 75% which is lower than was observed in this study. The high rate of transferability among related species may be depended on phylogenetic relationships and genetic similarity among the studied cucurbit species/genera (Oliveira et al., 2006).

On the other hand, low transferability may be due to failure in SSR amplification likely resulting from mutation such as small/large insertions/deletions (indels) or base nucleotide substitutions at primer binding sites and lack of specificity (Li et al., 2002; Varshney et al., 2005). Similarly, the high cross-transferability of the *C. pepo* SSRs to other four target genera was recorded in this study. Our results are in accordance with many previous studies that have shown the high inter-species transferability (90%) of SSRs developed from *C. pepo* and *C. moschata* (Gong et al., 2008); 27 out of 40 *Cucurbita pepo* and *C. moschata* SSR markers were transferable to gourds (Yildiz et al., 2015). The high transferable rate of SSR from pumpkin to four other genera in this study may be due to highly conserved SSR regions between genomes of closely related plant species like Cucurbitaceae (Kalia et al., 2011). The transferability of cucumber SSR markers to *L. siceraria* (bottle gourd) germplasm has been reported previously (Bhawna et al., 2015).

In the present study, the 12 SSR markers used for genotyping 24 cucurbits were polymorphic,

indicating that they can be useful for assessing the genetic variability in cucurbit genera/species. The 6 bottle gourds SSR produced number of alleles ranged from 3 (LSR112) to 19 (LSR077), this finding was higher than the number of alleles, 3 (LSR074) to 9 (LSR015) obtained in a previous study reported by Mashilo et al. (2016). The mean PIC value of 0.72 is higher than those from the study of Bhawna et al. (2015) who studied the transferability of 19 cucumber microsatellite markers in 42 bottle gourd, showed PIC value of 0.33. In another study, a mean allelic number of 8.72 per locus and PIC of 0.67 were observed within 191 wild and cultivated *Cucumis melo* L. using 36 SSR markers. The current study suggests that the reliability of the markers in the study of genetic relationships and cultivar identification in five Curcubita genera. It is noteworthy that the gourd SSRs used in this study amplified all the 11 *L. siceraria* accessions despite variations in fruit shapes, thus detecting polymorphism among the accessions.

The dendrogram revealed genetic diversity and relationships in the five genera studied. As for theaccessions of *C. lanatus* and *L. siceraria* (speckled gourds), they are seen to be closely related, thereby demonstrating some level of conservation of genomic regions across genera. Genetic similarities between *C. lanatus* and *C. pepo* have been reported in a study using RAPD markers (Al- Anbari et al., 2015). The *C. lanatus* were placed side by side to other *T. occidentalis* species in our analysis, deciphering a closer genetic relationship between the two genera. Low genetic diversity was observed in the *T. occidentalis* accessions in this study while similar findings were also observed for *T. occidentalis* (12 accessions) using 10 RAPD marker analysis (Adeyemo & Tijani, 2018). The possible explanation for the low genetic variation may be as a result of a genetic bottleneck during the evolution of *Telfairia* and its domestication selection processes involving frequent self-fertilization.

Moreover, the extensive similarity in grouping structure between *T. occidentalis* and *T. cucumerina* species suggests a common ancestral genetic relatedness between the two genera of Cucurbitaceae. Furthermore, the dendrogram resulted in the grouping of species of *C. pepo*, *T. occidentalis* and *T. cucumerina* in the same group. This may be that the accessions in this study have a close genetic relationship. However, very few reports are available for diversity at an inter-generic level. It has been suggested that *C. pepo* are somewhat genetically divergent based on previous studies (Harry et al*.,* 2015; Kazmi´nska et al*.,* 2017).

On the other hand, dendrogram analysis showed a clear separation among the bottle gourd accessions belonging to the genus *Lagenaria*, thus elucidating the level of genetic relationship and species variation within the genus. The previous study by Mashilo et al. (2016) reported that genetic diversification was present amongst *L. siceraria* (bottle gourd) landrace collections from South Africa. Besides, Yildiz et al. (2015) reported that Turkish *L. siceraria* accessions were separated from the other cucurbits analysed in their study. Interestingly, *L. siceraria* (speckled gourd) aligned with other *L. siceraria* (bottle) and *C. lanatus*, possibly because of species relative wide genetic diversity exists within the two gourds.

Conclusion

In conclusion, we have shown the transferability of the bottle *L. siceraria* and *C. pepo* genomic SSR markers across other genera in the Cucurbitaceae. The transferable markers detected a considerable level of inter/intraspecific genetic diversity and relationship among cucurbit species of the five genera under study. Although additional SSRs could be used, the highly informative markers studied here could be considered for assessing genetic relatedness and accession identification in cucurbit species in future studies.

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