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ORIGINAL PAPER

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Development, characterization and variability analysis of microsatellites in lychee (*Litchi chinensis* Sonn., Sapindaceae)

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Abstract We report 12 microsatellites enriched in CT repeats obtained from a genomic library of the lychee (*Litchi chinensis* Sonn.) cultivar Mauritius. The polymorphisms revealed by these microsatellites were evaluated in a collection of 21 lychee cultivars. A total of 59 fragments were detected with these 12 SSRs, with an average of 4.9 bands/SSR. Three primer pairs seem to amplify more than a single locus. The mean expected and observed heterozygosities over the 9 single-locus SSRs averaged 0.571 (range: 0.137–0.864) and 0.558 (range: 0.169–0.779) respectively. The total value for the probability of identity was 7.53×10^{-5}. In addition, the selected SSRs were used to amplify DNA from four longan cultivars. Eleven of the 12 SSRs produced amplification fragments in longan, and eight of these fragments were polymorphic. All except two of the products amplified from longan were the same size as those amplified from lychee, suggesting a close genetic proximity between the two species. The SSRs studied produced 22 different patterns, allowing the unambiguous identification of 16 lychee and the 4 longan cultivars studied. Discrimination was possible with just four selected microsatellites. Two groups with two and three undistinguishable cultivars were obtained, reflecting probable synonymies. Unweighted pair-group method of artimetic averages (UPGMA) cluster analysis divided the lychee cultivars studied into two main groups, one consisting of ancient cultivars and the other with more diverse recent cultivars. This is the first report of microsatellite development in the Sapindaceae, and the results demonstrate the usefulness of microsatellites for identification, similarity studies and germplasm conservation in lychee and related species.

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Introduction

The lychee, or litchi (*Litchi chinensis* Sonn.) is an evergreen tree native to southern China, northern Viet Nam, and the Malay peninsula, where it has been cultivated for centuries (Menzel and Simpson 1990). During the nineteenth and twentieth centuries its cultivation extended to other countries with tropical or subtropical climates (Morton 1987), although production in the Asia-Pacific region accounts for more than 95% of the world cultivation of about two million tonnes. The main producers are China, India, Thailand and Viet Nam (Menzel 2002). The lychee is a member of the soapberry or Sapindaceae family, which comprises over 2,000 species of tropical and subtropical trees, shrubs, herbs, and vines classified into about 150 genera (Menzel 2002). Other species of the family grown for their edible fruits, although to a lesser extent than the lychee, are the longan [*Euphoria longan* (Lour.) Steud.], the rambutan (*Nephelium lappaceum* L.) and the pulasan (*N. mutabile* Blume), all of which, including the lychee, belong to the subfamily Nepheleae (McConchie et al. 1994).

Due to the long history of lychee cultivation in China, where the first certain Chinese references date back 2,000 years (Morton 1987; Menzel and Simpson 1990), many cultivars are available in addition to seedling trees, although only about 15 are grown commercially (Menzel 2002). However, there is a lot of confusion regarding the naming of the cultivars since the current cultivar names derive from Cantonese and Mandarin names translated to English (Aradhya et al. 1995); consequently, problems derived from synonymies and/or homonymies are frequent in this species (Menzel and Simpson 1990). Moreover, the characterization of several cultivars is based on just a few fruit morphology and quality traits (Aradhya et al. 1995). Thus, a standardization of cultivar names and characterization should be carried out in this species, both for optimum germplasm management and for the establishment of appropriate breeding programs. Molecular markers can be of great interest to fulfill this objective but few molecular studies, restricted to isozyme

analysis (Aradhya et al. 1995; Degani et al., 1995a) and RAPDs (Anuntalabhochai et al. 2002), have been carried out in the lychee.

Among the molecular markers currently available, microsatellites or simple sequence repeats (SSRs) appear to be the marker of choice for genotype identification both in plants and animals due to their high polymorphism and reproducibility (Gupta and Varshney 2000; Wünsch and Hormaza 2002). Microsatellites are tandem repetitions of 1–6 bp nucleotide motifs found in all genomes analyzed to date, and have been widely used as genetic markers in different organisms since their first description in 1989 (Litt and Luty 1989; Tautz 1989; Weber and May 1989). The major drawback of microsatellite markers used to be the difficulty and cost of the isolation process which usually involves the construction and screening of genomic libraries to identify the sequences flanking the repeat regions. The low yield obtained using conventional library screening approaches has been greatly improved by the introduction of efficient library enrichment methods (Zane et al. 2002).

In this paper, we report the development for the first time of a set of 12 microsatellite markers for the lychee, using a genomic library enriched in CT repeats. Their polymorphisms have been studied in a group of 21 lychee and 4 longan cultivars, and their value for cultivar identification and variability analysis in these species is discussed.

Table 1 Lychee and longan cultivars studied. The first 21 cultivars correspond to lychee, and the last 4 to longan

Cultivar	Source	Reported origin
B2	Australia	-
Bengal	Australia	China[b]
Calcuttia Late	Canary Islands	India[b]
Chacapat	Australia	Thailand[c]
Fay Zee Siu	Australia	China[a]
Gee Kee	Australia	China[c]
Groff	Australia	USA (Hawaii)[a,b,c]
Haak-Yip	Australia	China[a,b]
Kaimana	Australia	USA (Florida)[b,c]
Kwai May Pink (B3)	Australia	China[a]
Kwai May Red (B10)	Australia	China[a]
Lenz	Canary Islands	USA[d]
Mauritius	Australia	China[a]
No Mai Chee 1	Canary Islands	China[b]
No Mai Chee 2	Canary Islands	China[b]
Sah Keng	Australia	Taiwan[c]
Salathiel	Australia	Australia[c]
Souey Tung	Australia	China[a,b]
Sum Yee Hong	Canary Islands	China[a,b]
Tai So	Australia	China[a]
Wai Chee	Australia	China[a,b]
Funk-How	Australia	Thailand
Biew Kiew	Thailand	Thailand
Choom Poo	Thailand	Thailand
Duan Yu	Australia	Thailand

[a] Watson et al. 1988
[b] Menzel and Simpson 1990
[c] Menzel and Simpson 1991
[d] Galán-Saúco et al. 2001

Materials and methods

Plant materials and genomic DNA extraction

Twenty-one lychee and 4 longan cultivars maintained at the Estación Experimental La Mayora-CSIC experimental orchards in Algarrobo-Costa, Spain, were analyzed. These genotypes were obtained from a collection in the ICIA (La Laguna, Canary Islands, Spain) or imported from Australia or Thailand (Table 1). In this paper, we have followed the standard cultivar names and spelling suggested by Watson et al. (1988) except for 'Seong Sue Wai' that has been shown to be different from 'Gee Kee' (Menzel and Simpson 1991).

Genomic DNA was extracted from fresh leaves following the method described by Fulton et al. (1995) modified with the addition of 1% sodium bisulfite to the extraction buffer.

Construction and screening of a microsatellite enriched library

A small-insert library was developed from DNA of the lychee cultivar Mauritius digested with *Mse*I and enriched with $(CT)_n$ sequences following the procedures of Billote et al. (1999) and Hamilton et al. (1999) with some modifications. The selective hybridization process involved the biotinylation of the $(CT)_n$ oligonucleotide and the selection of the hybridized DNA on streptavidin-coated magnetic beads. After selective hybridization, the captured fragments were recovered by PCR and cloned using the pGEM-T Easy plasmid (Promega, Madison, Wis.) in *Escherichia coli* ultracompetent cells (Epicurian coli XL1 Blue ultracompetent cells, Stratagene, La Jolla, Calif.) following the manufacturer's recommendations. The recombinant clones were PCR amplified and transferred to a nylon membrane that was hybridized with a $(CT)_{15}$ probe labeled with digoxigenin (DIG oligonucleotide 3′-end labeling kit, Roche Applied Science, Indianapolis, Ind.). Positive clones were then sequenced to identify the flanking regions that were used to design appropriate primer pairs with the program Primer3 (Whitehead Institute for Biochemical Research, Cambridge, Mass.).

SSR analysis

The primers obtained were initially used to study a reduced group of four lychee cultivars by PCR amplification in 15 μl volumes containing 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.01% Tween20, 2 mM $MgCl_2$, 0.1 mM of each dNTP, 0.4 μM of each primer, 25 ng genomic DNA and 0.5 units of BioTaq DNA polymerase (Bioline, London). Reactions were carried out in an I-cycler (Bio-Rad Laboratories, Hercules, Calif.) thermocycler using the following temperature profile: an initial step of 1 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, then a final step of 5 min at 72°C. Amplification products were resolved on 3% high resolution agarose gels (Hormaza 2002) and the primers that showed clear and scorable amplification patterns were selected for further SSR analysis. SSRs in all the lychee and longan genotypes were analyzed using a CEQ 2000XL capillary DNA analysis system (Beckman Coulter, Fullerton, Calif.). PCR reactions were performed as previously described, except that reverse primers of each primer pair were labeled with WellRED fluorescent dyes D2, D3 and D4 (Proligo, Paris). The annealing temperature for all the primer pairs was 50°C. The analyses were repeated at least three times to assure the reproducibility of the results.

The allelic composition of each accession and the number of total alleles was determined for each SSR marker. Putative alleles were indicated by the estimated size in bp. The genetic information was assessed for only the single locus microsatellites, using the following parameters: number of alleles per locus (A), observed heterozygosity (Ho), expected heterozygosity ($He=1-\sum pi^2$ where pi is the frequency of the ith allele), Wright's fixation index (F=1-

Ho/He) (Wright 1951) and the probability of identity (PI=1-$\sum pi^4$+ $\sum\sum(2pipj)^2$, where pi and pj are the frequency of the *i*th and *j*th alleles respectively) that measures the probability that two randomly drawn diploid genotypes will be identical assuming observed allele frequencies and random assortment (Paetkau et al. 1995). All the parameters were calculated with the program IDENTITY 1.0 (Centre for Applied Genetics, University of Agricultural Sciences, Vienna).

Genetic diversity

Genetic relationships among the genotypes studied were calculated using UPGMA cluster analysis of the similarity matrix obtained from the proportion of shared alleles from the single locus SSRs (Nei and Li 1979). The cophenetic coefficient was computed for the dendrogram after the construction of a cophenetic matrix. All those analyses were computed with the program NTSYSpc 2.11 (Exeter Software, Stauket, NY).

Results

Microsatellite development

A total of 380 clones from the enriched genomic library were randomly chosen and screened by hybridization with the probe $(CT)_{15}$. One hundred and ninety-nine gave a positive signal, an enrichment percentage of 52%. Forty-two of these clones were sequenced, producing a total of 30 readable sequences, all containing at least one $(CT)_n$/$(GA)_n$ repeat. Four sequences were rejected due to the proximity of the microsatellite to the end of the insert. Finally, specific primers were designed for 26 microsatellite sequences with melting temperatures ranging between 50ºC and 55ºC and producing amplification fragments of 90–350 bp. No redundant clones were detected.

The 26 selected primer pairs were pre-screened on four individual plants. From those, 18 (69%) produced clear repeatable amplification patterns and were used to analyze all the lychee and longan cultivars. Five of these primer pairs were discarded because they gave unreadable patterns, 1 produced a monomorphic pattern, and 12 (67%) detected polymorphisms among the lychee cultivars studied. These 12 microsatellites were classified into simple and compound, and each class into perfect or imperfect (Weber 1990; Varshney et al. 2000). Eleven microsatellites were simple and only one was compound. The compound microsatellite was imperfect, and from the group of 11 simple microsatellites, 6 were perfect, with 8–18 motifs, and 5 were imperfect, with 2–11 uninterrupted motifs (Table 2). Microsatellite sequences have been deposited in Gen Bank and sequences of primer pairs are available at www.eelm.csic.es.

SSR polymorphisms in the lychee cultivars

The level of polymorphism of these 12 SSRs was investigated in all 21 lychee cultivars. The parameters of variability analyzed for these SSRs are presented in Table 2. A total of 59 bands were detected, with an average of 4.9 bands/SSR. With LMLY1 and LMLY2 most of the genotypes had more than two bands, suggesting the amplification of at least two different loci. LMLY10 behaved as a single locus in all but four cultivars ('Kway May Red', 'Gee Kee', 'No Mai Chee 1' and 'No Mai Chee 2'). For the remaining nine SSRs, although the absence of segregation data makes it impossible to attribute the alleles to their corresponding locus, the banding patterns obtained were consistent with their inheritance as single locus. These SSRs detected 35 alleles, ranging from 2 to 7, with an average of 3.9 alleles per locus. Allele frequencies ranged from 0.023 to 0.910 (mean=0.257). Eleven (31%) were rare alleles ($P<0.1$) and only one (3%) was almost fixed ($P>0.9$) with a probability of 0.910. The mean of the probabilities was 0.238 when this allele was discarded. Some alleles were exclusive to certain cultivars; thus, 'Fay-Zee Siu' presented a unique allele, 'Lenz' two unique alleles, and a group of three undistinguishable cultivars ('Kway May Red', 'No Mai Chee 1' and 'No Mai Chee 2') presented two exclusive alleles. The expected heterozygosity ranged from 0.137 in LMLY11 to 0.864 in LMLY3 (mean=0.571). The observed heterozygosity ranged from 0.169 in LMLY6 to 0.779 in LMLY7 (mean=0.558). The

Table 2 SSR description and variability parameters for 12 polymorphic microsatellites in 21 lychee cultivars

SSR	Repeat type	Number of alleles (A)	Allele size range (bp)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Wright's fixation index (F)	Probability of identity (PI)
LMLY1[a]	$(CT)_{11}TT(CT)_5$	10	132–214	-	-	-	-
LMLY2[a]	$(GA)_8$	8	154–183	-	-	-	-
LMLY3	$(GA)_{18}$	3	178–190	0.864	0.577	-0.497	0.383
LMLY4	$GAA(GA)GG(GA)_4$	4	204–210	0.273	0.621	0.560	0.299
LMLY5	$(GA)_9$	5	280–304	0.727	0.677	-0.074	0.280
LMLY6	$(GA)_9(CA)_2(GA)_4$	3	146–154	0.182	0.169	-0.077	0.910
LMLY7	$(CT)_{17}$	7	216–238	0.818	0.779	-0.050	0.135
LMLY8	$(GA)_9$	4	288–302	0.636	0.478	-0.331	0.432
LMLY9	$(GA)_3GGGAA(GA)_9$	3	92–96	0.818	0.641	-0.276	0.358
LMLY10[a]	$(CT)_{11}TT(CT)_5$	6	312–342	-	-	-	-
LMLY11	$(GA)_4GGAA(GA)_2G(GA)_4$	2	155–156	0.137	0.375	0.635	0.601
LMLY12	$(CT)_{11}$	4	204–209	0.682	0.705	0.033	0.262

[a] Indicates a multi-locus SSR in lychee cultivars

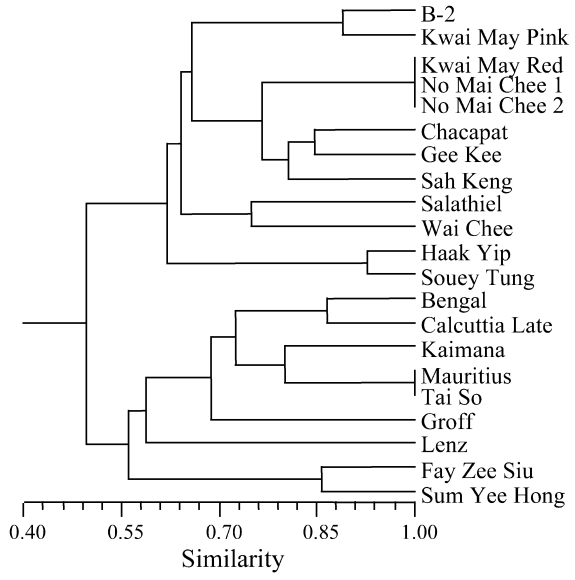

Fig. 1 Dendrogram of the 21 lychee cultivars studied based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient after amplification with 12 pairs of SSR primers

expected and observed heterozygosity values were compared using the fixation index (F), which had an average over all the markers of –0.009, with values between –0.497 (LMLY3) and 0.635 (LMLY11). For six loci this parameter was negative (excess of heterozygotes observed) and for three the F value was positive (excess of homozygotes observed). The maximum probability of identity was detected in LMLY6 (0.910), and the minimum in LMLY7 (0.135), while the total probability of identity (the probability of two cultivars sharing the same genetic profile by chance) was 7.53×10^{-5}.

The DNA of the four longan cultivars studied was correctly amplified with 11 of the 12 SSRs that were polymorphic in the lychee cultivars, and 8 of these detected variation among all 4 longan cultivars. Except for LMLY6, where two of the alleles amplified in the longan were absent in the lychee cultivars studied, the amplification products obtained in the longan were also present in the lychee. However, LMLY1, which shows more than one locus in the lychee, behaved as a single locus SSR in the longan cultivars analyzed.

Cultivar identification and genetic relationships among the different lychee accessions

Sixteen of the 21 lychee and the 4 longan cultivars analyzed could be unambiguously identified by the 12 polymorphic loci studied. Two groups of three ('Kway May Red', 'No Mai Chee 1' and 'No Mai Chee 2') and two ('Mauritius' and 'Tai So') lychee cultivars remained undistinguished. The differentiation of these 21 genotypes could be made with just 4 selected microsatellites (LMLY7, LMLY12, LMLY5 and LMLY4).

Similarity values between the different lychee cultivars ranged from 0.23 ('Chacapat'-'Fay Zee Siu' and 'Wai Chee'-'Fay Zee Siu') to 0.93 ('Souey Tung' - 'Gee Kee'). The dendrogram obtained after UPGMA analysis with the 21 lychee cultivars using the single locus SSRs is shown in Fig. 1. A single tree was obtained with a cophenetic correlation coefficient between the cophenetic matrix and the similarity matrix of 0.8. Two main groups were obtained.

Discussion

Microsatellite development

In this study, using a genomic library enriched for CT/AG repeats, 12 polymorphic microsatellites have been developed and screened in a sample of 21 lychee and 4 longan cultivars. This is the first report on the development of microsatellites in the Sapindaceae. The microsatellite enrichment procedure was highly successful, since over 50% of the clones obtained contained microsatellite sequences and, in consequence, large numbers of microsatellites could be obtained from a single enriched library.

The shortest perfect microsatellite contained eight repeats, whereas, among the imperfect microsatellites, the longest uninterrupted motif repeat was two repeats, in the polymorphic locus LMLY11. The same percentages (50%) of perfect and imperfect microsatellites were present among the polymorphic loci, but the majority were simple since only one of the 14 microsatellite sequences contained a compound repeat type, a $(CA)_n$ motif associated with the isolated motif (GA). It has been suggested that the percentage of compound microsatellites is related to the type of enrichment used (Van de Wiel et al. 1999) and the dinucleotide repeat isolated (Milbourne et al. 1998).

SSR polymorphism and cultivar identification

All the variability parameters calculated for the SSRs described in this work were higher than those previously reported for isozymes in lychee (Aradhya et al. 1995; Degani et al. 1995a). The high values of the proportion of polymorphic loci (67%), average number of alleles per locus (A=3.9), expected heterozygosity (He=0.571) and total probability of identity (7.53×10^{-5}) indicate that SSR markers will become a useful tool for genetic variation studies and for genotype identification and similarity analyses in the lychee.

The mean F obtained was close to zero (–0.009), indicating that the collection of 21 lychee cultivars analyzed behaved similarly to a random mating population. This suggests that the narrow genetic base present in commercial lychee cultivars has not resulted in high levels of homozygosity. One explanation for this could be that although lychee appears to be self-compatible,

outcrossing is important in this species. Lychee flowers are functionally unisexual and the overlap between male and female flowers is only partial within a cultivar (Stern and Gazit 1996). However, a higher survival of hybrid versus selfed fruits has been reported (Degani et al. 1995b). Most of the cultivars analyzed in this study are ancient, and only some of them are selections obtained after open-pollination (Menzel and Simpson 1991). Thus, the high levels of observed heterozygosity could be due to empirical selection in favor of heterozygous genotypes, which usually show wider adaptability and yield stability in traditional agro-ecosystems (Aradhya et al. 1995). Those selected genotypes would have been maintained later by vegetative propagation.

Cross-species amplification of longan DNA with lychee SSR primers

Eleven of the 12 polymorphic lychee SSRs produced scorable amplification fragments in the 4 longan cultivars analyzed under the same PCR conditions. Eight of these SSRs were polymorphic among the longan cultivars. All the alleles detected by these 11 SSRs were of the same size in longan and in lychee, except 2 (136 and 138 bp in LMLY6), which were shorter in the longan cultivars. It has been suggested that a greater genetic distance implies a decrease both in the ability to amplify the SSR loci (Steinkellner et al. 1997) as well as a shortening in the length of the alleles obtained (Ellegren et al. 1995), probably due to the bias in favor of longer repeat regions during the process of microsatellite development (Perry and Bousquet 1998). The high conservation of fragment sizes between lychee and longan indicates that lychee microsatellites will also be useful as markers for genetic studies in longan. Moreover, this conservation of allele size between the two species probably reflects the taxonomic proximity between them. In fact, the longan is also native to China, with a distribution pattern similar to that of the lychee (Morton 1987). Both species are classified in the same subfamily, Nepheliaeae, and intergeneric hybridization between them is possible (McConchie et al. 1994).

Cultivar identification and genetic relationships in lychee and longan

Eighteen different genotype profiles were obtained with a minimum combination of 4 SSRs allowing the unambiguous identification of 16 lychee cultivars and the 4 longan cultivars studied. Only two groups of lychee cultivars remained undistinguishable. One of them is formed by ‘Mauritius’ and ‘Tai So’ that have been described as the same cultivar or sport mutations (Watson et al. 1988; Menzel and Simpson 1986). The other group is formed by ‘Kwai May Red’, and two accessions of ‘No Mai Chee’. The results obtained indicate that these two accessions could, in fact, belong to the same cultivar. Moreover, it seems that nearly all older introductions of ‘No Mai Chee’ in Australia may in fact be ‘Kwai May Red’ (Watson et al. 1988). It is thus likely that those three genotypes indeed correspond to ‘Kwai May Red’ (also called B-10) (Fig. 1). An analysis of the original individuals of each cultivar should be carried out to confirm this point.

On the other hand, other cultivars frequently considered as synonyms (Menzel and Simpson 1986) present clearly different patterns (‘Gee Kee’/‘Wai Chee’/‘Salathiel’, and ‘Haak Yip’/‘Souey Tung’) even if they are close in the dendrogram. This genetic proximity could be the reason behind their phenotypic homology. Our results also confirm, in our collection, differences between additional cultivars frequently treated as homonymies. This is the case for ‘Souey Tung’, called ‘Groff’ in Australia (Watson et al. 1988), or ‘Fay Zee Siu’ called ‘Haak Yip’ (Aradhya et al. 1995). Similar results have been obtained with these cultivars using isozymes (Degani et al. 1995a).

UPGMA cluster analysis of the nine single locus SSRs separated the lychee cultivars into two groups (Fig. 1). In general, the first major cluster includes ancient cultivars with a very long history of cultivation (500–600 years) in China as ‘No Mai Chee’, ‘Kway May Red’, ‘Kway May Pink’, ‘Gee Kee’, ‘Wai Chee’, ‘Haak Yip’ and the most recent, ‘Souey Tung’, only 100 years old (Menzel and Simpson 1990). This cluster also includes recent cultivars related to the ancient cultivars, such as ‘Sah Keng’, developed in Taiwan in the1970s from seedlings of ‘Haak Yip’ (Menzel and Simpson 1986), and ‘Salathiel’, of unknown origin, but frequently related to ‘No Mai Chee’ (Menzel 2002). The presence of ‘Chacapat’, introduced from Thailand, in this group, supports its probable Chinese origin (Menzel and Simpson 1991).

The second major cluster contains more diverse and recent cultivars. One subcluster contains four different cultivars related to ‘Mauritius’-‘Tai So’: the two Indian cultivars, ‘Bengal’, (a seedling of the Indian cultivar Purbi) and ‘Calcutta Late’ and two selections from Hawaii, ‘Kaimana’ and ‘Groff’ (Menzel and Simpson 1991). The most distant cultivars in this cluster were the Chinese cultivars ‘Fay Zee Siu’, also reported by Degani et al. (1995a) as one of the most distant cultivars in their study, and ‘Sum Yee Hong’, and a cultivar from the United States called ‘Lenz’.

Some of the cultivars analyzed in this work have been described as selection of other cultivars (Menzel 2002, Menzel and Simpson 1991). Three are possible seedlings of ‘Haak Yip’: ‘Sah Keng’, developed in Taiwan in the1970s, ‘Groff’ and ‘Kaimana’, both from Hawaii. The availability of codominant molecular markers, such as microsatellites, opens the possibility to confirm those relationships. Thus, genotypes of five SSRs in ‘Groff’, two in ‘Kaimana’ and one in ‘Sah Keng’ do not support the paternity of ‘Haak Yip’ although previous studies with isozymes (Degani et al. 1995a) supported the possible paternity of ‘Haak Yip’ for those cultivars. The differences between the results obtained using isozyme analysis

and SSRs could be due to at least two reasons. First, the lower overall polymorphism revealed by isozyme analysis is perhaps not sensitive enough to accurately establish the paternity of the cultivars. Second, since several genotypes known as 'Haak Yip' have been shown to be genetically different (Degani et al. 1995a), 'Kaimana' and 'Sah Keng' could reflect the heredity of alleles present in the different accessions known as 'Haak Yip'. In the case of 'Groff', we found a high number of alleles which differed from those of 'Haak Yip' present in our collection, in agreement with the study of Aradhya et al. (1995) who also discarded the paternity of 'Haak Yip'. In fact, 'Groff' was selected from a population of 500 seedlings of open-pollinated 'Tai So', 'Brewster' and 'Haak Yip' cultivars (Menzel and Simpson 1990). Our results show that 'Groff' could indeed be a descendant of 'Tai So' whereas the 'Haak Yip' cultivar used to produce 'Groff' would be different from that in our collection.

The mislabeling of lychee cultivar names and/or homonymies and synonymies in different places, and even within a given country, leads to confusion for lychee researchers, resulting in misunderstandings of the genetic relationships among cultivars. Despite the large number of lychee cultivars available, only about 15 are grown commercially in China while in other countries commercial orchards are based on a single or a small number of cultivars (Menzel and Simpson 1990). Only a few cultivars have been developed in the last 60 years by selection among existing cultivars or among open-pollinated seedlings from such cultivars (Menzel and Simpson 1990). Consequently, all currently grown cultivars are related to a few traditional Chinese cultivars, and are relatively few generations removed from their wild ancestors, resulting in a narrow genetic base. As suggested by Aradhya et al. (1995) most lychee germplasm collections are, in fact, collection of genotypes, irrespective of their cultivar names or other designations. One of the origins of this confusion is the movement of cultivars among different countries. Since, as most fruit tree species, the lychee is vegetatively propagated, cultivar identification allows a standardizable reference for the identification and control of the propagation of any genotype, independently of the factors that may limit or influence traditional phenotypic characterization. Furthermore, the possibility of studying the genetic diversity among cultivars and populations will benefit lychee breeding programs, by facilitating decisions on parental genotypes for crosses, and for germplasm management to maximize the conserved diversity, especially important in the lychee due to the narrowness of the genetic base. The use of neutral, codominant, and highly informative markers, such as microsatellites, is essential to fulfill those objectives. Moreover, the transportability of these markers to other members of the Sapindaceae, such as the longan, will be useful to extend these approaches to other species genetically related to lychee.

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