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Transferability of Simple Sequence Repeat (SSR) Markers Developed in *Litchi chinensis* to *Blighia sapida* (Sapindaceae)

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Abstract Ackee (*Blighia sapida*, Sapindaceae) is a multipurpose fruit tree species of high economic importance, native to the Guinean forests of West Africa, and belongs to the same family as that of lychee (*Litchi chinensis*). In this study, a set of 12 primer pairs for simple sequence repeats (SSRs) previously developed for lychee has been evaluated for polymorphism in 16 ackee trees from different populations. Seven primer pairs have been found to be transferable, and four have revealed polymorphisms. However, the average number of alleles per locus has dropped from 4.9 for lychee to 3.7 for ackee. Characterization of the four polymorphic markers in 279 individuals belonging to14 different ackee populations from Benin has revealed that the numbers of alleles per locus range from two to 14 with a mean number of 5.8. The observed and expected heterozygosities range between 0.020 to 0.359 and 0.020 to 0.396, respectively.

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Abbreviations

HWE	Hardy–Weinberg equilibrium
ICRAF	World Agroforestry Centre
LD	linkage disequilibrium
SSRs	simple sequence repeats
EST	expressed sequence tag
cDNA	complementary DNA

Introduction

Ackee (*Blighia sapida* K. Konig) is a multipurpose fruit tree species native to the Guinean forests of West Africa. Although overlooked by researchers in the region, the tree is highly valued by farmers and is an important component of different agroforestry systems in Benin. Recently, ackee has emerged as a high-priority species for domestication in Benin after a national survey and ranking of nontimber forest products (Codjia et al. 2003). *B. sapida* is widely cultivated in Jamaica where it has been introduced during the eighteenth century (ICRAF 2008) with an annual turnover of approximately $400 million in 2005 for the trade of arils of fruits (Pen 2006). Seeds and capsules of fruits are used for soap-making and for fishing, and all parts of the tree have medicinal uses (Ekué et al. 2004, Dossou et al. 2004; Ekué 2005).

To develop strategies for domestication and sustainable management of this species, information on the level of genetic variation and the extent of genetic diversity within and between natural and managed populations is vital. The development of microsatellites or simple sequence repeats (SSRs) provides an ideal tool to investigate genetic variation patterns due to their

codominant, multiallelic, and highly polymorphic properties. However, given the time-consuming and expensive process of isolating SSRs, it is advantageous to utilize primer sequences already identified in one species in other closely related species. Cross-species amplification and utility of molecular markers depend on the conservation of priming sites within flanking sequences and on the maintenance of arrays long enough to promote polymorphisms (FitzSimmons et al. 1995). Several studies have demonstrated the utility of using primer pairs designed from one species to other species of the same genus (Rallo et al. 2003; Pandey et al. 2004; Mottura et al. 2005; Takayama et al. 2008) and even to species of other genera (e.g., White and Powell 1997; Zucchi et al. 2002; Viruel and Hormaza 2004; Barreneche et al. 2004). Cross-species amplification was previously reported in longan (*Euphoria longan* (Lour.) Steud.) and pulasan (*Nephelium rambutan-ake* L.), both belonging to the subfamily Nepheleae as lychee (Viruel and Hormaza 2004; Sim et al. 2005).

In this study, cross-species transferability of 12 SSRs developed for *Litchi chinensis* Sonn. (Viruel and Hormaza 2004) to a new target species, *B. sapida*, belonging to the Sapindoideae subfamily was investigated.

Materials and Methods

Plant Material and DNA Analysis

Leaves were collected from 279 individual trees from 14 natural and planted populations covering the whole range of the species in Benin (between 6°54′ to 11°09′ N and 0°59′ to 3°28′ E) and dried in silica gel.

Total genomic DNA was extracted from ~1 cm^2 of dried leaf tissue using a DNeasy® Plant Kit (Qiagen, Hilden, Germany) and adding up to 2.6% polyvinylpyrrolidone K30 to the AP1 lysis buffer. Polymerase chain reaction (PCR) amplification was conducted in a 10-µL volume containing 1 µL of genomic DNA (10 ng), 5 µL of HotStarTaq Master Mix, 0.1 U/µL *Taq* DNA polymerase, 3 mM $MgCl_2$, 400 µM dNTPs (Qiagen, Hilden, Germany), and 5 pmol of each primer. The PCR protocol consisted of an initial denaturation step of 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, annealing for 1 min, 72°C for 1 min, and a final extension step of 72°C for 10 min. For primers LMLY4 and LMLY6, the duration of denaturation and annealing during 35 cycles was 1 min. All PCRs were conducted in a Peltier Thermal Cycler (PTC-200 version 4.0, MJ Research).

Amplified products were resolved electrophoretically on an ABI Genetic Analyzer 3100 together with an internal size standard GeneScan 500 ROX (fluorescent dye ROX) from Applied Biosystems. Fragment sizes were determined using software packages GENESCAN 3.7 and GENOTYPER 3.7 (Applied Biosystems).

The sequences of all 12 lychee primer pairs used are presented in Table 1. A total of 16 ackee genotypes from different populations were initially used to evaluate the amplification and polymorphisms of these lychee primer pairs, and those markers found to be polymorphic were then used to amplify all trees.

Table 1 Transferability of 12 SSR markers from *L. chinensis* to *B. sapida*

SSR marker	Repeat type	Presence of		T_a (°C)	Number of alleles			Allele size range (bp)		
		PCR band	Polymorphism		*Litchi chinensis*	*Blighia sapida*	*Nephelium rambutan-ake*	*Litchi chinensis*	*Blighia sapida*	*Nephelium rambutan-ake*
LMLY1	$(CT)_{11}TT(CT)_5$	No	No	–	10	–	–	132–214	–	–
LMLY2	$(GA)_8$	No	No	–	8	–	3	154–183	–	154–168
LMLY3	$(GA)_{18}$	Yes	No	48	3	1	–	178–190	156	–
LMLY4	$GAA(GA)GG(GA)_4$	Yes	Yes	45	4	2	1	204–210	182–183	182
LMLY5	$(GA)_9$	Yes	Yes	50	5	14	1	280–304	289–315	290
LMLY6	$(GA)_9(CA)_2(GA)_4$	Yes	Yes	39.4	3	2	4	146–154	122–135	132–154
LMLY7	$(CT)_{17}$	Yes	Yes	50	7	5	1	216–238	241–247	240
LMLY8	$(GA)_9$	Yes	No	48	4	1	1	288–302	282	285
LMLY9	$(GA)_3GGGAA(GA)_9$	No	No	–	3	–	–	92–96	–	–
LMLY10	$(CT)_{11}TT(CT)_5$	No	No	–	6	–	–	312–342	–	–
LMLY11	$(GA)_4GGAA(GA)_2G(GA)_4$	No	No	–	2	–	–	155–156	–	–
LMLY12	$(CT)_{11}$	Yes	No	48	4	1	3	204–209	203	195–217

All lychee SSRs were developed from a genomic DNA library enriched in CT repeats. Annealing temperature (T_a) for ackee, the number of alleles, and the allele size range for *L. chinensis* and *N. rambutan-ake* are adopted from Viruel and Hormaza (2004) and Sim et al. (2005), respectively

Table 2 Amplification frequency of *L. chinensis* SSR markers across three species of the Sapindaceae family

Species	Successful amplification of lychee primers		Polymorphic lychee SSR markers		Source
	Number	Percent	Number	Percent	
Blighia sapida	7	58.3	4	57.1	This study
Nephelium rambutan-ake	7	58.3	3	42.9	Sim et al. (2005)
Euphoria longan	11	91.7	8	72.7	Viruel and Hormaza (2004)
Litchi chinensis	12	100	12	100	

Data Analysis and Interpretation

An SSR marker was deemed transferable if the amplification of a PCR band of the expected size was observed (González-Martinez et al. 2004). The program MICRO-CHECKER (van Oosterhout et al. 2004) was used to check for scoring errors, allele dropouts, and null alleles. Observed (H_O) and expected (H_E) heterozygosity were computed for each polymorphic locus. Deviation from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were tested for all loci using GENEPOP 3.4 (Raymond and Rousset 1995). Diversity parameters were computed separately for natural and planted stands of ackee.

Results and Discussion

SSR Transferability

Out of 12 lychee primer pairs tested, seven yielded amplification products of expected sizes (58.3%; Table 2). A similar frequency of amplification was observed in *N. rambutan-ake* (Sim et al. 2005), while a higher frequency was observed in *E. longan* (91.7%; Viruel and Hormaza 2004) using the same set of primers.

This frequency of cross-genus transferability of SSR loci within the Sapindaceae family is much higher than that, 2.9% of 346 primer pairs, observed in the Myrtaceae family, from *Eucalyptus* spp. to *Eugenia dysenterica*. It is within the range (36.4% to 63.6%) observed in the Meliaceae family (from *Switenia humilis* to six different genera; White and Powell 1997; Zucchi et al. 2002). Similar to the presented results, transferability of SSRs developed in *Quercus robur* to *Castanea sativa* and vice versa within the Fagaceae family was possible with 47% (25) of the *Quercus* SSRs and 63% (19) of the *Castanea* SSRs showing amplification products in the nonsource species (Barreneche et al. 2004).

In general, cross-species transferability differs highly among taxa especially in flowering plants. It seems to be highest in species with long generation time and with an outcrossing breeding system like *B. sapida* (Barbará et al. 2007).

Among all 12 primer pairs tested, 11 were deemed simple markers and one (LMLY6) was deemed a compound marker (Table 1; Viruel and Hormaza 2004). Among those 11 simple SSRs, six were perfect and five were imperfect markers. Five of the six perfect markers and only one of the imperfect SSRs were transferable to ackee. A similar finding was observed in pulasan (Table 1) as reported by Sim et al. (2005). This apparent association between transferability and perfectness of the repeat sequence was previously suggested by Kutil and Williams (2001). It was reported that markers based on imperfect repeats were likely to be less conserved than those harboring perfect repeats as the "death" of a microsatellite was accompanied by the accumulation of interruptions and/or large deletions, and thus compound SSRs could, therefore, represent a late stage in microsatellite evolution.

Among those seven transferable lychee SSR markers to *B. sapida*, four were polymorphic (57.1%), while only three out of seven (42.9%) were polymorphic for *N. rambutan-ake* and eight out of 11 (72.7%) were polymorphic for *E. longan* (Table 2). When transferring 346 SSRs from *Eucalyptus* spp. to *E. dysenterica*, the percentage of polymorphic loci dropped to 70% (Zucchi et al. 2002). Rosetto (2001) summarized data from 19 studies and found that 58% of microsatellites were polymorphic within the same family and 78% within the same subgenus. Therefore, the results obtained in this study were within the range reported previously.

Table 3 Characteristics of four polymorphic SSR markers in *B. sapida*

Locus	Natural stands (n=109)			Planted stands (n=170)		
	N_a	H_O	H_E	N_a	H_O	H_E
LMLY4	2	0.149	0.137	2	0.082	0.095
LMLY5	13	0.359	0.396	10	0.328	0.364
LMLY6	2	0.020	0.020	2	0.078	0.071
LMLY7	5	0.084	0.103	5	0.064	0.062

N_a number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

In this study, a total of 26 alleles has been detected (3.7 alleles per marker). This is lower than those 59 alleles detected in lychee (4.9 alleles per marker), but considerably higher than those 14 alleles observed in *N. rambutan-ake* (two alleles per marker). In general, both numbers of alleles per locus and allele size were lower in ackee than in lychee, but with a few exceptions. The number of alleles obtained with SSR marker LMLY5 in ackee is three times that found in lychee, and sizes of alleles obtained with SRR markers LMLY5 and LMLY7 are higher in ackee than in lychee (Table 1).

The relatively high frequency of cross-transferability observed in this study is rather unexpected as ackee belongs to the Sapindoideae subfamily, while lychee, pulasan, and longan belong to the subfamily Nepheleae and are, therefore, more closely related. It is known that microsatellites derived from expressed sequence tags (ESTs), complementary DNA, and low-copy or undermethylated DNA libraries are more conserved and exhibit higher transferability than those derived from noncoding regions (Shepherd et al. 2002). In this study, lychee SSRs have been developed from a genomic DNA library enriched in CTs and are, therefore, expected to display lower frequencies of transferability compared to EST-SSRs. However, it has been previously reported that the conserved nature of SSRs derived from coding regions limits their observed levels of polymorphisms (Shepherd et al. 2002).

Characterization of Polymorphic Markers

Genetic variation estimates of the four polymorphic loci detected in this study are listed in Table 3. In natural stands of ackee, observed and expected heterozygosities ranged from 0.020 to 0.359 and from 0.020 to 0.396, respectively. Higher numbers of alleles were detected in natural stands (from two to 13 with a mean of 5.5) than in planted stands (from two to ten with a mean of 4.8) of ackee. Moreover, there was also a drop in both H_O and H_E in planted stands for all detected loci, except for LMLY6. A significant deviation from the HWE was observed in two natural stands of ackee, in Pobe for locus LMLY5 (P=0.007) and in Adjahonmey for locus LMLY7 (P=0.017). For a single planted stand (Kandi), deviation from HWE was observed for locus LMLY5 (P=0.009). Presence of null alleles, attributed to excess of homozygotes for most allele size classes, were detected for loci LMLY5 in Kandi and LMLY7 in Adjahonmey. This might explain the observed deviation from HWE for the Adjahonmey stand. Linkage disequilibrium among markers was significant (P<0.05) between LMLY4 and LMLY5 in a single planted stand (Glazoue) and between LMLY5 and LMLY7 in a single natural stand (Adjahonmey).

In summary, these findings provide additional evidence for the potential transferability of SSRs across genera. Previous studies covering many plant families have revealed that generally only a low percentage of markers amplify fragments from species belonging to different genera (Weising et al. 2005). The relatively high percentage of cross-transferability of lychee SSR markers to ackee observed in this study renders these markers useful for investigating the genetic diversity and structure of *B. sapida* populations.

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