Transferability of SSR markers from lychee (*Litchi chinensis* Sonn.) to pulasan (*Nephelium ramboutan-ake* L.)

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Transferability of SSR markers from lychee (*Litchi chinensis* Sonn.) to pulasan (*Nephelium ramboutan-ake* L.).

Abstract — Introduction. Pulasan and lychee are from the same family and closely related, therefore the SSR markers are expected to be highly transferable between these two taxa. We investigated the transferability of 12 lychee (*Litchi chinensis* Sonn.) simple sequence repeat (SSR) loci to pulasan (*Nephelium ramboutan-ake* L.). **Materials and methods**. Genomic DNA was extracted from 20 accessions of pulasan for the PCR amplification of the SSR loci using 12 pairs of SSR primers derived from lychee. The PCR products were resolved on denaturing polyacrylamide gels. **Results**. The percentage of SSR transferability from lychee to pulasan was 58.3% and the percentage of polymorphic SSR markers was 25%. **Discussion**. The moderate transferability and low polymorphism rates suggest the possibility of interruptions within the repeat motif and mutations in the flanking sequences of SSR repeat motifs. Our results did not reveal a high rate of transferability between lychee and pulasan. However, this study showed that the SSR markers developed in lychee are a good source of molecular markers for pulasan.

Malaysia / *Litchi chinensis* / *Nephelium ramboutan-ake* / molecular biology / genetic markers / PCR / genetic polymorphism

Transférabilité de marqueurs SSR du litchi (*Litchi chinensis* Sonn.) au kapulasan (*Nephelium ramboutan-ake* L.).

Résumé — Introduction. Kapulasans et litchis sont de la même famille et étroitement liés, on s'attendrait donc à ce que les marqueurs SSR soient fortement transmissibles entre ces deux taxa. Nous avons testé la transférabilité de 12 marqueurs SSR du litchi (*L. chinensis* Sonn.) au kapulasan (*N. ramboutan-ake* L.). **Matériel et méthodes**. De l'ADN génomique a été extrait de 20 accessions de kapulasan afin d'amplifier des loci SSR en utilisant 12 paires d'amorces issues de litchi. Les produits issus de PCR ont été révélés sur gels de polyacrylamide. **Résultats**. Le pourcentage de la transférabilité des marqueurs SSR du litchi au kapulasan a été de 58,3 % et le pourcentage de marqueurs SSR polymorphes a été de 25 %. **Discussion**. La transférabilité modérée et les faibles taux de polymorphisme obtenus suggèrent que des interruptions aient pu intervenir dans le processus de répétition des séquences et que des mutations aient pu avoir lieu dans les séquences de motifs répétés des SSR. Nos résultats n'ont pas révélé un taux élevé de transférabilité entre le litchi et le kapulasan. Cependant, notre étude a prouvé que les marqueurs SSR développés pour le litchi sont une bonne source de marqueurs moléculaires pour le kapulasan.

Malaisie / *Litchi chinensis* / *Nephelium ramboutan-ake* / biologie moléculaire / marqueur génétique / PCR / polymorphisme génétique

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1. Introduction

Pulasan (*Nephelium ramboutan-ake* L.) and lychee (*Litchi chinensis* Sonn.) are members of the soapberry in the family Sapindaceae, which comprises over 1 350 species of tropical and subtropical trees, shrubs, herbs and vines, classified into 140 genera [1]. In Malesian regions, 42 genera with approximately 235 species have been recorded [1]. Pulasan and lychee are grouped together with longan (*Dimocarpus longan* Lour.) and rambutan (*Nephelium lappaceum* L.) to form the tribe Nephelieae under the subfamily Sapindoideae [1]. Pulasan is said to originate from Java, Borneo and the Philippines [2]. It is now cultivated as a fruit crop throughout Malaysia, Thailand and Indonesia, though it is less common compared with rambutan. Pulasan is a dioecious plant and, to avoid having male plants in a plantation, it is mainly cultivated by means of grafting. Pulasan has a basic chromosome number, (x = 11), compared with that of lychee, (x = 14 or 15) [1]. The occurrence of polyploidy in Sapindaceae is rather scarce.

Simple sequence repeats (SSRs), or microsatellites, are DNA fragments containing tandem repeats of a short sequence (1 to 6 nucleotides). The characteristics of SSR markers are abundance, co-dominance, high polymorphism, high reproducibility and ease of assay by polymerase chain reaction (PCR). However, the major drawbacks of SSR markers are the laborious procedures and high cost of the isolation process, which usually involves the construction and screening of genomic DNA libraries to identify the sequences flanking the repeat regions for primer design.

Recently, comparative genetics revealed that gene content and order are highly conserved among closely related species [3]. Thus, primer pairs designed on the basis of the sequences obtained from one species could be used to detect SSRs in related species. This process depends on the conservation of priming sites within the flanking sequences to enable amplification, and on the maintenance of repeat arrays long enough to promote polymorphism [4]. SSR transferability across related species and genera makes these markers powerful for comparative genetic studies [5, 6]. Genetic studies using SSR markers have been carried out in various important crops such as *Triticum* [3], *Olea* [7], *Medicago* [8], *Oryza* [9], *Saccharum* [10] and *Picea* [11]. However, study of the development of SSR markers in the family Sapindaceae, particularly the subfamily Sapindoideae, is still scarce.

Recently, Viruel and Hormaza, using a genomic DNA library enriched in CT repeats, developed twelve SSR markers for lychee [12]. These SSR markers were found to be highly polymorphic in lychee [12] and, though limited in number, represent the only SSR markers currently available for lychee. Lychee and pulasan are closely related as they are from the tribe Nephelieae. Therefore, there is a high probability of application of the lychee SSR markers in pulasan. In this study, we report the transferability of the twelve SSR markers derived from lychee to pulasan.

2. Materials and methods

2.1. Plant materials

Ten pulasan accessions were sampled from the MARDI (Malaysia Agriculture Research and Development Institute) pulasan germplasm collections in Kemaman (Terengganu) and ten other pulasan accessions were sampled in Serdang (Selangor). The accession numbers of the 20 pulasans are T4, T11, T12, T15, T20, T23, T25, T28, T30, T32, T35, T38, T40, T44, T48, T50, T52, T54, T58 and T60. These pulasan accessions sampled in the germplasms were collected from various parts of Malaysia.

2.2. DNA extraction

Genomic DNA was isolated from dried leaf materials using the CTAB method [13] with minor modifications. Two grams of silica-gel-dried leaf materials and 4 mL 2× CTAB buffer (2% CTAB, 1.4 mM NaCl, 20 mM EDTA, 100 mM Tris-HCL at pH = 8, 1% PVP–40, 0.2% β-mercaptoethanol) were adopted in the modifications. The crude DNA extract was then purified by phenol-chloroform extraction following the method of Sambrook

Table I.
Results of transferability of SSR markers from lychee to pulasan with twelve SSR markers developed for lychee using a genomic DNA library enriched in CT repeats [12].
Yes or no: indicate successful or non-successful amplification or polymorphism of PCR product.

SSR marker	Repeat type	Presence of PCR band	Presence of polymorphism	Number of alleles[1]		Allele size range[1] (bp)	
				Lychee	Pulasan	Lychee	Pulasan
LMLY1	$(CT)_{11}TT(CT)_5$	No	No	10	–	132–214	–
LMLY2	$(GA)_8$	Yes	Yes	8	3	154–183	154–168
LMLY3	$(GA)_{18}$	No	No	3	–	178–190	–
LMLY4	$GAA(GA)GG(GA)_4$	Yes	No	4	1	204–210	182
LMLY5	$(GA)_9$	Yes	No	5	1	280–304	290
LMLY6	$(GA)_9(CA)_2(GA)_4$	Yes	Yes	3	4	146–154	132–154
LMLY7	$(CT)_{17}$	Yes	No	7	1	216–238	240
LMLY8	$(GA)_9$	Yes	No	4	1	288–302	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	Yes	4	3	204–209	195–217

[1] The number of alleles and the allele size range for lychee are adopted from Viruel and Hormaza [12].

and Russel [14]. The purity and concentration of the DNA were determined by comparison with λ*Hind*III DNA Marker (Promega, USA) through gel electrophoresis.

2.3. SSR transference

Transference is defined here as the positive amplification of a PCR band of the expected size [15]. Twelve pairs of SSR primers (LMLY 1 to LMLY 12) from lychee [12] were synthesized and employed to screen the 20 accessions of pulasan by means of PCR amplification. PCR amplification was carried out in 12.5 µL reaction mixture containing 25 ng template DNA, 2.5 µM SSR primers, 200 µM each of the dNTPs, 2.5 mM $MgCl_2$, 1 × PCR buffer and 0.5 U *Taq* DNA polymerase (Invitrogen, USA). The PCR amplification was carried out in a GeneAmp® PCR System 2400 thermocycler (Perkin Elmer, USA) using the same thermal profiles as those of lychee [12] with a minor modification in the annealing temperature to obtain the optimum amplification. The annealing temperature ranged from (50 to 53.5) °C.

Products from PCR amplification were initially assessed on 1.5% agarose gel. Products of positive amplifications were then resolved on a denaturing polyacrylamide gel (7% acrylamide/bisacrylamide [19:1], 7 M urea and 1 × TBE). The gel was run at constant voltage of 250 V for (3.5 to 4) h using PROTEAN® II XI (Bio-Rad, USA). The gel was stained following the silver staining method [16].

3. Results

Our study was carried out to determine the transferability of lychee SSR markers to pulasan. Of the twelve lychee SSR markers, seven (LMLY 2, LMLY 4, LMLY 5, LMLY 6, LMLY 7, LMLY 8 and LMLY 12) were able to amplify in all the 20 pulasan accessions (*table I, figure 1*). This gave the percentage of successful amplification of lychee SSR markers on pulasan of 58.3%. This value was considered moderate compared with the transferability of the same set of SSR markers from lychee to longan, *i.e.*, 91.7% [12], and

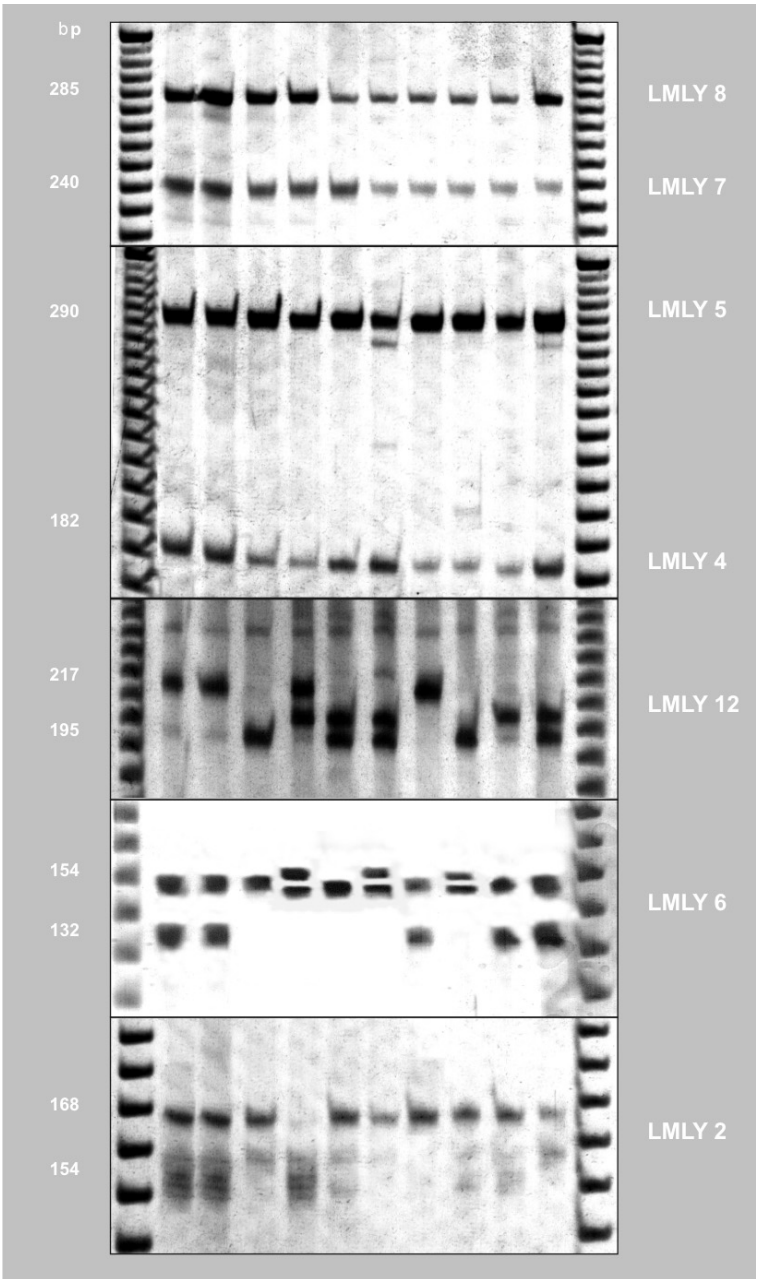

Figure 1.
Silver-stained denaturing polyacrylamide gel electrophoresis of PCR products of seven lychee SSR markers on ten pulasan accessions. A 10-bp ladder (Invitrogen, USA) was loaded on the first and last lanes of the gels. The number on the right indicates the size range of fragments for each SSR marker.

from *Quercus* to *Castanea* and *vice versa*, respectively, 47% and 63% [17]. The seven SSR markers were amplified well in the pulasan though stutter bands appeared in LMLY 2.

A total of 14 bands/alleles was detected in the 20 pulasan accessions (*table I*). Generally, the sizes of the alleles in pulasan, for each of the amplified SSR markers, did not differ much from those of lychee. Four SSR markers (LMLY 4, LMLY 5, LMLY 7 and LMLY 8) showed monomorphic characteristics in pulasan. On the other hand, the SSR markers LMLY 2, LMLY 6 and LMLY 12 were polymorphic, with LMLY 6 exhibiting the highest number of alleles, *i.e.*, four. Three alleles were detected each in LMLY 2 and LMLY 12. In general, the amplified SSR markers in pulasan showed a reduced number of alleles compared with lychee (*table I*), except LMLY 6. For this marker, LMLY 6, four alleles with a size range of 132–154 bp were detected in pulasan compared with only three alleles in lychee.

Of the seven amplified SSR markers, three (LMLY 2, LMLY 6 and LMLY 12) were polymorphic in the 20 pulasan accessions (*figure 1*). Therefore, the percentage of the polymorphic SSR markers in pulasan was 25.0% (3 out of 12) compared with 66.7% (8 out of 12) in longan [12].

4. Discussion

The cross-transferability of SSR markers showed contrasting results, depending largely on the evolutionary distance and the complexity of the genome [17]. For example, the cross-transferability from *Pinus taeda* to *P. sylvestris* ranged from 36% to 53% [15]; from *Festuca arundinacea* to different forage and cereal species ranged from 59% to 92% [18]; and from lychee to longan was 91.7% [12].

Nevertheless, it is widely accepted that the successful cross-amplification of a SSR locus does not prove the maintenance of the repeat motif in the non-source species. This is clearly shown in this study as, out of the seven amplified SSR markers (LMLY 2, LMLY 4, LMLY 5, LMLY 6, LMLY 7, LMLY 8 and LMLY 12), only three SSR markers (LMLY 2, LMLY6 and LMLY 12) maintained the polymorphic characteristics in pulasan. The four monomorphic SSR markers (LMLY 4, LMLY 5, LMLY 7 and LMLY 8) might have lost their sequence repeat regions or reduced the number of the repetitive unit and subsequently lost the polymorphic character. This may due to interruptions within the repeat motif, leading to monomorphism and shortening [19]. The monomorphic condition

could also be caused by a low level of variability in the pulasan germplasms. Pulasan is commonly cultivated by grafting. The pulasan accessions in the germplasms were collected from rural areas in various parts of Malaysia, where grafting is commonly used by villagers to propagate the plant. Therefore, the pulasan germplasms may have a low degree of polymorphism. Other molecular markers such as AFLP and RAPD could be used as a comparison in the germplasm evaluation to verify if the pulasan germplasms have a narrow genetic base.

The rest of the SSR markers (LMLY 1, LMLY 3, LMLY 9 and LMLY 10) failed to amplify in pulasan. This might be due to base substitutions and indels occurring in the flanking sequences of SSR repeat motifs, causing the specific primers to fail to bind and amplify the SSR motifs. The lack of a repeat motif as well as modification on both the repeat and the flanking regions clearly suggest the need for caution in using the same SSR loci across different taxa [20].

SSR markers transferred from lychee, in general, showed a smaller average length or size in the pulasan (all the amplified SSR markers, except LMLY 7 and LMLY 12). This is in agreement with Peakall *et al.* [20] (research on *Glycine max*) and Gonzáles-Martinez *et al.* [15] (research on *Pinus* spp.). LMLY 2 and LMLY 12 are perfect simple microsatellites in lychee. However, the number of alleles detected in the pulasan for both the SSR markers decreased. The interruptions in the SSR repeat motifs might have occurred in the pulasan, which may lead to imperfect microsatellites. Interruptions in the stretch of the SSR repeat could prevent slippage of DNA polymerase during DNA replication, and a decrease in the degree of polymorphism would be observed [21]. On the other hand, LMLY 6 showed an increase in allele number in pulasan compared with lychee. It is not certain if the interrupted repeat motif (*table I*; caused by CA) exhibited in lychee did not occur in the pulasan. If the interruption did not occur, LMLY 6 in the pulasan might have contained a perfect microsatellite that may lead to a higher degree of polymorphism in the SSR locus.

Stutter bands appeared in LMLY 2, indicating non-specific amplification in the pulasan. A few suggestions have been proposed to solve this problem, such as the use of high-fidelity DNA polymerase AmpliTaq Gold (Invitrogen, USA), a higher annealing temperature [18] and touch-down PCR [20].

At this point, it is useful to know that, if SSR markers are available from closely related species, then utilization of the SSR markers on related species is possible. Our results did not reveal a high rate of transferability between lychee and pulasan (58.3%) compared with between lychee and logan (91.7%). The transferability rate may indicate that lychee and logan are more closely related genetically than lychee and pulasan. It is noted that the lychee SSR markers were developed using a genomic DNA library enriched in CT repeats instead of expressed sequence tags (ESTs). Therefore, the transferability rate of SSRs would be lower. ESTs are coding sequences and are more conserved across species. SSR markers developed from ESTs are expected to have a higher rate of transferability than genomic SSR markers [22]. However, the conserved nature of the EST-SSRs may limit their polymorphism [18].

In conclusion, due to the difficulty and cost involved to develop specific SSR markers in species with limited commercial value, cross-amplification of markers among genetically closely related species would seem to be an adequate strategy. Although the transferability of lychee SSR markers to pulasan is not high, it does not cost much to test and use these readily available SSR markers for pulasan.

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References

[1] Adema F., Leenhouts P.W., van Welzen P.C., Flora Malesiana, Series I, Spermatophyta: flowering plants, Vol. 11, part 3: Sapindaceae, Rijksherbarium, Leiden, Netherlands, 1994, pp. 419–768.

[2] Aman R., Buah-buahan nadir Semenanjung Malaysia, Dewan Bahasa dan Pustaka, Kuala Lumpur, 1992.

[3] Kuleung C., Baenziger P.S., Dweikat I., Transferability of SSR markers among wheat, rye and triticale, Theor. Appl. Genet. 108 (2004) 1147–1150.

[4] Fitzsimmons N.N., Moritz C., Moore S.S., Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution, Mol. Biol. Evol. 12 (1995) 432–440.

[5] Szewc-McFadden A.K., Kresovich S., Bliek S.M., Mitchell S.E., McFerson J.R., Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species, Theor. Appl. Genet. 93 (1996) 534–538.

[6] Smulders M.J.M., Bredemeijer G., Ruskortekaas W., Arens P., Vosman B., Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species, Theor. Appl. Genet. 94 (1997) 264–272.

[7] Rallo P., Tenzer I., Gessler C., Baldoni L., Transferability of olive microsatellite loci across the genus *Olea*, Theor. Appl. Genet. 107 (2003) 940–946.

[8] Eujayl I., Sledge M.K., Wang L., May G.D., Chekhovskiy K., Zwonitzer J.C., Mian M.A.R., *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp., Theor. Appl. Genet. 108 (2004) 414–422.

[9] Brondani C., Rangel P.H.N., Borba T.C.O., Brondani R.P.V., Transferability of microsatellite and sequence tagged site markers in *Oryza* species, Hereditas 138 (2003) 187–192.

[10] Cordeiro G.M., Pan Y.B., Henry R.J., Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm, Plant Science 165 (2003) 181–189.

[11] Yazdani R., Scotti I., Jansson G., Plomion C., Mathur G., Inheritance and diversity of simple sequence repeat (SSR) microsatellite markers in various families of *Picea abies*, Hereditas 138 (2003) 219–227.

[12] Viruel M.A., Hormaza J.I., Development, characterization and variability analysis of microsatellites in lychee (*Litchi chinensis* Sonn., Sapindaceae), Theor. Appl. Genet. 108 (2004) 896–902.

[13] Doyle J.J., Doyle J.L., Isolation of plant DNA from fresh tissue, Focus 12 (1990) 13–15.

[14] Sambrook J., Russel D.W., Molecular cloning: a laboratory manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2001.

[15] González-Martínez S.C., Robledo-Arnuncio J.J., Collada C., Díaz A., Willioms C.G., Alía R., Cervera M.T., Cross-amplification and sequence variation of microsatellite loci in Eurasian hard pines, Theor. Appl. Genet. 109 (2004) 103–111.

[16] Joseph S.S., The analysis of microsatellites by silver staining of nondenaturing polyacrylamide gels, in: Tietz D., Nucleic acid electrophoresis, Springer-Verlag, Berlin, Germany, 1998.

[17] Barreneche T., Casasoli M., Russell K., Akkak A., Meddour H., Plomion C., Villani F., Kremer A., Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs), Theor. Appl. Genet. 108 (2004) 558–566.

[18] Saha M.C., Mian M.A.R., Eujayl I., Zwonitzer J.C., Wang L., May G.D., Tall fescue EST-SSR markers with transferability across several grass species, Theor. Appl. Genet. 109 (2004) 783–791.

[19] Taylor J.S., Durkin J.M.H., Breden F., The death of a microsatellite: a phylogenetic perspective on microsatellite interruptions, Mol. Biol. Evol. 16 (1999) 567–571.

[20] Peakall R., Gilmore S., Keys W., Morgante M., Rafalski A., Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants, Mol. Biol. Evol. 15 (1998) 1275–1287.

[21] Pépin L., Amigues Y., Lepingle A., Berthier J.L., Bensaid A., Vaiman D., Sequence conservation of microsatellites between *Bos Taurus* (cattle), *Capra hircus* (goat) and related species: examples of use in parentage testing and phylogeny analysis, Heredity 74 (1995) 52–61.

[22] Scott K.D., Eggler P., Seaton G., Rossetto M., Ablett E.M., Lee L.S., Henry R.J., Analysis of SSRs derived from grape ESTs, Theor. Appl. Genet. 100 (2000) 723–726.

Transferibilidad de los marcadores SSR del litchi (*Litchi chinensis* Sonn.) al pulasán (*Nephelium ramboutan-ake* L.).

Resumen — Introducción. Los pulasanes y los litchis son de la misma familia y están estrechamente ligados. Por ello, sería de esperar que los marcadores SSR fuesen altamente transmisibles entre ambos taxa. Testamos la transferabilidad de 12 marcadores SSR del litchi (*L. chinensis* Sonn.) al pulasán (*N. ramboutan-ake* L.). **Material y métodos**. Se extrajo ADN genómico de 20 adhesiones de pulasán con el fin de ampliar los loci SSR utilizando 12 pares de iniciadores resultantes de litchi. Los productos resultantes de PCR resultaron de los geles de poliacrilamida. **Resultados**. El porcentaje de la transferibilidad de los marcadores SSR del litchi al pulasán fue de un 58,3% y el porcentaje de los marcadores SSR polimorfos fue de un 25%. **Discusiones**. La transferibilidad moderada y los bajos índices de polimorfismo obtenidos sugieren que han podido intervenir unas interrupciones en los procesos de repetición de secuencias y que han podido tener lugar unas mutaciones en las secuencias de motivos repetidos de los SSR. Nuestros resultados no han mostrado un alto índice de transferibilidad entre el litchi y el pulasán. No obstante, nuestro estudio probó que los marcadores SSR desarrollados para el litchi son una buena fuente de marcadores moleculares para el pulasán.

Malasia / *Litchi chinensis* / *Nephelium ramboutan-ake* / biología molecular / marcadores genéticos / PCR / polimorfismo genético