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Full Length Research Paper

Cross-genera transferability of (simple sequence repeat) SSR markers among cassava (*Manihot esculenta* Crantz), rubber tree (*Hevea brasiliensis* Muell. Arg.) and physic nut (*Jatropha curcas* L.)

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Cross-genera transferability of simple sequence repeat (SSR) markers among three economically important plants of family *Euphorbiaceae* has been proposed. A set of SSR loci generated from cassava (199), rubber tree (49) and physic nut (42) were used to determine transferability with five accessions each of cassava, rubber tree and physic nut. The results revealed that cross-genera transferability among these species was observed. Of the 290 markers, 144 could amplify DNA of at least one non-donor species and 34 markers could amplify DNA of all tested species. A total of 57, 120 and 59 alleles were detected in cassava, rubber tree and physic nut, respectively, by transferable markers. The highest transferability (59.18%) was observed from cassava to rubber tree, followed by from rubber tree to cassava. Low transfer rates were found between cassava and physic nut, and between rubber tree and physic nut. These identified transferable markers for cassava, rubber tree and physic nut (37, 61 and 46, respectively) will be useful for comparative mapping and genomic studies. In addition, this finding is an important initial knowledge on cross-genera transferability of SSR markers in these three commercial species.

Key words: Microsatellites, transferability, Euphorbiaceae, cassava, rubber tree, physic nut.

INTRODUCTION

The *Euphorbiaceae* family is a large and diverse family of flowering plants (Zeng et al., 2010). It includes several economically important plants of the world including cassava (*Manihot esculenta* Crantz) a primary staple food and industrial crop (Ceballos et al., 2004), rubber tree (*Hevea brasiliensis* Muell. Arg.) the main resource of natural rubber (Leitch et al., 1998) and physic nut

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(*Jatropha curcas* L.) a high oil content crop with important applications in biodiesel production (Kumar and Sharma, 2008). Given both the diversity of this family, as well as the considerable economic importance of some members of the family, the development of common genetic tools will greatly aid studies that seek to undertake genomic analysis with an aim to improve or conserve these species.

In modern genomic studies, DNA-based molecular markers have become an effective tool with applications in genome mapping, DNA fingerprint analysis, genetic diversity analysis and phylogeny and evolution studies (Sharma et al., 2008). Among the different classes of molecular markers, simple sequence repeat (SSR) markers are one of the most favorable molecular markers because of their co-dominant inheritance, multi-allelic nature, reproducibility, relative abundance and good genome coverage (Powell et al., 1996). In recent years, a large number of SSR markers developed for these species have been published (Anderson et al., 2004; Feng et al., 2009; Kumar et al., 2010; Kunkeaw et al., 2011; Le Guen et al., 2010; Lokko et al., 2007; Phumichai et al., 2010; Raji et al., 2009; Sraphet et al., 2011; Tangphatsornruang et al., 2008; Wen et al. 2010). While these markers were very useful in studies applied to the species in which they were developed, some of them may be useful in other related taxa, which not only reduces the high cost and time of marker development, but also may provide significant insights into comparative genome mapping analyses.

Comparative mapping is a powerful tool for integrating genetic data among related taxa (Nadeau and Sankoff, 1998; Paterson et al., 2000). It is the process of identifying conserved chromosome segments across taxa, to evaluate genome evolution or how the genome has been rearranged through time and to determine the functions of genes and non-coding regions of the genome (Nadeau and Sankoff, 1998). Genetic maps constructed in one genus/species can be compared by means of common markers with closely related genus/species. The application of common markers developed from one species to another, called “transferability” has been observed in many species, for example in *Brassiceae*, *Fabaceae*, *Solanaceae* (Paterson et al., 2000), *Olea* (Rallo et al., 2003) and Poaceae (Kuleung et al., 2004). These studies have revealed that chromosome segments are conserved among related taxa.

In *Euphorbiaceae*, a few studies on cross-genera transferability have been undertaken (Feng et al., 2009; Kumar et al., 2010; Raji et al., 2009; Wen et al., 2010), with the evaluation of rubber tree markers in *Ricinus communis*, *Manihot utilissima* and *Phyllanthus emblica* (Feng et al., 2009), the evaluation of cassava markers in *R. communis*, *Euphorbia esula* (Raji et al., 2009) and *Manihot esculenta* (Wen et al., 2010), and the evaluation of physic nut markers in *R. communis* (Kumar et al., 2010) and *M. esculenta* (Wen et al., 2010). However, the complete cross-genera transferability among cassava, rubber tree and physic nut markers has not yet been investigated. Cross-genera transferable markers would be extremely useful for comparative genome studies among these three species. In addition, these sets of primer will increase the number of available markers in cassava, rubber tree and physic nut which can reduce the cost and time of marker development. Therefore, this project aimed to examine the transferability of SSR markers originating from cassava, rubber tree and physic nut amongst these three crop species.

MATERIALS AND METHODS

Plant materials and genomic DNA

Five accessions of cassava, rubber tree and physic nut were used in this study. The list of each accession is shown in Table 1. Genomic DNA of each sample was isolated from young leaf tissue using the DNeasy Plant Mini Kit (QIAGEN, Hilden Germany). DNA concentrations were evaluated using a NanoDrop™ 1000 Spectrophotometer (Thermoscientific).

Polymerase chain reaction (PCR) amplification

DNA of each sample was amplified with 199 cassava SSR primer pairs (Sraphet et al., 2011), 49 rubber tree SSR primer pairs (Feng et al., 2009) and 42 physic nut SSR primer pairs (Phumichai et al., 2010) which were able to amplify in the donor species. The PCR reactions were carried out in 20 µl final volume containing 50 ng of genomic DNA, 1 × PCR buffer (Promega, USA) with 1.5 mM $MgCl_2$, 0.2 µM of each PCR primer, 200 mM of each dNTP and 1 U of *Taq* DNA-polymerase (Promega) and amplification was peformed in a MyCycle™ Thermal Cycle (BioRad, USA). The PCR program for SSR amplification consists of the following steps: 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 50°C for 45 s and 72°C for 1 min, then a final step of 72°C for 5 min. The amplified products were gel fractionated on 5% denaturing polyacrylamide gels (PlusOne ReadySol DNA/PAGE 40% T, 5% C, Amersham

Table 1. List of the *Euphorbiaceae* taxa included in this study and origin of each accession.

Species	Number of accessions and tags	Origin
M. esculenta Cranzt	'Hanatee'	Thailand
	'Hauy Bong 60'	Thailand
	'MEARG2'	Argentina
	'MCOL1186A'	Colombia
	'MECU144'	Cuba
H. brasiliensis Muell. Arg.	'RRII 105'	India
	'RRII 203'	India
	'PR 217'	Indonesia
	'PB 235'	Malaysia
	'RRIM 600'	Malaysia
J. curcas L.	'1'	Thailand
	'17'	Thailand
	'25'	Thailand
	'33'	Thailand
	'41'	Thailand

Biosciences, Sweden) using the GIBCO BRL Sequencing System (Gibco BRL, USA). As a marker, 50 ng of 100 bp DNA ladder marker +1.5 kb (SibEnzyme, Russia) was loaded into the same gels. The gels were visualized by silver staining according to the protocol of Benbouza et al. (2006)

Data analysis

The amplified fragments were scored for presence or absence of alleles and the number of allele per locus. Positive amplification and percentage of transferability in a species were determined and calculated according to Kuleung et al. (2004). Genetic similarity among the different taxa was established from 34 SSR markers amplifiable in all species. Cluster analysis and construction of dendrogram were performed with the unweighted pair-group method (UPGMA) using TFPGA package v 1.3 (Miller, 1997).

RESULTS AND DISCUSSION

Cross-genera transferability is an important step to identify markers for comparative mapping. Given the ad-vantages of identification of transferable markers among cassava, rubber tree and physic nut which are econo-mically important crop belonging to *Euphorbiaceae*, a set of 290 SSR markers (199, 49 and 42 SSR markers ampli-fiable in cassava, rubber tree and physic nut, respec-tively) have been used to determine transferability among them. Transferability was determined in five accessions each for cassava, rubber tree and physic nut, and revea-led that 144 (49.66%) markers could amplify DNA of at least one non-donor species and 34 (11.72%) could amplify DNA of all tested species (Figure 1). The cross-genera transferability among these species revealed that transferability was observed in all sets of markers (cassava, rubber tree and physic nut markers). A total of 57, 120 and 59 alleles were detected in cassava, rubber tree and physic nut, respectively, by transferable markers (Table 2). The details of transferable SSR markers are

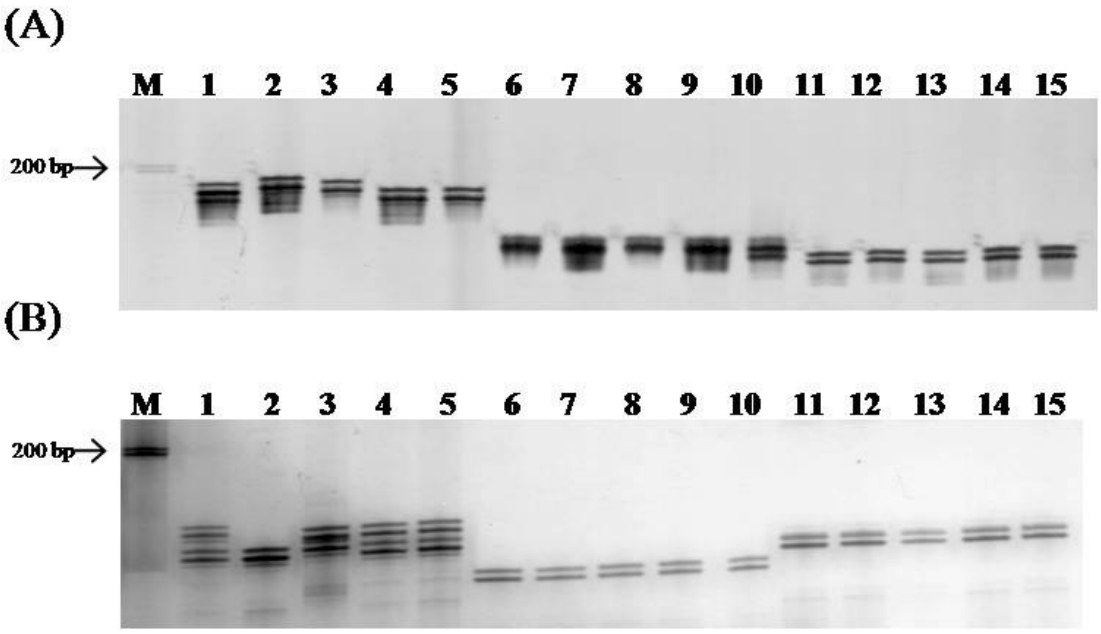

Figure 1. Patterns of SSR alleles generated by PCR using CA094 (A) and CA508 (B) loci. DNA bands were analyzed on 5% denaturing polyacrylamide gel and visualized by silver staining (M refers to 100 bp DNA ladder marker +1.5 kb. Lanes 1 to 5 represent DNA samples of cassava varieties 'Hanatee', 'Hauy Bong 60', 'MEARG2', 'MCOL1186A' and 'MECU144', respectively. Lanes 6 to 10 represent DNA samples of rubber tree varieties 'RRII 105', 'RRII 203', 'PR 217', 'PB 235' and 'RRIM 600', respectively. Lanes 11 to 15 represent DNA samples of physic nut accession number '1', '17', '25', '33' and '41', respectively.

Table 2. Cross-genera transferability of SSR markers among cassava (*M. esculenta* Cranzt), rubber tree (*H. brasiliensis* Muell. Arg.) and physic nut (*J. curcas* L.).

Donor species of markers	Number of markers	Number of amplified markers (percentage of amplification)			Number of alleles		
		Cassava	Rubber tree	Physic nut	Cassava	Rubber tree	Physic nut
Cassava	199	-	57 (28.64%)	38 (19.09%)	674	116	50
Rubber tree	49	29 (59.18%)	-	8 (16.33%)	46	121	9
Physic nut	42	8 (19.04%)	4 (9.52%)	-	11	4	56

shown in Table 3. This indicates that there is a relationship between these species as expected. The highest transferability (59.18%) was found when amplifying cassava DNA with rubber tree markers and the second highest, when amplifying rubber tree DNA with cassava markers, suggesting that cassava and rubber tree are more closely related than physic nut. Transferability of cassava markers in physic nut and the reciprocal were almost the same and quite low {a usual occurrence in cross-genera transferability (Kuleung et al., 2004)), similarly, the transferability of rubber tree markers to physic nut and its reciprocal was also low, probably for

Table 3. Details of transferable markers.

Donor species of markers	Primer name	Number of alleles			References
		Cassava	Rubber tree	Physic nut	
Cassava	CA2	3	3	(-)	Sraphet et al., 2011
	CA5	1	(-)	1	
	CA8	2	1	(-)	
	CA23	4	2	(-)	
	CA27	4	1	1	
	CA28	4	3	3	
	CA36	3	1	(-)	
	CA37	4	2	(-)	
	CA42	2	1	(-)	
	CA44	4	3	(-)	
	CA55	7	(-)	1	
	CA59	4	3	1	
	CA60	4	(-)	1	
	CA64	3	(-)	1	
	CA68	4	3	(-)	
	CA70	2	6	1	
	CA76	4	1	2	
	CA83	5	(-)	1	
	CA86	6	(-)	2	
	CA94	5	4	1	
	CA104	6	3	(-)	
	CA113	5	4	(-)	
	CA118	4	2	(-)	
	CA125	6	(-)	1	
	CA135	4	1	(-)	
	CA138	2	1	1	
	CA140	2	1	(-)	
	CA143	4	(-)	1	
	CA172	3	(-)	1	
	CA204	3	2	(-)	
	CA206	3	1	(-)	
	CA219	3	1	1	
	CA227	3	2	(-)	
	CA236	3	2	2	
	CA241	5	1	1	
	CA258	3	2	(-)	
	CA268	3	(-)	1	
	CA291	4	1	1	
	CA293	4	3	(-)	
	CA358	4	1	1	

Table 3. Contd.

Donor species of markers	Primer name	Number of alleles			References
		Cassava	Rubber tree	Physic nut	
Cassava	CA361	1	1	2	Sraphet et al., 2011
	CA364	6	1	(-)	
	CA368	6	3	(-)	
	CA376	4	2	(-)	
	CA377	6	1	3	
	CA430	3	4	(-)	
	CA436	3	(-)	1	
	CA442	3	1	(-)	
	CA443	3	2	1	
	CA444	4	5	(-)	
	CA449	3	5	1	
	CA450	3	5	1	
	CA455	3	(-)	1	
	CA460	5	1	(-)	
	CA481	3	(-)	1	
	CA482	3	2	1	
	CA486	3	3	1	
	CA488	4	1	(-)	
	CA504	4	1	(-)	
	CA505	2	1	1	
	CA508	4	1	1	
	CA514	4	1	(-)	
	CA560	4	1	(-)	
	CA565	2	1	2	
	CA572	2	1	(-)	
	CA585	4	1	(-)	
	CA591	3	2	1	
	CA614	3	2	(-)	
	CA619	3	2	2	
	CA674	3	3	3	
Rubber tree	HBE4	1	2	1	Feng et al., 2009
	HBE9	(-)	3	1	
	HBE17	1	3	(-)	
	HBE19	1	3	1	
	HBE23	3	2	(-)	
	HBE32	1	2	(-)	
	HBE33	1	2	(-)	
	HBE35	2	2	(-)	
	HBE37	2	2	(-)	

Table 3. Contd.

Donor species of markers	Primer name	Number of alleles			References
		Cassava	Rubber tree	Physic nut	
	HBE41	2	4	(-)	
	HBE97	2	3	(-)	
	HBE101	2	2	(-)	
	HBE112	2	2	1	
	HBE114	2	3	(-)	
	HBE117	2	3	(-)	
	HBE123	1	2	1	
	HBE132	3	5	(-)	
	HBE136	1	2	(-)	
	HBE139	2	2	(-)	
	HBE153	1	2	(-)	
	HBE155	2	3	1	
	HBE160	1	3	(-)	
	HBE161	1	2	(-)	
	HBE173	1	2	(-)	
	HBE180	3	3	(-)	
	HBE201	2	4	2	
	HBE236	1	2	1	
	HBE240	1	1	(-)	
	HBE250	1	1	(-)	
	HBE264	1	2	(-)	
Physic nut	JCT10	1	1	1	Phumichai et al., 2010
	JCT12	1	1	2	
	JCT18	1	1	1	
	JCT23	1	(-)	1	
	JCT28	2	(-)	1	
	JCT35	1	(-)	2	
	JCT45	2	(-)	1	
	JCT50	(-)	(-)	1	
	JCT59	(-)	1	1	
	JCT76	2	(-)	1	

(-) no PCR product or non specific amplification.

the same reason as already mentioned. In order to test this assumption, the genetic similarity between the species was calculated based on UPGMA. The genetic similarity coefficient is shown in Figure 2. The result confirmed the assumption, indicating that the smallest genetic distance was observed between cassava and rubber tree (coefficient = 1.2). The high relationship between cassava and rubber tree can also be considered based on chromosome number, cassava has the same chromosome number as rubber tree (2n = 36) and known as allopolyploid (De Carvalho and Guerra, 2002; Leitch et al., 1998), whereas the physic nut chromosome number

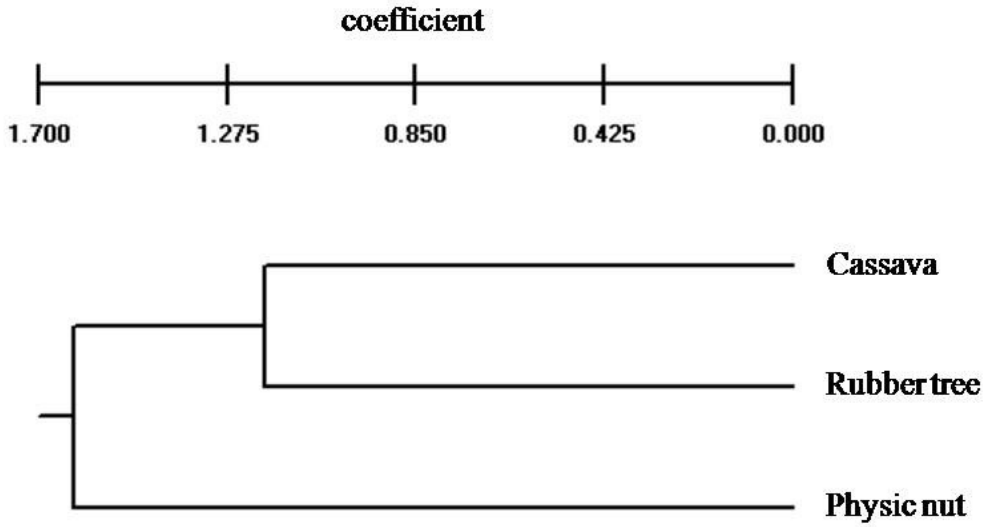

Figure 2. Dendrogram showing relationship among cassava, rubber tree and physic nut. The dendrogram was based on UPGMA cluster analysis using 34 SSR loci.

is smaller (2n = 22), and the genome size is also smaller than that of other species of the Euphorbiaceae family (Carvalho et al., 2008). Realistically, the possibility of utilization of cassava markers in comparative genomics or molecular genetic studies in rubber tree is higher than in physic nut.

Recently, Raji et al. (2009) have reported transferability of cassava markers to *R. communis* and *E. esula* as 15 and 11%, respectively. Here, we reported a higher percentage of transferability of cassava marker, in addition, the species *H. brasiliensis* (28.64%) and *J. curcas* (19.09%), and up to 57 and 38 more markers can be utilized in *H. brasiliensis* and *J. curcas* genomic studies, respectively. In the study by Feng et al. (2009), the transfer rate of rubber tree markers to *M. utilissima*, *R. communis* and *P. emblica* ranged from 58.64 to 68.39%. According to our results, the transfer rate of rubber tree to *M. esculenta*, a closely related species to *M. utilissima*, was 59.18%. We also investigated the transfer rate of rubber tree to *J. curcas* which was 16.33%, and eight markers derived from rubber tree are now available for *J. curcas*. Recently, Wen et al. (2010) attempted to increase number of markers for *J. curcas* by identification of transferability from cassava markers. They documented high transferability which is in contrast to the results presented here, and may reflect the different sets of primers used. Cross-genera transferability of physic nut markers has been previously reported only in *R. communis* (Kumar et al. 2010), but at this time, we reported additionally on transferability to *M. esculenta* and *H. brasiliensis*.

In conclusion, cross-genera transferability is possible among cassava, rubber tree and physic nut but transferability between cassava and rubber tree is more feasible than between cassava or rubber tree and physic nut. The number of available markers for cassava, rubber tree and physic nut were increased by 37, 61 and 46, respectively. These findings provide opportunities for comparative genomics and genome evolution studies in these commercial crops, as well as increasing broad utility markers for further research.

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