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Genetic Variability and Out-crossing rate in open pollinated Duku 'Kumpe' (*Lansium parasiticum* (osbeck) K.C.Sahni & Bennett), a Potential Type of Duku from Jambi, Indonesia

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ABSTRACT

Duku 'Kumpe' is one of the potential local type cultivars of duku (*Lansium parasiticum* (osbeck) K.C. Sahni & Bennett) fruit species, which is found in the Jambi. For sustainable utilization of this local population of duku fruit, understanding of reproductive system and genetic information of the plant is needed. The reproductive system of a plant species is a factor that directly determines the potential and success of conservation program for the future. Until now, the breeding system about this plant still has been unknown. There were different opinions whether duku produce sexually (apomixis) or sexually (outcrossing). Based on this fact, it is necessary to clarify this problem. A total of 60 seeds from three parental trees (20 seeds from Kumpeh and 20 seeds from Duku Lusi and 20 seeds from Hutan Pagar) were germinated in the greenhouse for obtaining DNA samples. DNA samples were extracted using CTAB method and amplified using Intra Sample Sequence Specific Amplification (ISSR) primers. Four selected primers (IC1, IC2, IC3, and IC4) were used to amplify all DNA samples and estimate the magnitude of genetic variability and out-crossing rate. Based on analysis of genetic variability and assessment of level out-crossing rate of three parental and six progenies from three populations proved that local cultivar 'Kumpeh' were facultative apomixis breeding system. The crossing parental of all progenies in Kumpeh population with three parental were very important for sustainable utilization of this local type duku 'Kumpeh'. The detection genetic variability even with low levels of fixation (Fst) and Heterozygosity (Ht) in Kumpeh population were very important and should be genetic conservation and genetic improvement of these genotypes.

Keywords: Apomixis, duku 'Kumpeh', genetic variability, ISSR, *Lansium parasiticum*, out-crossing rate.

INTRODUCTION

Duku (*Lansium parasiticum* (osbeck) K.C.Sahni & Bennett) is very popular and important tropical fruit belonging to the Meliaceae family. But it is scarcely grown on a plantation scale, most of the fruits seen in markets being collected from village trees. It has been in cultivation for a long time, and in 1915 year being recorded on by the Chinese traveler Ma Huan (4). Duku is a native and its distribution mainly in South-East Asia especially Indonesia (Southern Sumatra, Tenggara, Malacca, Sumatra, Thailand, and Philippines, especially in Southern region of Sumatra) (5). This tropical plant is not only important economically and very popular edible fruit and snack eaten fresh as dessert but also it can also be used in cosmetics due to its extract has antioxidant property as well as anti-inflammatory and lightening effects with a good safety profile (2).

The accepted scientific name of duku is *Lansium parasiticum* (osbeck) K.C. Sahni & Bennett and there are eleven variations of this species. Although it is not appropriate, the scientific name of duku is often referred to *Lansium domesticum* (art), one of its synonym. These major groups of duku and its related were categorized three

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Submission date: 16-Dec-2018 12:08AM (UTC+0800)

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File name: nurainas_PAK15_de_pharmacia_letre.pdf (530.16K)

Word count: 3808

Character count: 21093



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Der Pharmacia Lettre, 2016, 8 (18):185-191
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Genetic Variability and Out-crossing rate in open pollinated Duku 'Kumpeh' (*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet.), a Potential Type of Duku from Jambi, Indonesia

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ABSTRACT

Duku 'Kumpeh' is one of the potential local type cultivars of Duku *Lansium parasiticum* (Osbeck) K.C. Sahni & Bennet., from specific area in the Jambi. For sustainable utilization of this local germplasm of tropical fruit, understanding of reproduction and genetic information of the plant is needed. The reproductive system of a plant species is a factor that directly determine the potential and success of conservation germplasm for the future. Until now, the breeding system about this plant still unclear although there were different opinions whether duku produce asexually (apomictic), sexual or both. Based on this fact, it is necessary to clarify this problem. A total of 60 seeds from three parental trees (20 seeds from Kumpeh and 22 seeds from Dusun Tuo and 18 seeds from Muaro Panco) were germinated in the greenhouse for obtaining DNA samples. DNA samples were extracted using CTAB method and amplified using Inter Simple Sequences Repeat (ISSR) primers. Four selected primers: (CCT)₈ (HQ200181), (AAT)₁₀ (HQ200186), (AAG)₆ (HQ200182) and (AG)₁₀ (DQ453906) were used to amplify all DNA samples and estimate the magnitude of genetic variability and out-crossing rate. Based on analysis of genetic variability and assessment of level out-crossing rate of three parental and sixty progenies from three populations proved that local cultivar 'Kumpeh' were facultative apomictic breeding system. The existing identical of all progenies in Kumpeh population with their parental were very important for sustainably utilization of this local type duku 'Kumpeh'. The detection genetic variability (even with low level) at Dusun Tuo and Muaro Panco population were very useful and strategic for genetic conservation and genetic improvement of these germplasm.

Keywords : Apomictic, duku 'kumpeh', genetic variability, ISSR, *Lansium parasiticum*, out-crossing rate.

INTRODUCTION

Duku (*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet) is very popular and important tropical fruit belonging to the Meliaceae family, but it is scarcely grown on a plantation scale, most of the fruits seen in markets being collected from village trees. It has been in cultivation for a long time and in 1413 year being remarked on by the Chinese traveller Ma Huan (4). Duku is a native and its distribution mainly in South East Asia especially Indonesia (Southern Sumatra), Peninsular Malaysia, Southern Thailand and Philippines, especially in Southern region of Sumatra (13). This tropical plant is not only important economically and very popular edible fruit and widely eaten fresh as dessert but also it can also be used in cosmetics due to its extract has antioxidant property as well as moisturizing and lightening effects with a good safety profile (25).

The accepted scientific name of duku is *Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet and there are eleven synonym of this species. Although it is not appropriate, the scientific name of duku is often referred to *Lansium domesticum* Corr, one of its synonym. Three major groups of duku and its related were recognized, there

were duku with small ellipsoid, pale yellow fruits without latex from trees and small flowers; bidjitan or langsung with larger ellipsoid, glabrescent pale yellow fruits with a little latex from trees with larger flowers; kokossan with smaller globose, orange yellow fruits with latex and a tough pericarp from trees with the largest flowers and most pubescent leaves (4).

Mabberley (1985) recorded that some types of same species *L. domesticum* in Malaysia show difference in characters of fruit and tree that become a problem in their taxonomic status due to the inconsistency vernacular name for different type in different regions (13, 23). Related to the type of duku, in Kumpeh (Jambi, southern Sumatra) there is a very potential local type namely duku 'kumpeh' and now widely cultivated for mainly income generating of local people Jambi. That why, now Jambi become number two of the center production of local fruit duku in Indonesia. The understanding about the mating system as basic for shaping the genetic diversity is very important for conservation purpose and sustainability utilization of this potential local germplasm of duku in the future. The mating system is a directly factor that affects the magnitude and distribution of genetic variation within and between populations of a plant species (24) and genetic structure and dynamics of the population a plant species (6). Until now, information about mating system of local type duku 'Kumpeh' from Jambi was still unclear, whether its asexually (apomixis) or sexual reproduction or both of those reproduction. The production of apomictic fruit were detected in duku due to the lack of viable pollen grains. Salma and Razali (1987) reported that the characteristics of bisexual flowers, anther tetrasporangia and cracked longitudinally but no pollen found and their fruits still produced were detected in duku from Peninsular Malaysia (21). In contrary fact, this plant has a perfect flowers and cross-pollinated reproductive system with honey bee as their pollinators (1).

Generally, two approaches were adopted to clarify the genetic patterns of apomictic plants i.e. via variation analysis on parental plants and their progeny or molecular analysis (8). Conventional technique by crossing test can be used to clarify the mating system of a plant species, but it is difficult to apply especially for the tree species. ISSR technique is a potential marker for clarify genetic variability of plant species. This marker is PCR primers based the microsatellite sequences, where repeat motifs are anchored either at 5' or 3' end with one or few specific nucleotides and amplify the sequences between the two microsatellite loci referred to as inter simple sequence repeat (ISSR) markers. In addition, ISSRs can be targeted towards particular sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP and RAPD (19). Application of dominant marker ISSR were successful to reveal genetic variability of a apomictic tropical fruit, mangosteen from different region in Sumatra (14) and effective to show the occurrence of out-crossing in olive plants (2). In this study, we applied the ISSR marker to clarify mating system and estimate magnitude of out-crossing rate of the potential local type duku 'kumpeh' from Jambi (*Lasium parasiticum*) by using ISSR marker in open pollinated populations.

MATERIALS AND METHODS

The samples of pollinated seeds from three parental trees in Kumpeh (position site at S: 01°34.254' - E: 103°51.350') Muaro Panco (position site at S: 02°07.971' - E: 102°01.701') and Dusun Tuo (position site at S: 01°23.112' - E: 102°20.922' Jambi province Indonesia) were collected during a fruiting season in January 2015 (see the collection sites at Figure 1). The sixty seeds (twenty seeds from Kumpeh, twenty-two seeds from Dusun Tuo and eighteen seeds from Muaro Panco) were germinated in the green house to obtain young seedling for DNA sources. Genomic DNA was extracted from young leaves of parental plants and their progenies using CTAB (hexadecyltrimethylammonium bromide) method (3). About 0.1 mg fresh young leaves were grinded for total DNA extraction by addition 1% of polyvinylpyrrolidone (PVP). DNA concentration was measured by subjecting the samples into 0.85% agarose gel electrophoresis, staining with ethidium bromide and visualization on ultraviolet (UV) transilluminator.

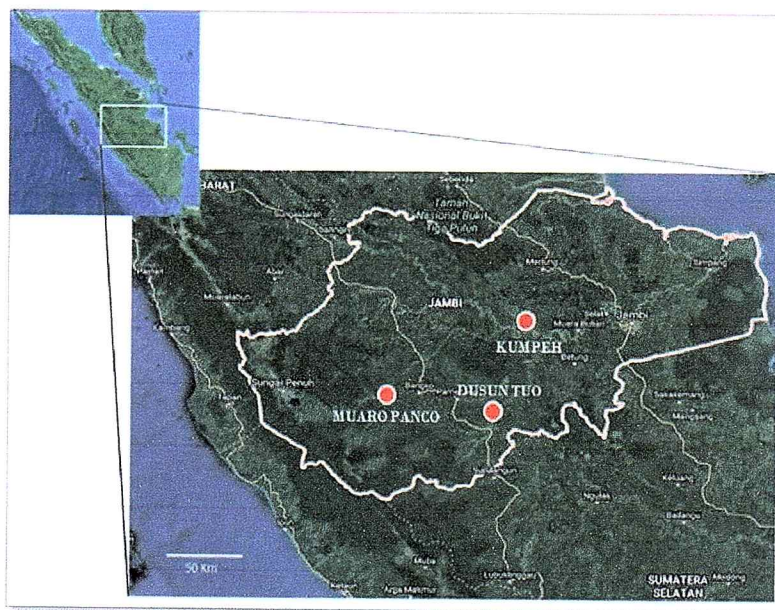


Figure 1. The Collection **Site** of three parental trees from Dusun Tuo, Kumpoh and Muaro Panco, Jambi Province, Indonesia (Modification from: <https://www.google.com/maps/place/Jambi,+Indonesia/>)

In general, the success of application of ISSR primer for genetic characterization is very specific for a plant species. For this reason, the selection ISSR primers is very crucial prior to apply for genetic characterization of a plant species. Ten of ISSR primer from some references related to species studied (10, 11, 12, 22) were screened to obtain the appropriate ISSR primers for study genetic variability and out-crossing rate of duku (see Table 1).

Table 1. Ten candidates of ISSR Primers for DNA amplification of local type duku 'Kumpoh' (*Lansium parasiticum*) from Jambi

No.	sequences	Primer name	References
1	AAC _n	HQ200181	Sexton <i>et al.</i> (2010)
2	(AAG) _n	HQ200182	Sexton <i>et al.</i> (2010)
3	(CTT) _n	HQ200189	Sexton <i>et al.</i> (2010)
4	(AAT) ₁₀	HQ200186	Sexton <i>et al.</i> (2010)
5	(GTC) _n	HQ200190	Sexton <i>et al.</i> (2010)
6	(GA) _n	DQ778303	Liu <i>et al.</i> (2012)
7	(CT) _n	DQ453907	Liu <i>et al.</i> (2014)
8	(TC) _n	DQ453914	Liu <i>et al.</i> (2014)
9	(AG) _n	DQ453906	Liu <i>et al.</i> (2014)
10	(AG) ₁₀	HM041045	Lemes <i>et al.</i> (2011)

PCR amplification of DNA samples using ISSR primer were conducted with a total volume of 12.5 ml solution containing 6.25 mL of Taq Go Green Master Mix Promega, 4.25 mL of dd H₂O (Nuclease Free Water), 1 mL of ISSR primer, and 1 mL of sample DNA (template). Amplification using PCR thermocycler, GSX1 nexus Eppendorf Master cycler with 45 cycles. The reactions were programmed with an initial denaturation step at 95 ° C for 1 min, and denaturation step at 95 ° C for 1 minute, annealing at temperatures appropriate optimization for 1 minute, extension at 72 ° C for 2 minutes, and final extension at 72 ° C for 10 minutes. The results of PCR amplification were subjected to electrophoresis on a 1.2% agarose gel immersed in TBE buffer and running with 110 v. Furthermore, applied electrophoresis gel was stained with 1% ethidium bromide for 10 minutes and washed by soaking in distilled water for 15 minutes. Finally viewed under ultraviolet light and photographed using Polaroid camera.

Data Analysis

Because of the ISSR is a dominant marker so each band was scored as present = 1 and or absent = 0. Based on Genetic relationship between progenies and their Parental trees were analyzed using Jaccard similarity index and continued by constructing A UPGMA cluster analysis using Program PAST Version 2.10 (5). If all progenies have similar alleles with their mother it assumed that no out-crossing in this plant species or the progenies were resulted from asexual reproduction or apomictic. Detection of different allele between progenies with parental trees

suggested that there were pollens transferred from other(s) donor individuals to parental trees, so they are not apomictic. Additional genetic diversity parameters were calculated using Program TFPGA Version 1.3 (15).

The magnitude of out-crossing rates were calculated per population of the three parental trees with sixty their offsprings using the software multilocus system MLRT version 3.4 (20). This program freedownload at <http://www.genetics.forestry.ubc.ca/ritland/program.html>. Based on the correlated-mating model proposed by Ritland (2002) assuming a mixed mating system model, the following mating system indices were obtained: the (minimum variance) single-locus population outcrossing rate (*ts*), the multilocus population outcrossing rate (*tm*), biparental inbreeding (*tm-ts*), multi locus correlation of outcrossed paternity (*rp*), and correlation of selfing (*rs*)(20).

RESULTS

A total ten ISSR primers were screened and four primer were selected to amplify DNA of 60 individuals samples resulted from seeds germination. Twenty one bands with high density resulted from amplification samples with four ISSR primers. The six bands detected to primer (AAC)₆, seven bands at primer of (AAG)₆, five bands at (AAT)₁₀, and four bands detected in (AG)₁₀. The nucleotide of size of each band was revealed at Table 2.

Table 2. Repeated pattern of four Primer Inter Simple Sequences Repeat (ISSR), bands number, and nucleotide size amplified DNA

Primer	Sequences	Number of Bands	Nucleotide size (bp)
HQ200181	(AAC) ₆	6	5290; 1940; 1360; 1070; 640; 410
HQ200182	(AAG) ₆	7	1600; 1310; 960; 820; 720; 570; 450
HQ200186	(AAT) ₁₀	5	2830; 2000; 1700; 1180; 930
DQ453906	(AG) ₁₀	4	1060; 920; 740; 450

Based on examination in twenty-one locus, all progenies (100%) from Kumpeh (K1-K20) possessed the identical genetic pattern with their parental (individu PK) with the average of Jaccard Similarity in the population =1.0. Selected example of identical genetic pattern amplified with ISSR marker (AAC)₆ was shown in Figure 2.

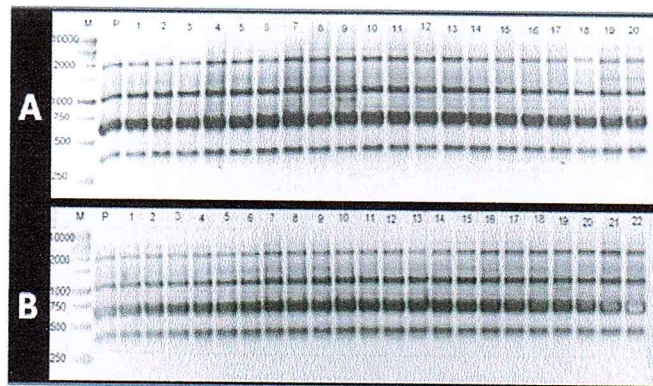


Figure 2. Similar pattern of ISSR marker (AAC)₆ in parental tree with twenty progenies from Kumpeh (A) and twenty two progenies from Dusun Tuo (B)

Fourteen or 78% of total progenies from Muaro Panco had identical genetic pattern with their parental (individual PMP). Four progenies differed to parental on one-two loci. Two progenies (MP11 and MP12) differed to parental on locus 4 of (AAG)₆, and one progeny each of MP13 and MP16 differed to parental PMP on Locus 2 and 3 of (AAC)₆ and on locus 3 of (AAG)₆, respectively. For Dusun Tuo population, Twenty one progenies (DT1-DT22, except DT7) or 96% of its total progenies had identical genetic pattern with their parental (individual PDT), and one progeny (DT7) differed to its parental (individual PDT) on Locus 3 of (AAT)₁₀. Variation of genetic pattern of the progenies and parental on unique loci was shaped their genetic structure (Figure 3).

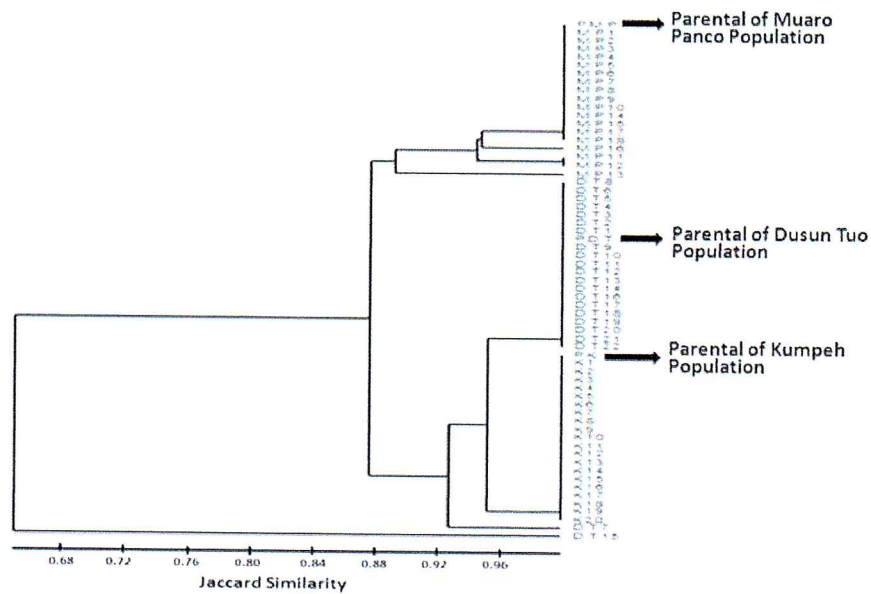


Figure 3. The genetic structure of sixty progenies and their Parental of Lansium parasiticum, duku Kumpoh using ISSR marker

Because the ISSR marker is dominant marker, the each band reflected homozygote or heterozygote a dominant allele and the absent band at same position indicated homozygote recessive allele. Parental and Progenies from Kumpoh and Muaro Panco had a dominant allele at locus 5 of (AAG)_n with band at 820 bp but it was not found in Dusun Tuo. So, it means that Dusun Tuo population had other unique recessive allele at same locus 5 of (AAG)_n. Other unique recessive allele of locus 2 (AAC)_n (band at 1940 bp) and locus 3 & 5 of (AAG)_n (band at 720 bp) were detected at Dusun Tuo and Muaro Panco populations. Unique recessive allele of locus 2 of (AAT)₁₀ (band at 1940 bp) was detected at Dusun Tuo population. The different unique allele from the population was very importance as marker and potential evolution of population or a plant species.

From the analysis sixty samples using MLTR version 3.4, indicated that the multi locus out-crossing rate (t_m) population ranged from 0.001 ± (0.000) in Dusun Tuo Population to 0.065 ± (0.515) in Muaro Panco population. The average single locus outcrossing rate population was very low and also varied from 0.001 ± (0.000) in Dusun Tuo to 0.076 ± (0.459) in Muaro Panco population (Table 1). The magnitude difference between multilocus out-crossing rate (t_m) with single locus out-crossing rate (t_s) indicated the level of mating among relative. But very low these difference suggested no possibility of biparental inbreeding or breeding among related plants.

Table 1. Estimates of multilocus out-crossing rates t_m , single locus out-crossing (t_s), correlated paternity (r_p) and fixation index (f_m) of maternal generation on ISSR markers

Parameters	Estimate value ± (standard deviation)
Dusun Tuo	
t_m	0.001 ± (0.000)
t_s	0.001 ± (0.000)
r_p	0.000 ± (0.000)
f_m	0.000 ± (0.499)
Kumpoh	
t_m	0.001 ± (0.168)
t_s	0.001 ± (0.168)
r_p	0.000 ± (0.000)
f_m	0.000 ± (0.487)
Muaro Panco	
t_m	0.065 ± (0.515)
t_s	0.076 ± (0.459)
r_p	-0.011 ± (0.059)
f_m	-0.999 ± (0.458)

Out crossing rate range from zero (obligate selfing) to one (obligate outcrossing)

DISCUSSION

The difference genetic between some progenies resulted from open population suggested gene flow still occur or some level out-crossing already conducted between different individual(s) with Parental three. Based on these genetic patterns of three populations suggested that local germplasm *Lansium parasiticum* (duku Kumpeh) facultative apomictic. The sexual reproduction determined the genotypic variation in population of apomictic plant *Hieracium pilosella* using ISSR (7).

The mating system of a plant species reflect to level of genetic diversity. The out-crossing species possesses a high genetic diversity (24) and selfing species tend to have a lower level of genetic diversity. Related to this, analysis of genetic variability between individuals from open pollinated populations and magnitude of the out-crossing rate should be estimated. The results of analysis genetic diversity of three populations (Dusun Tuo, Kumpeh and Muaro Panco) indicated that all those populations had very low genetic diversity (Heterozigosity, He ranged from 0.00-060; polymorphic loci, p ranged from 0.00-19.1). This level of genetic diversity was lower than those the genetic diversity of the plant species with similar trait breeding system i.e. selfing species (18). This facts also revealed that the *Lansium parasiticum* from Kumpeh population produced their offspring by asexual reproduction via apomictic or obligate selfing. Although level of genetic variability of progenies in Dusun Tuo and Muaro Panco were relatively very low, but small portion of pollens donor for out-crossing were detected.

Song *et al.* (2000) reported the relationship between some accessions of *Lansium domesticum* from five state in Peninsular Malaysia and identified some different types of duku (23). Some accessions had identical genotype due to the seedling arose apomictical from the parental plants and they may have been produced by asexual, grafting. The genetic variability of obligate apomictic tropical fruit mangosteen (*Garcinia mangostana* L.) that assessed by ISSR technique was also occurred due to many genetic resources from different locality in Sumatra (14).

The very low out-crossing rate with statistically was different from zero ($P < 0.05$) revealed that this *Lansium parasiticum* was assumed mostly a self fertilizing species. The $I_m - t_i$ values were not different from zero assuming that biparental inbreeding was not occurred in Kumpeh and Muaro Panco populations. So low level out-crossing rate of those populations was not resulted from selfing but due to asexual reproduction. The very low level of f_m (0.000--0.009) reflected that the offspring were not produced from inbreeding or crossing between related individuals but resulted from agamospermy (producing seeds without fertilization. According to Bayer *et al.*, (1990), the variation sexual and asexual reproduction in some populations of an alpine perennial species, *Antennaria media* (asteraceae), were occurred. These populations contained mixture sexual individuals, partial apomictic and obligate apomictic.

The concordance magnitude of multilocus out-crossing rate with the trait of breeding system were reported in self incompatible plant, *Olea europaea* subsp. *europaea* (2), mixed mating system plant, *Moringa oleifera* (17), *Warburgia ugandensis* (16) and *Koombassia malaccensis* (9). So, the level of out-crossing rate of duku 'Kumpeh' concordance to its breeding system.

Finally, from the results of analysis genetic variability and out-crossing rate suggested that the breeding system of the local cultivar 'Kumpeh' from Jambi was the mixed mating system i.e. sexual reproduction and asexual apomictic (partial apomictic). The understanding mixed mating system and their difference between populations was very important for conservation, management and enhance the utilization of this germplasm in the future. The existing identical accessions cultivar 'kumpeh' in their habitat were very useful for sustainably utilization. Event of the low genetic variability detected at Dusun Tuo and Muaro Panco, these evidence were very strategic for genetic conservation and useful for genetic improvement these germplasm.

CONCLUSION

Based on analysis of genetic variability and assessment of level out-crossing rate of sixty progenies from 3 populations proved that local cultivar 'kumpeh' of *Lansium parasiticum* were facultative apomictic breeding system.

Acknowledgments

I would like to thanks Dr. Agus Susanto from [Research Centre of Tropical Fruit, Solok, West Sumatra](#) for providing research facilities. Mr. Siddiq and Mr. Dam for sample collection. Part of this study supported by Cluster Research Grant of Professor Andalas University No. 42/UN.16/HKRGB/LPPM 2016.

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