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Cross-species Amplification and Characterization of *Shorea* Microsatellites in *Shorea contorta* Vidal (Dipterocarpaceae)

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Abstract

The tropical rainforests in Southeast Asia consist of diverse Dipterocarpaceae species. In the Philippines, *Shorea contorta* is one of the most economically and ecologically important dipterocarps. Presently, its long-term survival is critically endangered and the remaining genetic resources are assumed to be under pressure. In this study, we cross-amplify 23 *Shorea* microsatellites in *S. contorta*. The polymorphic loci were selected and characterized using planted and natural populations of the studied species. Results revealed a high success rate for cross-species amplification (78.26%). The ten (10) polymorphic microsatellites showed a mean number (N*a*) and effective number (N*e*) of alleles of 6.00 and 2.75, respectively. The mean values for observed (H*o*) and expected (H*e*) heterozygosities were 0.45 and 0.47, respectively. In general, these microsatellites are useful for population genetic studies in *S. contorta.*

Keywords: Transferability, Cross-amplification, Dipterocarps, Simple Sequence Repeats, Tropical Forest

Introduction

Species of the timber family Dipterocarpaceae dominate the majority of the Southeast Asian forests. In the Philippines, dipterocarps are mostly found in groups with other species occurring in relatively dense stands (Lomibao, 1973) and they are considered to be economically and ecologically significant tree species (Lamprecht, 1989). *Shorea contorta* is one of about 45 dipterocarp species in the country and it is endemic (Ashton, 1982). The species is critically endangered (IUCN, 2011) and the genetic resources are least explored. This investigation is one of the few attempts for genetic study in *S. contorta*. We use microsatellites (or Simple Sequence Repeats: SSRs) to provide tools in assessing the species population genetic status.

SSRs are species-specific genetic markers (Chabane *et al.,* 2005); their development is costly and time-consuming. Hence, a simple, cost efficient technique is the cross-amplification or transfer of SSRs from closely related species. Some SSRs show high rates of transferability across closely related taxa (Gaitan-Solis *et al.,* 2002; Saha *et al.,* 2004, Akkak *et al.,* 2009). This was reported in many plants, from short-lived agronomic crops (e.g., Hempel and Peakall, 2003; Kuleung *et al.,* 2004; Gao *et al.,* 2005) to long-lived timber species (e.g., Isagi and Suhandono, 1997; Gonzalez-Martinez *et al.,* 2004).

Transferability of SSRs has frequently been reported within Dipterocarpaceae species. Some *Shorea* SSRs were successfully transferred to *Parashorea malaanonan*

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(Villarin, 2015; Abasolo *et al.,* 2009). Further, SSRs developed for *Shorea cordifolia* (Stacy *et al.,* 2001), *S. curtisii* (Ujino *et al.,* 1998), *S. laevis* (Masuda *et al.,* 2010) , *S. leprosula* (Lee *et al.,* 2004a; Ng *et al.,* 2009), *S. platyclados* (Ng *et al.,* 2009), *Hopea bilitonensis* (Lee *et al.,* 2004b), *Neobalanocarpus heimii* (Iwata *et al.,* 2000) and *Dryobananops aromatica* (Nanami *et al.,* 2007) were cross-amplified to *S. maxwelliana* (Masuda *et al.,* 2010), *S. megistophylla* (Stacy *et al.,* 2001), *S. parvifolia* (Lee *et al.,* 2004a), *S. robusta* (Pandey and Geburek, 2009) and to other species of different genera within the family (Ujino *et al.,* 1998; Ng *et al.,* 2009).

In this investigation, we aim to explore cross-amplification of the 23 *Shorea* SSRs to *S. contorta* as used in the previous study of Villarin (2015). Further, we would like to select and characterize a set of polymorphic SSR as tools for genetic studies in *S. contorta* populations.

Materials and Methods

Plant Material

We collected leaf samples from natural and planted populations of *Shorea contorta* (Table 1).The samples were stored in sealed plastic bags with silica gel prior to DNA extraction. Fourteen leaf samples from different populations were used for cross-species amplification. Negative and positive control samples were included following the method used by Villarin (2015). Further, we randomly select 94 samples from different populations to characterize the polymorphic SSRs.

Laboratory Method and Data Analysis

DNA isolation, cross-species amplification of *Shorea* SSRs, and interpretation of peaks followed the method described by Villarin (2015). The 23 *Shorea* SSRs used in this study were those used in a related study conducted for *Parashorea malaanonan* (Villarin, 2015). For characterization of microsatellites, we screened data from polymorphic SSRs as to the number of alleles (Na), effective number of alleles (Ne; Nei, 1975), observed and expected heterozygosities (Ho and He, respectively; Nei, 1975) and fixation index (F; Wright, 1965). The calculations were performed using GenAlEx 6 (Peakall and Smouse, 2005).

Tests for Hardy-Weinberg expectations (HWE: exact probabilities) at each microsatellite locus and linkage disequilibrium between loci (LD: exact probabilities) were performed using Arlequin (ver.3.1: Excoffier *et al.,* 2005). The software MICRO-CHECKER (ver. 2.2.3: Van Oosterhout *et al.,* 2004) was further used to check for scoring errors, allele drop-outs, and presence of null alleles.

Results and Discussion

Cross-amplification of *Shorea* SSRs in *Shorea contorta*

Five SSRs were not amplified in *S. contorta (*Table 2). From the successfully amplified SSRs, three were monomorphic (*Sle*02, *Sle*10, and *Sle*280), one revealed stutter peaks (*Sle*111a), two have weak amplification (*Sle*17 and *Sle*303a), and two showed many missing data (*Sle*01 and *Sle*566) due to complete failure of amplification in many samples. Failure in cross-amplification of the *Shorea* SSRs within genus was also reported in a related study conducted for *S. macrophylla* (Ng *et al.,* 2009). This can be explained by the locus-specific characteristic of markers, which was confirmed and discussed in the study conducted by Schlotterer *et al.,* (1997) and Harr *et al.,* (1998). In general, 78.26% of the Shorea SSRs developed for *S. leprosula* (Lee *et al.*, 2004a; Ng *et al.,* 2009) and *S. curtisii* (Ujino *et al.,* 1998) were successfully amplified in the investigated species (Table 2). High transferability rates of SSRs in closely related

Table 1: *Location of S. contorta populations*

Population	**Latitude (N)**	**Longitude (E)**	**Population**	**Latitude (N)**	**Longitude (E)**
Planted Populations			**Natural Populations**		
*Molave Hill, CP	10^O44.58'	124^O48.03'	*Cienda, CP	10^O44.49'	124^O50.62'
*FORY, CP	10^O44.72'	124^O48.23'	*Pangasugan, CP	10^O45.47'	124^O48.86'
*Marcos, CP	10^O45.93'	124^O47.43'	*Patag, CP	10^O44.53'	124^O49.60'
*Pangasugan,CP	10^O45.26'	124^O47.63'	Bohol, CP	9^O41.94'	124^O07.52'
*Arboretum, CP	10^O44.81'	124^O47.90'	Samar, CP	11^O09.77'	125^O16.42'
*Mailhi,CP	10^O38.06'	124^O54.52'	*[3]Makiling, NP	14^O09.22'	121^O14.08'
*[2]Licuma, CP	11^O3.74'	124^O31.57'	*[4]Tanay, NP	14^O33.52'	121^O22.32'
*[2]Catmon,CP	11^O4.93'	124^O34.41'	*[4]LandGrant, NP	14^O23.95'	121^O32.58'
*Patag, CP	10^O44.17'	124^O48.27'	Quezon, NP	13^O59.30'	121^O48.79'
*Cienda, CP	10^O43.63'	124^O48.73'	Subic, NP	14^O45.60'	120^O15.23'
Bohol, CP	9^O42.95'	124^O06.29'			
Nueva Viscaya, NP	16^O28.91'	121^O07.91'			
Surigao, SP	8^O14.87'	126^O16.80'			

NP=Northern Philippines; CP= Central Philippines; SP= Southern Philippines;*Baybay, Leyte; *[2]Ormoc, Leyte; *[3]Laguna; *[4]Rizal

Table 2: *Cross-species amplification profile of Shorea SSRs in S. contorta*

LOCUS	**Annealing Temp. (^{o}C)**	**Amplification**	**Polymorphism**	**Allele Size Range (base pairs)**
*Sle*01	48	yes	yes^{MD}	182-194
*Sle*02	45	yes	no	150
*Sle*05	-	no	no	-
*Sle*07	53	yes	yes	170-174
*Sle*08	45	yes	yes	200-209
*Sle*10	45	yes	no	193
*Sle*14	53	yes	yes	204-215
*Sle*15	-	no	no	-
*Sle*16	-	no	no	-
*Sle*17	56-45^{TD}	yes^W	no	-
*Sle*20	-	no	no	-
*Sle*111a	56-45^{TD}	yes	SP	-
*Sle*118	52	yes	yes	157-164
*Sle*267	55	yes	yes	105-109
*Sle*280	52	yes	no	102
*Sle*303a	56-45^{TD}	yes^W	-	-
*Sle*392	55	yes	yes	181-195
*Sle*562	52	yes	yes	156-160
*Sle*566	54	yes	yes^{MD}	77-96
*Shc*01	52	yes	yes	126-142
*Shc*04	-	no	no	-
*Shc*07	54	yes	yes	130-156
*Shc*09	54	yes	yes	186-189

TD=touchdown; SP=stutter peaks; MD=missing data>50%; W=weak amplification

Table 3: *Genetic diversity indices of Shorea SSRs in S. contorta*

Locus	N	Na	Ne	Ho	He	F
*Sle*07	94	3	1.411	0.33	0.293	-0.132
*Sle*08***	87	14	9.497	0.736	0.9	0.178
*Sle*14	91	5	1.724	0.451	0.422	-0.073
*Sle*118*	94	7	1.659	0.34	0.399	0.143
*Sle*267	94	3	1.066	0.064	0.062	-0.028
*Sle*392	93	2	1.776	0.409	0.439	0.065
*Sle*562	92	6	2.989	0.598	0.669	0.102
*Shc*01	94	4	1.432	0.298	0.303	0.013
*Shc*07	94	13	4.203	0.819	0.766	-0.075
*Shc*09	94	3	1.764	0.426	0.435	0.017
Mean	92.7	6	2.752	0.447	0.469	0.021
SE	0.716	1	0.804	0.07	0.078	0.032

N=sample size; Na=total no. of alleles; Ne=effective no. of alleles; Ho=observed heterozygosiy; He=expected heterozygosity; F=fixation index; SE=standard error of the mean; significant departure from Hardy-Weinberg expectation: *$P=<0.05$, **$P=<0.01$, ***$P=<0.001$*

species of tropical trees have been reported in many studies (e.g. Mottura *et al.,* 2005; Ouinsavi *et al.*, 2006). For dipterocarps, 73.91% of the 23 *Shorea* SSRs were successfully amplified in *Parashorea malaanonan* (Villarin, 2015). Abasolo *et al.* (2009) also reported high success rate in cross-amplification (75%) of the 16 *Shorea* SSRs in *P. malaanonan*. Further, Pandey *et al.* (2009) obtained a 90% success rate in *S. robusta* cross-amplified with 27 *Shorea* SSRs (Lee *et al.,* 2004ab, Ujino *et al.,* 1998, Stacy *et al.,* 2001). SSRs are often transferable among closely related species because of the conservation of DNA sequences within the flanking regions of microsatellite motives of phylogenetically related taxa (Kijas *et al.,* 1995; Dayanandan *et al.,* 1997).

Characterization of *Shorea* SSRs in *S. cortorta*

Analysis of microsatellite data using the polymorphic *Shorea* SSRs showed no evidence for scoring errors and allele drop-outs for all loci. Characterization of these SSRs revealed that gene diversity of most loci (Table 3) was lower in the investigated non-focal species (species for which SSRs were transferred) than in the focal species (species for which SSRs were developed). However, *Sle*08 in this study showed a higher He (0.90) compared to the He in the focal species (*Sle*08: 0.70, Ng *et al.,* 2009). This result was also evident in a similar study conducted in *S. parvifolia* (*Sle*118: 0.90, Lee *et al.,* 2004a; He in the focal species for *Sle*118: 0.8, Lee *et al.,* 2004a). This can be explained by an ascertainment bias, which was observed in several reciprocal transference studies (Ellegren *et al.,* 1995; Cooper *et al.,* 1998; Crawford *et al.,* 1998; Hutter *et al.,* 1998; Matsuoka *et al.,* 2002). Different microsatellite evolution could also be assumed in the different species resulting in lower He in non-focal species (Taylor *et al.,* 1999; Zhu *et al.,* 2000; Gonzalez-Martinez *et al.,* 2004). In this study, the diversity ranges of most loci investigated (Table 3) are confirmed to be mostly lower in several non-focal *Shorea* species in comparison to the source species (Ujino *et al.,* 1998; Lee *et al.,* 2004a; Ng *et al.,* 2009; Pandey *et al.,* 2009).

Some loci showed significant departures from HWE, which can be explained by locus-specific and biological characteristics. Thus, null alleles were detected at *Sle*08 explaining corresponding significant departures from HWE. Small sample size is another explanation for significant departures

from HWE (Chen *et al.,* 2007).

High fixation indices (F) were estimated for several loci (*Sle*08, *Sle*118, and *Sle*562) known to cause deviations from HWE. High F values can be explained by the Wahlund effect since the total population analyzed is structured in several subpopulations (Hartl and Clark, 1989). Similarly, null alleles can also cause high F values (Pemberton *et al.,* 1995) which were observed at *Sle*08 (F=0.178).

Linkage disequilibrium (LD) tests were significant (P¡0.05) for the locus pairs *Sle*08: *Sle*562, *Sle*14: *Shc*01, *Sle*118: *Sle*562, *Sle*118: *Shc*01, *Sle*118: *Shc*09, *Sle*392: *Shc*01, and *Sle*562: *Shc*01. Samples from planted populations were included in the analyses which putatively experienced bottlenecks contributing to non-random associations of alleles among loci (Frankham *et al.,* 2002). Hence, LD is evident.

Conclusion

It can be concluded that successful cross-species amplification of *Shorea* SSRs within genera of the family offers opportunity to study genetic status of the *S. contorta* populations without cost and time in the development of SSR markers. The neutral markers can be used for ecology, evolution, and conservation studies of the highly endangered dipterocarps in the Philippines. We suggest the 10 polymorphic set of markers to be used for genetic studies in *S. contorta*. Multiplexing few, if not most, of these loci is also recommended considering economic and time constraints.

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