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GENETIC DIVERSITY IN *Daemonorops draco* (Willd.) Blume (*Arecaceae*) AMONG WILD AND CULTIVATED POPULATIONS INFERRED BY RAPD MARKERS

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SUMMARY

The genetic diversity of a plant is very important, because genetic variation affects its existence in the natural populations. Analysis of genetic diversity among three wild populations (Bengayoan of 8 accessions, Tebo of 10 accessions, and Sepintun of 8 accessions) and two cultivated populations (Nunusan of 9 accessions and Mandiangin of 8 accessions) of *Daemonorops draco* (Willd.) Blume were inferred by using random amplified polymorphic DNA (RAPD) markers. Based on the 40 RAPD primers screening, 6 primers showed clear and reproducible bands. The results of the binary character in a data matrix were analyzed by using POPGENE software version 3.2. The results indicated that the genetic diversity in wild populations (H = 0.204) is higher than that of the cultivated populations (H = 0.174). The highest genetic diversity is found in Sepintun population (0.085) which is found in a secondary forest. This population is recommended as a germplasm for the cultivation of *D. draco* in the future. This study showed that a conservation area such as a national park will not always be a potential source of germplasm as the behavior of indigenous people living in this area greatly affects the genetic diversity of *D. draco*.

Key words: RAPD, *Daemonorops draco*, genetic diversity, wild and cultivated populations

Key findings: The study found that the genetic diversity of *Daemonorops draco* in wild populations is higher than the cultivated populations. The highest genetic diversity is found in Sepintun population. The last population is recommended as a germplasm for cultivation of *D. draco* in the future.

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INTRODUCTION

Daemonorops draco (Willd) Blume is a rattan species with high economic value in Sumatra (Asra *et al*., 2014). Within the periods of 2014 to 2017, the price of red resin from *D. draco* at the local market is quite high at around 2.500.000 to 3.000.000 IDR per kg (US $ 192.31 to 230.77; 1 US $ = 13.000 IDR) that mostly depends on the level of purity (Asra, 2017). Population number of the species is decreased because of several factors including legal or illegal logging, land conversion into to palm oil, and rubber plantations. The cultivation of *D. draco* by the local people has not developed because it is difficult to obtain *D. draco* seeds, since the fruits are usually harvested as young fruit. The mature *D. draco* fruits, which are the only source of seeds are usually not available. *D. draco* is one of their sources of income and is increasingly difficult to find in natural forests. For that reason, *D. draco* conservation program should be prioritized.

One of the peculiar characteristics of *D. draco* is the generation of red resin on the surface of the fruit's skin. The red colored resin is obtained from *D. draco* named dracorubin and dracorhodin (Bechtold and Mussak, 2009). This resin has long been used in China (Chinese Pharmacopoeia Commission, 2015) as medicine for diarrhea, anti-tumor, antimicrobial, antivirus, to stop bleeding, allergic dermatitis, and antioxidant activities *in vivo* and *in vitro* (Gupta *et al.*, 2007; Xin *et al.*, 2011; Hu *et al.*, 2011; Chang *et al.*, 2014). The natural distribution of *D. draco* is found in Malay Peninsula, Thailand and the Western part of Indonesia (Sumatra and Borneo) (Rustiami *et al.*, 2004).

The success of a conservation program depends on the genetic diversity information of both within and between populations. The data on the genetic diversity of a plant is very important, because the genetic variation affect its existence in the natural population. Those plants with high genetic diversity could easily adapt to environmental changes. Kang and Chung (1997) argued that the genetic diversity data and gene flow mechanisms are supposed to be a measure to determine the effectiveness of *in situ* and *ex situ* conservation program.

The analysis of genetic diversity within and among populations could be made by using genetic markers, such as random amplified polymorphic DNA (RAPD) method (Al-Khalifah *et al.*, 2012). It is used to study the genetic variation within species, to determine the relationship between closely related species and genotypes within species, and to study the clonal structure (Vierling and Nguyen, 1992). The advantages of using RAPD markers are: (1) a small quantity of DNA is needed, (2) low cost, (3) easy to learn, (4) primers are easily obtained, and (5) reveals a high level of polymorphism (Azrai, 2005; Emoghene *et al.*, 2015). The principle of RAPD markers is based on genetic differences in amplification during

polymerase chain reaction (PCR) of DNA samples (Williams *et al.*, 1990). The disadvantage of a dominant marker is that it is unknown whether the genotype resulting from amplification reflects a heterozygous or homozygote (Williams *et al.*, 1990; Welsh and McClelland, 1990). RAPD method has been used in some palm species i.e. *Calamus palustris* (Changtragoon *et al.*, 1995), *Pinanga javana* (Witono *et al.*, 2006), *Elaeis guineensis* (Sathish and Mohankumar, 2007), *Phoenix dactylifera* (Younis *et al.*, 2008; Haider *et al.*, 2012; Al-Khalifah *et al.*, 2012; Mirbahar *et al.*, 2014; Khierallah *et al.*, 2014; Bahraminejad *et al.*, 2015; Elmeeret al., 2016), and *Satakentia liukiuensis* (Witono and Kondo, 2010).

The objective of this study was to analyze the genetic diversity level of *D. draco* in wild and cultivated populations in Jambi and Riau and also to identify the population with high genetic diversity. Those populations with high genetic diversity have the potential as germplasm sources for cultivation.

MATERIALS AND METHODS

Plant material

The young leaf tissues of *Daemonorops draco* were collected from 3 wild populations, i.e. Bengayoan of 8 accessions, Tebo of 10 accessions, and Sepintun of 8 accessions; and cultivated status from 2 populations, i.e, Nunusan of 9 accessions and Mandiangin of 8 accessions (Figure 1).

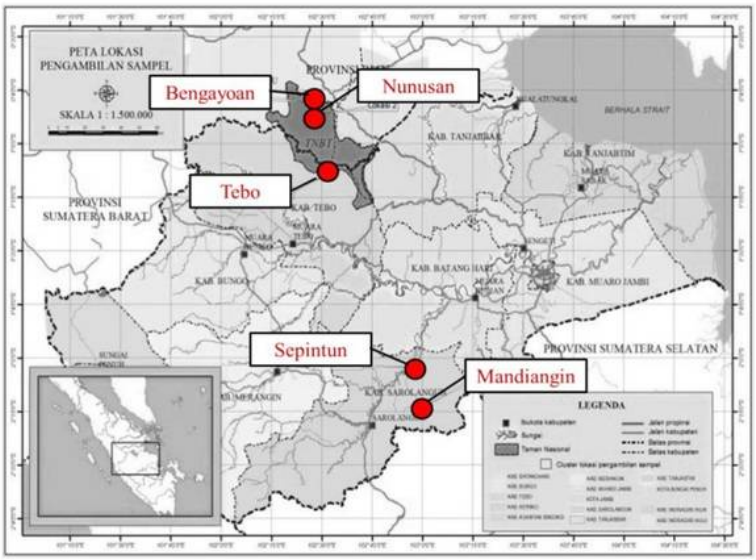

Figure 1. Source of sample populations used in the study: 1) Bengayoan, 2) Nunusan, 3) Tebo, 4) Mandiangin and 5) Sepintun.

DNA isolation

The chemicals required were: buffer extract (consist of 10% CTAB, 1M Tris-HCl (pH 8.0), 0,5 M EDTA (pH 8,0), and 5 M NaCl), liquid nitrogen, chloroform, 99% ethanol, 70% ethanol, and TE. Modification of DNA isolation protocol of Carmen del Castillo *et al.* (2006) (Asra *et al.*, 2013b). The pieces of leaves were crushed in a mortar with liquid nitrogen, then 1 ml extract buffer was added, vortexed, and incubated in water bath at 65 ^{0}C. Subsequently, 750 µl chloroform was added and centrifuged at 13,000 rpm for about 10 minutes, and the supernatant was transferred into new tube. Those steps were repeated once. The supernatant was added with cold ethanol 99% (0.8 ml), incubated at -20 ^{0}C for 1 hour, centrifuged at 10,000 rpm for 5 minutes, and the supernatant was discarded. The pellet was washed with 400 µl of 70% cold ethanol, centrifuged at 10,000 rpm for 5 minutes, and the ethanol was discarded. The pellet at room temperature, dissolve the DNA pellet in 50 µl of TE.

PCR amplification

The DNA amplification was done using Go Taq ® Green Master Mix kit. The RAPD primers used were six primers selected from the 40 primers (Changtragoon *et al.*, 1995; Sreekumar and Renuka, 2005; Witono and Kondo, 2010), i.e. OPA 11, OPO 10, OPE 18, OPAQ 05, OPZ 13, and UBC 499. Amplification was performed in a PCR merk German-Biometric. PCR was performed with the following stages: 1) initial denaturation temperature at 96 ^{0}C for 2 minutes in 1 cycle, (2) amplification at 94 ^{0}C for 30 seconds, 36 ^{0}C for 1 minutes, and 72 ^{0}C for 2 minutes by 45 cycles, and (3) final extension at 72 ^{0}C for 5 minutes in 1 cycle, followed by cooling at 4 ^{0}C (Williams *et al.*, 1990).

Data analysis

The bands were scored from the DNA profiles generated by each primer. Then, the presence or absence of each DNA band was treated as a binary character in a data matrix (code 1 and 0, respectively). The results of the binary were analyzed through the data matrix by using population genetics (POPGENE) software version 1.32 (Yeh *et al.*, 1997). A dendrogram was constructed based on the genetic similarity matrix (Nei, 1978) using the PAST program version 2.10 (2011).

RESULTS AND DISCUSSION

Marker polymorphisms generated by RAPD markers

The reproducible polymorphic bands generated by the 6 primers were detected in the 150 to 3,000 bp range, with a total number of 86 amplified bands (average of 14 bands per primer). Amplified polymorphic and percentage of polymorphic bands produced by each primers are shown in Table 1. The highest number of bands is found at UBC 499 and the highest percentage of polymorphic bands is found on primers UBC 499 and OPO 10. RAPD marker profiles of *Daemonorops draco* in all populations are shown in Figure 2.

Genetic diversity

A dendrogram was constructed by using the PAST software version 2.10

Table 1. Primer sequences with amplification numbers and polymorphic bands.

Primer	Sequences	Number of markers amplified	Number of polymorphic markers	Percentage of polymorphic bands (%)
OPA 11	CAATCGCCGT	14	13	92.86
OPAQ 05	ACGGAGCTGA	13	10	76.92
OPE 18	GGACTGCAGA	14	13	92.86
OPO 10	TCAGAGCGCC	13	13	100
OPZ 13	GACTAAGCCC	14	12	85.71
UBC 499	GGCCGATGAT	18	18	100
Total		86	79	548.35
Average		14.33	13.12	91.39

and showed that the populations of *D. draco* are divided into 3 groups with similarity index among 0.015 and 0.975 (Figure 3). Group 1 consists of 2 contiguous geographic subgroups within the population, i.e. Bengayoan (8 accessions) and Nunusan (9 accessions). The second group consist of Tebo (10 accessions) and group 3 consists of two subgroups, i.e. Sepintun (8 accessions) and Mandiangin (8 accessions). Cultivated population of Mandiangin is grouped with wild population of Sepintun. This is caused by the origin of seeds Mandiangin derived from Sepintun. Cultivated population of Bengayoan is closely related to the wild population of Nunusan, and based on the dendrogam, it turns out that the two populations are in one group, because the habitat of these population are not far from each other (Figure 1).

Genetic diversity generated by the six RAPD primers among the accessions in cultivated populations in Nunusan and Mandiangin are lower (H = 0.174) than the wild populations in Bengayoan, Tebo, and Sepintun (H = 0.204) (Table 2). Based on the information from the local people, cultivated populations of *D. draco* in Mandiangin came from the wild populations in Sepintun, while Nunusan population came from the forest in Bukit Tigapuluh National Park. The genetic diversity of *D. draco* is higher in wild populations because of some reasons: a) cross-breeding among individuals, since *Trigona* as a pollinator (Asra, 2015) are quite abundant in the wild forest than rubber plantations as a habitat of cultivated population, b) cultivated population came from a certain mother individual of wild population, and c) total number of individuals within the wild population is higher (Hiebert and Hamrick, 1983).

The small individuals within the population are likely to lead to inbreeding, and as a result, genetic variation would be low. Inbreeding increases the proportion of homozygous individuals in a population (Klug *et al.*, 2012). According to Shi *et al.* (2007) and Klug *et al.* (2012), the genetic consequences of small population size are increased genetic drift, inbreeding, and reduced gene flow. The genetic drift caused by the collection of seeds from wild plants, some of which are limited and usually found in a population of cultivation, leads to a population that when cultivated deviate significantly from the gene pool of the ancestor (Zohary, 2004) and causes low variability in a population (Shi *et al.*, 2007).

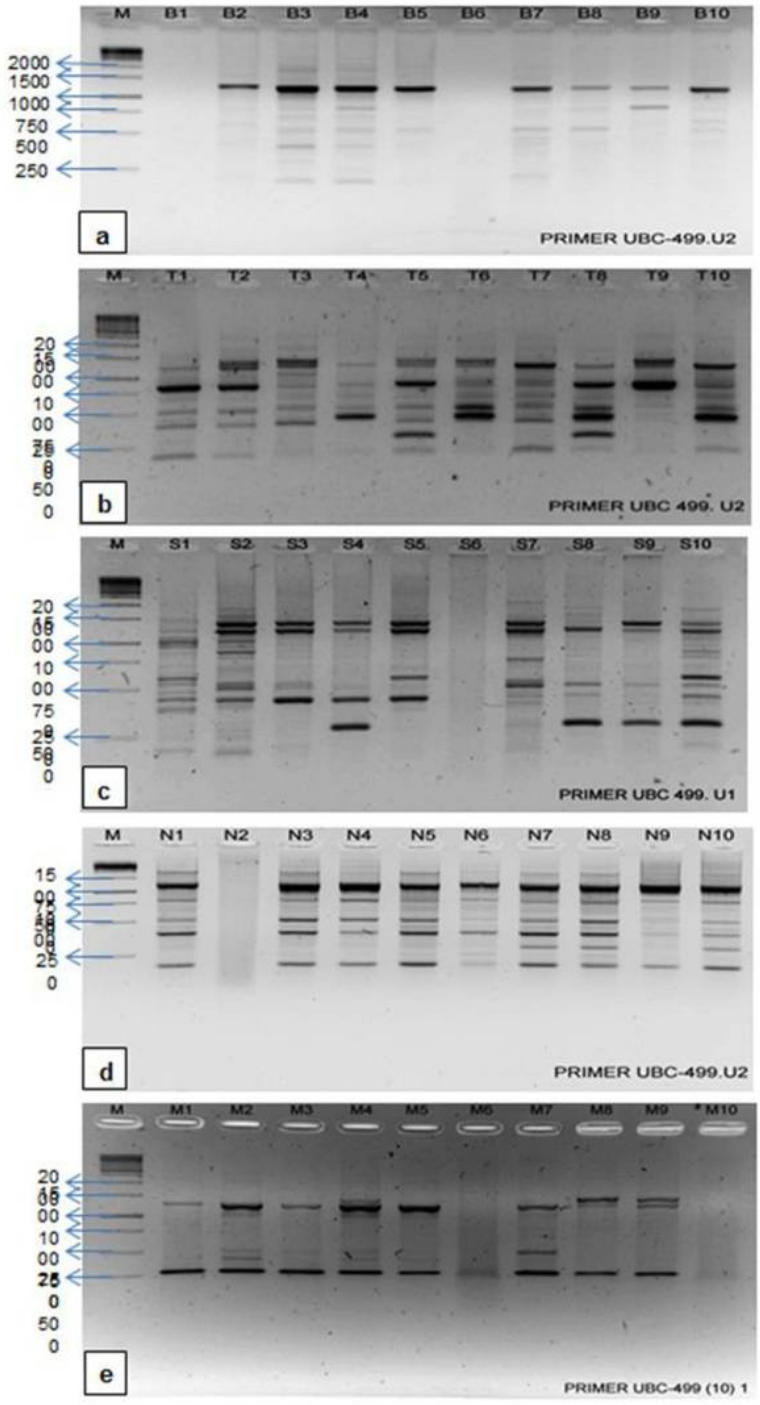

Figure 2. RAPD profile for populations of *D. draco* generated by UBC 499: (a) Bengayoan,(b) Tebo, (c) Sepintun, (d) Nunusan, (e) Mandiangin.

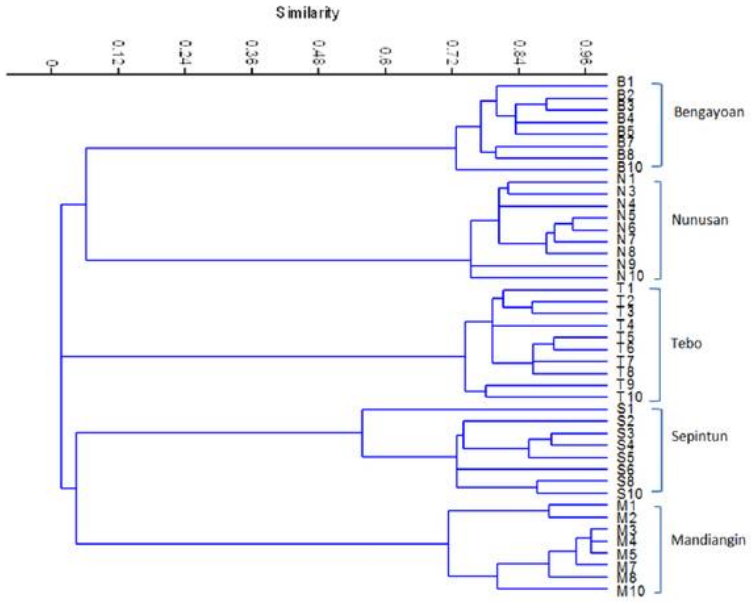

Figure 3. Dendrogam indicating genetic similarity between individuals from wild and cultivated populations.

Table 2. Genetic diversity analysis of *D. draco* in wild and cultivated populations.

Populations	Total accessions	Na	Ne	*H*	*I*	Pp (%)	N
Wild	26	1.697 ± 0.002	1.335 ± 0.002	0.204 ± 0.001	0.314 ± 0.001	69.74	159
Cultivated	17	1.474 ± 0.002	1.309 ± 0.002	0.174± 0.001	0.257± 0.001	47.37	108

Pp (%): Percentage of polymorphic loci; N: Number of polymorphic loci; Na: The average number of alleles observed; Ne: The average number of effective alleles; H: The average heterozygosity/Nei's genetic diversity; I: Mean Shannon index (Lewontin, 1972)

Another possibility is that low genetic diversity in cultivated populations may be caused by the apomictic characters of the seed (Asra *et al.*, 2013a). Most of the seeds in cultivated populations are obtained from sampling in the forest floor, and caused genetic variation similar with its ancestor. Similar results are shown in a study of genetic diversity in cultivated plants compared with their wild relatives of *Rehmannia glutinosa*, a medicinal plant in China (Zhou *et al.*, 2005; Shi *et al.*, 2007).

Based on the results, we recommend that the seed should come from wild populations for cultivation, because genetic diversity in wild population is higher (H = 0.204) than cultivated ones (H = 0.174). The plants with a high genetic diversity could adapt easily to the environmental changes including pest and disease.

Table 3. Genetic diversity of *D. draco* in 5 populations

Population	Number of sample	Na	Ne	*H*	*I*	Pp (%)	N
Bengayoan (wild population)	8	1.219±0.002	1.142±0.001	0.081±0.001	0.119±0.001	21.93	50
Nunusan, BTNP (cultivated population)	9	1.180±0.002	1.120±0.001	0.069±0.001	0.101±0.001	17.98	41
Tebo, BTNP (wild population)	10	1.184±0.002	1.128±0.001	0.072±0.001	0.106±0.001	18.42	42
Sepintun (wild population)	8	1.224±0.002	1.151±0.002	0.085±0.001	0.125±0.001	22.37	51
Mandiangin (cultivated population)	8	1.167±0.002	1.126±0.001	0.069±0.001	0.100±0.001	16.67	38

Pp (%): Percentage of polymorphic loci; N: Number of polymorphic loci; Na: The average number of alleles observed; Ne: The average number of effective alleles; H: The average heterozygosity/Nei's genetic diversity; I: Mean Shannon index (Lewontin, 1972)

The highest percentage of polymorphic loci and number of polymorphic loci are shown by population of Sepintun 22.37% and 51, respectively, followed by Bengayoan population 21.93% and 50, Tebo population 18.42% and 42, Nunusan population 17.98% and 41, and the lowest in Mandiangin population 16.67% and 38. One indicator of genetic diversity is the percentage of polymorphic loci. According to Klug *et al.* (2012), percentage of polymorphic loci by profiles of different DNA bands in different individuals can be calculated to show the level of genetic diversity in the population.

Other common methods to estimate diversity in the population is the expectation of heterozygosity (He) (Nybom, 2004). Based on the heterozygosity value, the highest population was Sepintun (H = 0.085), followed by Bengayoan (H = 0.081), Tebo (H = 0.072), Mandiangin (0.069), and Nunusan (H = 0.069) (Table 3).

Sepintun population has high total mature individuals than other populations. It is about 33 mature individuals. According to Asra *et al.* (2014), genetic diversity of the *D. draco* using ISSR markers in Bukit Tigapuluh National Park (BTNP) is lower than the Sepintun Secondary Forest (H = 0.0969). Based on the information from local people, Sepintun area is the center of red resin production. The total number population supported by out-crossing, causes those populations to have a high genetic diversity. This is in accordance with the opinion of Hamrick and Godt (1996) which states that in out-breeding species have high levels of genetic diversity in the population. The pollinator insects (*Trigona* spp.), suspected of being a pollinator, are mostly found in Sepintun compared to other populations, due to the presence of many flowering plants around this population (Asra *et al.*, 2013a). Stehlik and Holderegger (2000) stated that the proportion of out-crossing depends on the degree of conformity, the availability of matching pairs, and the type of pollinator. When the pollinator is overflowing, the food search pattern becomes directed to

genetically affect the plants of being pollinated and eaten.

The sex ratio in dioecious plants is important to study (Opler and Bawa 1978). Sex ratio of *D. draco* in wild population i.e. Bengayoan 1:5, Tebo is 1:5.4, and Sepintun is 1:2.3 whereas cultivated population in Nunusan 1:1.4 and Mandiangin 1:1.5 (Asra *et al.*, 2012). The indigenous tribes such as Talang Mamak, Old Malay (in Bengayoan, Tebo, and Nunusan), and the local people in Mandiangin always cut down and burn male individuals of *D. draco* because they consider them useless, since it does not produce fruits (Asra *et. al.*, 2014). However, in Sepintun population, the male individuals are allowed to grow, and they will cut them down to make baskets for their daily needs. The habits of indigenous people directly affected the reproductive biology of *D. draco.* We recommended to the management authority of Bukit Tigapuluh National Park that education awareness is important for the indigenous people to maintain the male individuals of *D. draco.* The national park in Bengayoan, Tebo, and Nunusan is the home of *D. draco* population.

CONCLUSION

The genetic diversity of *Daemonorops draco* in wild populations (H = 0.204) is higher than the cultivated populations (H = 0.174), because the total number of individuals within wild population is low. The highest genetic diversity is presented in Sepintun population. This population is recommended as the germplasm source for cultivation of *D. draco* in the future.

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