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Short Communication: Cross-species amplification of microsatellite markers developed for *Jatropha curcas* within five species of *Jatropha*

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Abstract. *Saptadi D, Heliyanto B, Sudarsono. 2020. Short Communication: Cross-species amplification of microsatellite markers developed for* Jatropha curcas *within five species of* Jatropha*. Biodiversitas 21: 5072-5076.* The transferability of SSR markers can be used to access the genetic diversity of related species. There are four close relatives of *Jatropha curcas* L in Indonesia, which can be utilized as a new diversity source through the interspecific crossing. This research was conducted to determine the ability of cross-species amplification of SSR markers developed from *J. curcas* to other *Jatropha* species (*J. integerrima, J. multifida, J. gossypifolia, J. podagrica*). It also investigated the relationship between these species. Out of 28 primers checked,11 primers showed cross-species amplification in all the species tested. Primer pairs EU099519, EU099528, and EU099525 have no transferability to other species. The overall percentage of polymorphism (PP) among all species tested was 95%, with the mean genetic similarity (GS) was 0.34. Least PP (17.35%) and highest GS (0.60) was found between pairs of *J. podagrica* and *J. multifida*. The correlation between the PP with GS was relatively high (0.75). The farthest and closest genetic distance was found between *J. curcas/ J. gossypifolia* and *J. podagrica/ J. multifida*, respectively. Further, selected primers from this study can be utilized in species differentiation, molecular identification of interspecific hybrids, and exploiting the genetic resource.

Keywords: Cross-species amplification, SSR marker, interspecific crossing

INTRODUCTION

The *Jatropha* genus has diverse phenotypes that include various growth habits and either monoecious or dioecious flowering types. The genus is divided into subgenera: subgenus *Jatropha* and subgenus *Curcas* (Heller 1996). In the tropics, 150–175 species of the woody and shrub types of *Jatropha* exist (Dehgan 1982). In the last decades, *Jatropha curcas* has become an important crop because it has high oil content seeds making them biodiesel source (Devappa et al. 2011). Further development of this currently underutilized crop could significantly impact the biodiesel industry. To this end, breeding varieties of *J. curcas* with high yield and high seed oil content are required to increase productivity.

Unfortunately, numerous studies have shown low-level genetic diversity among the *J. curcas* accessions (Saptadi et al. 2011; Saptadi et al. 2017). Breeding for improved *J. curcas* varieties requires the availability of more diverse genetic materials than those available in the germplasm collection; thus, increasing the genetic diversity of *J. curcas* is a priority. Increased genetic diversity could be made possible by interspecific hybridization among *Jatropha* species. Therefore, the availability of genetic information of related *Jatropha* species helps validate interspecific F1 hybrid production successes.

There are five species of *Jatropha* in Indonesia, including *J. curcas*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, and *J. podagrica*. Members of the *Jatropha* sub-genus have some superior characters, such as high oil content, biotic and abiotic stress tolerance (Asbani 2008; Dhillon et al. 2009). However, those members of the *Jatropha* sub-genus are generally cultivated as ornamental plants, such as bottle-shrub plants (*J. podagrica* Hook.), coral plant (*J. multifida*), bellyache bush (*J. gossypifolia* L.), spicy *Jatropha* (*J. integerrima* Jacq.), and red physic nut (*J. montana* Willd.) (Terryana et al. 2019). Taxonomically, *J. podagrica*, *J. multifida*, *J. integerrima*, and *J. gossypifolia* belong to the *Jatropha* subgenus, whereas *J. curcas* belongs to the *Curcas* subgenus (Dehgan 1982). Currently, among these species, only *J. curcas* has been genetically characterized in Indonesia; therefore, genetic evaluations of *J. podagrica*, *J. multifida*, *J. integerrima*, and *J. gossypifolia* existed in Indonesia are necessary before they can be fully utilized in interspecific hybridization to introgress the target traits into *J. curcas*.

Simple Sequence Repeat (SSR) markers or microsatellites are widely used in genetic diversity analysis. SSR is a short repeating sequence abundant in the eukaryotic genome, codominant, and easy to apply (Wang et al. 2014). SSR markers have been widely used for evaluation of the *Jatropha* spp. germplasm genetic

diversity (Mastan et al. 2012; Maurya et al. 2013; Montes et al. 2014; Sanou et al. 2015; Saptadi et al. 2017). Even though these SSR markers were developed based on the *J. curcas* genome, they may also be useful for other *Jatropha* species (Sudheer et al. 2011; Kumar Yadav et al. 2011). Previous studies have indicated that SSR markers may be used for genetic diversity analysis of *Jatropha* species (Whankaew et al. 2011; Wen et al. 2010). However, we need to determine the evaluated SSR markers' efficiency and effectiveness in evaluating Indonesia's *Jatropha* species. The study aims to evaluate the transferability of the SSR markers developed from the *J. curcas* genome to genetically evaluate the accessions of *J. multifida*, *J. integerrima* (red and pink flowers), *J. podagrica*, and *J. gossypifolia* that exist in Indonesia and determine their genetic similarity to *J. curcas*.

MATERIALS AND METHODS

Molecular analysis of *Jatropha* species was carried out in the Plant Molecular Biology Laboratory, Faculty of Agriculture, IPB University, Indonesia. *Jatropha* leaf samples were collected from various places in Bogor, Indonesia (Table 1). The evaluated SSR primers consisted of 28 primer pairs previously used for *J. curcas* genetic studies (Saptadi et al. 2011).

The total nucleic acid was isolated from the fresh young leaf (0.1 g) using CTAB methods described by Sudheer et al. (2009). PCR was performed using a total volume of 25 µL, comprised of 0.2 µM of each primer, 1.25 U Taq polymerase (Real Biotech Corporation), 1 X PCR buffer, 0.1 µM dNTPs (10 mM mix), and 1 µL of DNA template. PCR cycles were run as follows: one cycle of denaturation at 95°C for 5 min, 36 cycles of denaturation at 94°C for 30 s, primer annealing at the appropriate temperature for each primer for 30 s, primer elongation at 72°C for 1 min, and one final cycle extension at 72°C for 5 min. The PCR amplified products were separated using 6% polyacrylamide gel electrophoresis (PAGE) in a Dedicated Height Sequencer (Cole-Parmer). The PAGE separation results were visualized by silver staining, and a 100-bp DNA ladder was used as DNA fragment size controls.

A binary system was applied by scoring 1 for a DNA band and 0 for the absence. The analysis was performed on the binary data to estimate genetic similarity values, as Nei and Li (1979). The polymorphism percentage (PP) was calculated using the following formula: PP = total number of polymorphic loci/total number of loci × 100. A dendrogram was produced using Unweight Pair Group Method Arithmetic (UPGMA) with DARwin 6.0.021 (Perrier & Jacquemoud-Collet 2006). The transferability percentage was calculated as follows: the number of alleles produced in one species/total number of alleles × 100.

RESULTS AND DISCUSSION

The 28 primers used in this experiment developed from the *J. curcas* genome and confirmed to generate amplified products using a *J. curcas* DNA (Saptadi et al. 2011). These 28 primers were tested for their ability to amplify SSR markers from four other *Jatropha* species (*J. multifida*, *J. podagrica*, *J. gossypifolia*, and *J. integerrima*). Of these 28 primer pairs, 11 primers (39.3%) yielded amplified products in four *Jatropha* species, eight primers (28.6%) in three species, and three primers (10.7%) in either two or one *Jatropha* species, respectively. The three remaining primers (EU099519, EU099528, and EU099525, 10.7%) did not generate any amplified product using the DNA template of either *Jatropha* species (Table 2). Examples of SSR marker loci generated using five different SSR primers were presented in Figure 1.

The EU586346 primer pairs yielded a monomorphic band in all species tested. The *J. podagrica* had the highest percentage of cross-species amplification (75%), while *J. multifida* had the lowest (57.1%) (Table 2). In total, 93 alleles were produced from six plants evaluated, including *J. curcas*. The number of alleles per primer pairs (Table 2) ranged from 0 (EU099519 primer) to 6 (EU586348) and with an average of 3.5 alleles. The total number of alleles per species ranged from 21 (in *J. multifida*) to 28 (in *J. gossypifolia*) (Table 2).

Polymorphic information content (PIC) calculated across *Jatropha* species for the SSR marker loci evaluated ranged from 0 (EU586346, EU099519, and EU099525) to 0.75 (EU099520). Based on their PIC values, five SSR loci (EU586345, EU099534, EU586346, EU099519 and EU099525) belonged to low, eleven were medium (EU586344, EU099527, EU099529, EU586351, EU586340, EU586347, EU099531, EU099521, EU099528, EU099518 and EU586349), and 12 were high PIC values (EU099520, EU099533, EU099526, EU586348, EF612741, EU099523, EU099530, AF469003, EU586343, EU099524, EF612739 and EU099522), respectively (Table 2). The average PIC value across *Jatropha* species and evaluated SSR loci was 0.48.

The values of calculated PP ranged from 17.4% to 62.2%, with an average PP of 38.3% (Table 3). The lowest PP value was calculated between *J. podagrica* and *J. multifida*, while the highest was between *J. curcas* and *J. gossypifolia* (Table 3). Moreover, the calculated genetic dissimilarity coefficient among the evaluated *Jatropha* species ranged from 0.32 (*J. multifida* vs. *J. gossypifolia*) to 0.8 (*J. curcas vs. J. podagrica* and *J. curcas* vs. *J. gossypifolia*) (Table 4).

Table 1. The five *Jatropha* species used in this study and their centers of origin

Species	Chromosome number[1)]	Center of origin
Jatropha curcas L.	2n = 22	Mexico, Central America[2)]
Jatropha multifida L.	2n = 22	Barbados[3)]
Jatropha podagrica L.	2n = 22	Panama, Honduras, Guatemala[3)]
Jatropha gossypifolia Hook	2n = 22	South America[3)]
Jatropha integerrima Jacq.	2n = 22	Complex natural crosses from Cuba[3)]

Note: [1)] Soontornchainaksaeng and Jenjittikul (2003); [2)] Heller, (1996); [3)] Dehgan (1982)

Table 2. PCR cross-amplification results from 28 SSR primers using four *Jatropha* species genomic DNA template

Primer codes	*Jatropha* species					Number of alleles per locus	PIC
	JM	JIR	JIP	JP	JG		
EU586348	-	+	+	+	+	6	0.73
EU586340	+	+	+	+	+	3	0.46
EU586346	+	+	+	+	+	1	0
EU586347	+	+	+	+	+	3	0.46
EU586343	+	+	+	+	+	5	0.68
EU586344	+	-	-	+	+	3	0.56
EU586345	+	+	+	+	+	2	0.14
EF612741	-	+	-	+	+	5	0.71
EF612739	-	-	-	+	+	4	0.62
EU099518	-	-	-	+	+	2	0.35
EU099519	-	-	-	-	-	0	0
EU099520	+	+	-	+	-	5	0.75
EU099521	+	+	+	-	+	3	0.41
EU099522	+	-	+	-	+	4	0.61
EU099523	-	+	-	-	-	4	0.70
EU099524	+	+	+	+	+	4	0.65
EU099525	-	-	-	-	-	0	0
EU099526	+	-	+	-	-	5	0.74
EU099527	-	-	-	-	+	3	0.56
EU099528	-	-	-	-	-	2	0.38
EU099529	-	+	+	+	+	3	0.50
EU099530	-	+	-	-	-	4	0.70
EU099531	+	+	+	+	+	3	0.42
EU099533	+	+	+	+	+	6	0.75
AF469003	-	-	+	+	+	4	0.70
EU586351	+	+	+	+	+	3	0.48
EU586349	+	+	+	+	+	2	0.35
EU099534	+	+	+	+	+	2	0.14
Transfer-ability (%)	57.1	64.3	60.7	67.9	75.0	-	
Total number of allele	21.0	27.0	22.0	22.0	28.0	93	

Note: JM: *J. multifida*, JIR*: J. integerrima* (red flower), JIP: *J. integerrima* (pink flower), JG: *J. gossypifolia*, JP*: J. podagrica;* (+): amplified product was presence; (-): amplified product was absence. The annealing temperatures for the primers have previously been reported by Saptadi et al. (2011)

Table 3. Polymorphism percentages calculated from cross-species amplification data of 25 polymorphic SSR marker loci analysis among five *Jatropha* species

	Species	1	2	3	4	5	6
1.	*J. curcas*	-					
2.	*J. integerrima* (red flower)	49.0	-				
3.	*J. integerrima* (pink flower)	50.0	24.5	-			
4.	*J. gossypifolia*	62.2	45.9	35.7	-		
5.	*J. multifida*	50.0	33.7	33.7	30.6	-	
6.	*J. podagrica*	49.0	32.7	28.6	31.4	17.4	-

Table 4. Dissimilarity matric calculated from the analysis of cross-species amplification data of 25 polymorphic SSR marker loci among five Jatropha species

	Species	1	2	3	4	5	6
1.	*J. curcas*	-					
2.	*J. integerrima* (red flower)	0.77	-				
3.	*J. integerrima* (pink flower)	0.75	0.41	-			
4.	*J. gossypifolia*	0.80	0.57	0.73	-		
5.	*J. multifida*	0.80	0.64	0.59	0.54	-	
6.	*J. podagrica*	0.77	0.57	0.52	0.61	0.32	-

When using *J. curcas* as an outgroup, the results of the phylogenetic analysis showed the five *Jatropha* species were divided into three different groups, consisted of Group I (*J. Gossypifolia, J. multifida and J. podagrica*), Group II (*J. integerrima* red or pink flowers) and Group III (*J. curcas*). The grouping of *Jatropha* species based on the dissimilarity distances was presented in Figure 2.

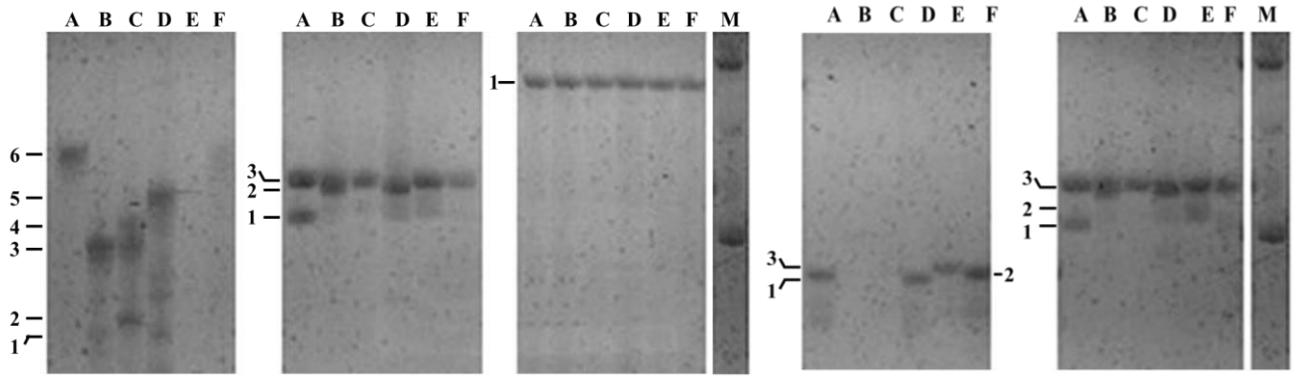

Figure 1. Gel photographs of cross-species amplification by 5 different SSR primer pairs (EU586348, EU586347, EU586346, EU586344, and EU586340) on five *Jatropha* species (1. *J. curcas*, 2. *J. integerrima* Pink Flower, 3. *J. integerrima* Red Flower, 4. *J. gossypifolia*, 5. *J. multifida* and 6. *J. podagrica*). M = 100 bp DNA ladder

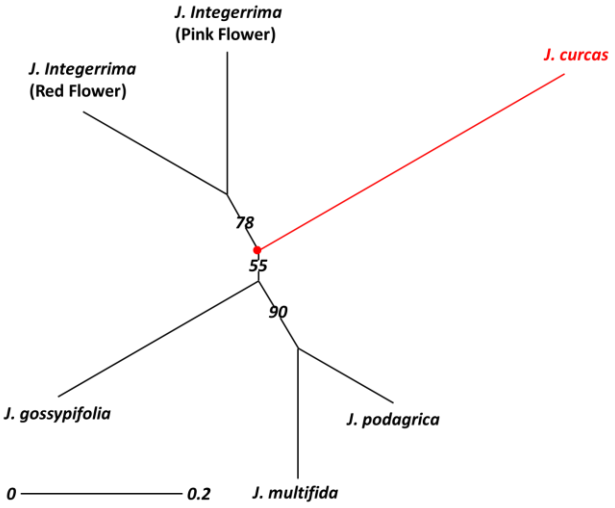

Figure 2. Results of phylogenetic analysis generated from the results of cross-species amplification data of 25 polymorphic SSR marker loci in five *Jatropha* species. The numbers on the branches showed the bootstrap analysis results

The development of species-specific SSR markers is very costly and time-consuming, so the information on SSR markers for economically less important species is still limited. A strategy that can overcome this problem is to utilize the cross-amplification ability of SSR markers to access the genetic diversity of the related species (Celiński et al. 2013; Aiello et al. 2020). Several studies on the SSR marker transferability were conducted among many taxonomic levels. For example, Datta et al. (2013) reported the successful transfer of SSR markers among Leguminoceae at a maximum rate of 47%. As many as 70 primers from 114 selected SSR markers of *Melilotus albus* can be used to amplify the markers from 18 other related species (Yan et al. 2017). Moreover, more than 50% of SSR primers developed from *Prosopia chilensis*, *P. alba*, and *P. flexuosa* can amplify the SSR in *P. cineraria* (Rai et al. 2017). In the *Jatropha* species study, EST SSR primers developed for *J. curcas* were effective for generating SSR markers of four related *Jatropha* species at the transferability rate of 75.6 – 85.2% (Laosatit et al. 2013).

In the present study, the transferability of *J. curcas* SSRs to other *Jatropha* species was only 57.1% to *J. multifida*, 62.5% to *J. integerrima*, 67.9% to *J. gossypifolia*, and 75.0% to *J. podagrica*. These results were lower than those reported by Kumar Yadav et al. (2011). In their study, Kumar Yadav et al. (2011) achieved transferability values of 76.0% to *J. multifida*, 95.6% to *J. integerrima*, 66.6% to *J. gossypifolia*, and 57.0% to *J. podagrica*, respectively. The rate of transferability for the SSR markers in both the present study and those reported by Kumar Yadav et al. (2011) indicated that the SSR flanking sequences used has a high level of conservation in the genus *Jatropha*. In general, an increase in the cross-amplification success of SSR markers isolated from the cultivated species to the wild species is concomitant with a decline in the genetic distances (Oliveira et al. 2013). This statement is consistent with the results obtained by Koppolu et al. (2010) in *Arachis* and by Lee et al. (2011) in *Allium*. The mean percentage of genomic SSR marker transferability in our study was 65%, which was smaller than those reported by Kumar Yadav et al. (2011) for EST-SSRs (an average of 73.65%). The higher transferability of EST-SSRs than G-SSRs reflected the coding sequences' conservation over the non-coding genomic sequences and the lower frequency of EST mutations over the non-coding genomic sequences (Wen et al. 2010).

The dendrogram presented in Figure 2 supports previous SSR markers analysis results (Sudheer et al. 2011), which grouped *J. multifida* and *J. podagrica* into a single group. Morphologically, the flowers of *J. multifida* and *J. podagrica* were very similar in shapes and colors. Moreover, *J. curcas* and *J. integerrima* were also more closely related, and this was in agreement with those reported by Terryana et al. (2019). Another report also showed that *J. curcas* and *J. integerrima* were able to cross-hybridized, which further indicated a taxonomically close relationship between these two species (Maurya & Yadav 2016).

Considering that the genetic distance between *J. podagrica* and *J. multifida* was closer than that between *J. curcas* and *J. integerrima*, it might be possible to generate interspecific hybrid among *J. curcas and J. podagrica* as well as *J. multifida*. Moreover, *J. curcas and J. podagrica*, as well as *J. multifida* was reported to have the same chromosome numbers (Soontornchainaksaeng et al. 2003). Our dendrogram also shows that *J. integerrima* (red flower) was genetically the closest to that of *J. integerrima* (pink flower), which was consistent with the previously reported karyotype study (Soontornchainaksaeng et al. 2003). Based on morphological and biochemical markers, Prabarakan and Sujatha (1999) reported that *J. curcas* and *J. gossypifolia* were able to generate a natural interspecific hybrid known as *Jatropha tanjorensis*. However, our study did not support Prabarakan and Sujatha's (1999) finding since the percentage of polymorphism was quite high between these species (62.2%), indicating the two species were distantly related and probably were not able to generate interspecific hybrid. On the other hand, our findings agreed with Avendaño et al. (2015), which showed 79.4 polymorphism percentages between *J. curcas* and *J. gossypifolia*.

In general, the SSR markers used in our study had high transferability values. Even though the genetic similarity values obtained in this study were relatively lower than previous studies, the percentages of polymorphism obtained between *J. curcas* and other *Jatropha* species were relatively high. Therefore, our results suggest that the tested SSR markers in this study may be used to estimate the degree of genetic relationship among *Jatropha* species. Thus, these markers could be used as supporting tools for evaluating the *Jatropha* species and identifying the interspecific hybrids' progenies among the *Jatropha* species.

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REFERENCES

Aiello D, Ferradini N, Torelli L, Volpi C, Lambalk J, Russi L, Albertini E. 2020. Evaluation of cross-species transferability of SSR markers in *Foeniculum vulgare*. Plants 9 (2): 7-9. DOI: 10.3390/plants9020175

Asbani N. 2008. Interspecific hybridization of *Jatropha curcas* L. and *Jatropha integerrima* Jaeq. for mites and thrips resistant crops. Pros Lokakarya Nasional III Inovasi Teknologi Jarak Pagar untuk Mendukung Program Desa Mandiri Energi 2008: 96-102. [Indonesian]

Avendaño R, Díaz EG, Valdez-Melara M, Solano NC, Villalobos AM, Cascante FA, Benavides BW, Solís-Ramos LY. 2015. Genetic diversity analysis of *Jatropha* species from Costa Rica using AFLP markers. Am J Plant Sci 6 (14): 2426-2438. DOI: 10.4236/ajps.2015.614245

Celiński K, Pawlaczyk EM, Wojnicka-Półtorak A, Chudzińska E, Prus-Głowacki W. 2013. Cross-species amplification and characterization of microsatellite loci in *Pinus mugo* Turra. Biologia (Poland) 68 (4): 621-626. DOI: 10.2478/s11756-013-0189-z

Datta S, Mahfooz S, Singh P, Choudhary AK, Chaturvedi SK, Nadarajan N. 2013. Conservation of microsatellite regions across legume genera increases marker repertoire in pigeon pea. Australian J Crop Sci 7 (13): 1990-1997.

Dehgan B. 1982. Novel *Jatropha*s for Florida Landscapes. Proc Fla State Hort Soc 95: 277-280.

Devappa RK, Makkar HPS, Becker K. 2011. *Jatropha* diterpenes: A review. J Am Oil Chem Soc 88 (3): 301-322. DOI: 10.1007/s11746-010-1720-9.

Dhillon RS, Hooda MS, Jattan M, Chawla V, Bhardwaj M, Goyal SC. 2009. Development and molecular characterization of interspecific hybrids of *Jatropha* curcas × *J. integerrima*. Indian J Biotechnol 8: 384-390.

Heller J. 1996. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. In: Physic nut. *Jatropha curcas* L. International Plant Genetic Resources Institute, Rome.

Koppolu R, Upadhyaya HD, Dwivedi SL, Hoisington DA, Varshney RK. 2010. Genetic relationships among seven sections of the genus *Arachis* studied by using SSR markers. BMC Plant Biol 10: 1-12. DOI: 10.1186/1471-2229-10-15.

Kumar Yadav H, Ranjan A, Asif MH, Mantri S, Sawant SV, Tuli R. 2011. EST-derived SSR markers in *Jatropha curcas* L.: Development, characterization, polymorphism, and transferability across the species/genera. Tree Genet Genomes 7 (1): 207-219. DOI: 10.1007/s11295-010-0326-6.

Laosatit K, Tanya P, Saensuk, C, Srinives P. 2013. Development and characterization of EST-SSR markers from *Jatropha* curcas EST database and their transferability across *Jatropha*-related species/genus. Biologia (Poland) 68 (1): 41-47. DOI: 10.2478/s11756-012-0143-5.

Lee GA, Kwon SJ, Park YJ, Lee MC, Kim HH, Lee JS, Lee SY, Gwag LG, Kim CK, Ma KM. 2011. Cross-amplification of SSR markers developed from *Allium sativum* to other *Allium* species. Sci Hortic 128 (4): 401-407. DOI: 10.1016/j.scienta.2011.02.014

Mastan SG, Sudheer PDVN, Rahman H, Ghosh A, Rathore MS, Prakash CR, Chikara J. 2012. Molecular characterization of intra-population variability of *Jatropha curcas* L. using DNA based molecular markers. Mol Biol Rep 39 (4): 4383-4390. DOI: 10.1007/s11033-011-1226-z

Maurya R, Gupta A, Singh SK, Rai KM, Chandrawati, Sawant SV, Yadav HK. 2013. Microsatellite polymorphism in *Jatropha curcas* L. A biodiesel plant. Ind Crops Prod 49: 136-142. DOI: 10.1016/j.indcrop.2013.04.034.

Maurya R, Yadav HK. 2016. Development of interspecific hybrid of *J . curcas x J . integerrima* and its molecular characterization using SSR markers. J Agroecol Nat Resour Manage 3 (1): 101-105.

Montes JM, Technow F, Martin M, Becker K. 2014. Genetic diversity in *Jatropha curcas* L. assessed with SSR and SNP markers. Diversity 6 (3): 551-566. DOI: 10.3390/d6030551.

Oliveira GAF, Pádua JG, Costa JL, de Jesus ON, de Carvalho FM, de Oliveira EJ, Oliveira JL. 2013. Cross-species amplification of microsatellite loci developed for *Passiflora edulis* Sims. in related *Passiflora* species. Brazilian Archive Biol Technol 56 (5): 785-792. DOI: 10.1590/S1516-89132013000500009.

Perrier X, Jacquemoud-Collet JP. 2006. DARwin Software. Retrieved from http://darwin.cirad.fr/

Rai MK, Shekhawat JK, Kataria V, Shekhawat NS. 2017. Cross-species transferability and characterization of microsatellite markers in *Prosopis cineraria*, a multipurpose tree species of Indian Thar desert. Arid Land Res Manag 31 (4): 462-471. DOI: 10.1080/15324982.2017.1338791.

Sanou H, Angulo-Escalante MA, Martinez-Herrera J, Kone S, Nikiema A, Kalinganire A, Hansen JK, Kjær ED, Graudal L, Nielsen LR. 2015. Loss of genetic diversity of *Jatropha curcas* L. through domestication: Implications for its genetic improvement. Crop Sci 55 (2): 749-759. DOI: 10.2135/cropsci2014.02.0165.

Saptadi D, Hartati RS, Setiawan A, Heliyanto B, Sudarsono. 2011. Development of simple sequence repeat markers for *Jatropha* spp. Jurnal Littri 17 (4): 140-149. DOI: 10.21082/littri.v17n4.2011.140%20-%20149.

Saptadi D, Hartati RS, Setiawan A, Heliyanto B, Sudarsono. 2017. Genetic diversity of Indonesian physic nut (*J. curcas*) based on molecular markers. Agrivitas 39 (60): 160-171. DOI: 10.17503/agrivita.v39i2.694.

Soontornchainaksaeng P, Jenjittikul T. 2003. Karyology of *Jatropha* (Euphorbiaceae) in Thailand. Thai For Bull Bot 31: 105-112.

Sudheer PDVN, Mastan SG, Rahman H, Ravi Prakash C, Singh S, Reddy MP. 2011. Cross-species amplification ability of novel microsatellites isolated from *Jatropha* curcas and genetic relationship with sister taxa : Cross-species amplification and genetic relationship of *Jatropha* using novel microsatellites. Mol Biol Rep 38 (2): 1383-1388. DOI: 10.1007/s11033-010-0241-9.

Terryana RT, Nugroho K, Mulya K, Tasma IM, Lestari P. 2019. Genetic diversity of *Jatropha* spp. Germplasm based on morphological and molecular markers. Jurnal AgroBiogen 15 (1): 23. DOI: 10.21082/jbio.v15n1.2019.p23-34.

Wang S, Liu Y, Liying M, Liu H, Tang Y, Wu L, Wang Z, Li Y, Wu R, Pang X. 2014. Isolation and characterization of microsatellite markers and analysis of genetic diversity in Chinese jujube (*Ziziphus jujuba* Mill.). PLoS One 9 (6): e0099842. DOI: 10.1371/journal.pone.0099842

Wen M, Wang H, Xia Z, Zou M, Lu C, Wang W. 2010. Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. BMC Res Notes 3: 42. DOI: 10.1186/1756-0500-3-42

Whankaew S, Kanjanawattanawong S, Phumichai C, Smith DR, Narangajavana J, Triwitayakorn K. 2011. 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.). Afr J Biotechnol 10 (10): 1768-1776. DOI: 10.5897/AJB10.2185.

Yan Z, Wu F, Luo K, Zhao Y, Yan Q, Zhang Y, Wang Y, Zhang J. 2017. Cross-species transferability of EST-SSR markers developed from the transcriptome of *Melilotus* and their application to population genetics research. Sci Rep 7 (1): 1-11. DOI: 10.1038/s41598-017-18049-8.