Name	Counterpart	Title
Revis Asra, Joko Ridho Witono, Izu Andry Fijridiyanto, Upik Yelianti	**B14**	Genetic diversity of Jernang (*Calamus* spp.) in Sumatra, using ISSR markers

Jernang rattan (Dragon's Blood Palm) belong to the Arecaceae family, which was previously included in the genus *Daemonorops*. Based on molecular and morphological phylogenetic evidence, this genus was transferred to the genus *Calamus* (Baker, 2015). There are more than 400 species of calamus (Baker, 2015), but only 12 species produce red resin. In Sumatra, five species of Jernang (*Calamus acehensis*, *C. brachystachys*, *C. draco*, *C. dracuncula*, and *C. dransfieldii*) have been found (Rustiami *et al.* 2004).
The red resin has a high economic value and is used as a natural dye and medicine (diarrhea, anti-tumor, anti-viral, anti-microbial, and bleeding. The population and production of Jernang decreased strongly (BKSDA Jambi 2010) due to illicit logging and the conversion of forests into oil palm and rubber plantationn (Sulasmi *et al.*, 2012a; Sulasmi *et al.*, 2012b). To support the conservation and cultivation of this Jernang species, it is necessary to know the genetic diversity of this plant. Molecular techniques are commonly used for the analysis of genetic diversity within and between populations. One of the molecular markers that can be used that has a high level of polymorphism is efficient, and inexpensive is the Inter Simple Sequence Repeat (ISSR) marker. ISSR is the most widely used marker to study clonal diversity and population genetic structure (Rossetto *et al.*, 1999).
The objective of this study was to examine the genetic diversity of four Jernang species in Sumatra by using ISSR markers in order to assess the extent of genetic variation and to discuss the conservation implications based on the genetic characteristics as conservation recommendations for those species.

Leaf samples of 10–25 individuals of four Jernang species in Sumatra (*Calamus Confusus*, *C. Longipes*, *C. Draco* and *C. Melanochaetes*) from Sarolangun Regency, Jambi Province (Sepintun, Lamban Sigatal, and Taman Bandung) and Aceh Utara Regency, Aceh Province (Riseh Teungoh Village, Sawang District) were collected and stored in silica gel prior to extraction.
All genomic DNA was extracted from silica gel-dried leaf tissues. DNA was isolated using the Quick-DNATM plant/seed MiniPrep Kit (Zymo Research) according to the manufacturer's manual. DNA samples from each plant were analyzed individually to detect intra- and inter-population variations as well as sex determination markers.
The DNA amplification was carried out following ISSR techniques. An initial screening of 20 ISSR primers that were successfully utilized in other plant species (Sarmah *et al.*, 2017; Asra *et al.*, 2014; Sarmah & Sarma, 2011; Younis *et al.*, 2008) was performed in order to test their readability and amplification profiles for polymorphism. Fourteen to fifteen markers were selected and used in this study for each Jernang species.
The amplified DNA fragments generated by ISSR markers will be processed using the electrophoresis with 5 µL of the standard DNA and a 1 kb DNA ladder (100 ng µL-1) in the first slot of 1.5% agarose gel in TBE 1X as the buffer solution. Then, the agarose gel was run using electrophoresis technique at 100 volts for 60 minutes at room temperature. The resulting amplified bands were observed and documented using the Gel Doc™ EZ Gel Documentation System (Bio-Rad). Each reaction was repeated at least twice to get reproducible bands.
DNA bands derived from PCR amplification of each sample at a marker locus were considered one allele. DNA bands that have the same migration rate were assumed to be homologous loci in the sample. The ISSR bands were scored as binary data, in which the presence and absence of bands in a given size class were converted to 1 and 0, respectively. The genetic relationships of each Jernang species were evaluated based on similarity coefficients as implemented in the NTSYS-PC software version 2.1 (Rohlf, 2000). Heterozygosity index (H), polymorphism information content (PIC), discriminating power (D), effective multiplex ratio (E), marker index (MI), arithmetic mean heterozygosity (Havp), and resolving power (R) were conducted using the iMEC software program online (Amiryousefi *et al.*, 2018).

Seven species of Jernang from Sumatra were collected in this study: Jernang Aceh (*Calamus confusus* (Furtado) W.J. Baker), Jernang Kalumuai (*Calamus longipes* Griff.), Jernang Rambai (*Calamus draco* Willd.), Jernang Umbut (*Calamus melanochaetes* (Blume) Miq.), Jernang Burung (*Calamus gracilipes* Miq.), Jernang Kelukup (*Calamus* sp.), and Jernang Landak (*Calamus* sp.). However, the three species (Jernang Burung, Jernang Kelukup, and Jernang Landak) were collected from fewer than five individuals, so these three species were excluded from the analysis. The other four Jernang (Jernang Aceh with 20 accessions, Jernang Kalumuai with 17 accessions, Jernang Rambai, and Jernang Umbut with 25 accessions and 10 accessions, respectively). In this study, selected 14 primers (Jernang Aceh, Jernang Rambai, and Jernang Umbut) and 15 primers (Jernang Kalamuai) used for genetic diversity analysis and relationship analysis of each Jernang species produced amplification products (scorable bands) and all resulted in polymorphic fingerprint patterns.

The lowest and highest polymorphism indices of four Jernang species from Sumatra. The heterozygosity index (H) ranged from 0.3200 (Jernang Umbut) to 0.5000 (Jernang Rambai). The polymorphism information content (PIC) of the primer ranged from 0.3209 (Jernang Kalamuai) to 0.4134 (Jernang Rambai). The effective multiplex ratio (E) ranged from 3.240 (Jernang Rambai) to 9.500 (Jernang Aceh and Jernang Umbut). The arithmetic means heterozygosity (Havp) ranged from 0.0011 (Jernang Kalamuai) to 0.0058 (Jernang Umbut). The marker index (MI) ranged from 0.0049 (Jernang Rambai) to 0.0296 (Jernang Umbut). The discriminating power (D) of the primer ranged from 0.3615 (Jernang Umbut) to 0.9438 (Jernang Kalamuai). The resolving power (R) of the primers varied from 1.2 (Jernang Umbut) to 12.5 (Jernang Kalamuai). The PIC values of all Jernang species showed that values higher than 0.25 and lower than 0.5 were included in the moderate category (Botstein *et al.*, 1980).

References

Amiryousefi A, Hyvönen J, Poczai P (2018) iMEC: Online Marker Efficiency Calculator. Applications in Plant Sciences 6: e1159

Asra R, Syamsuardi, Mansyurdin, Witono JR (2014) The study of genetic diversity of *Daemonorops draco* (Palmae) using ISSR markers. Biodiversitas 15: 109-114

Baker WJ (2015) A revised delimitation of the rattan genus *Calamus* (Arecaceae). Phytotaxa 197: 139–152

BKSDA Jambi (2010) Non-timber forest product of Jambi province. Department of Forestry, Jambi Area Office, Jambi, Indonesia

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32: 314–331

Rohlf FJ (2000) NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.1. Applied Biostatistics Inc.

Rossetto M, Jezierski G, Hopper SD, Dixon KW (1999) Conservation genetics and clonality in two critically endangered eucalypts from the highl y endemic south-western Australian. Biol Conserv 88: 321-331

Rustiami H, Setyowati FM, Kartawinata K (2004) Taxonomy and uses of *Daemonorops draco* (Willd.) Blume. J Trop Ethnobiol 1: 65-75

Sarmah P, Barua VJ, Nath J, Sarma RN, Kurian B, Hemanthkumar AS, Sabu KK (2017) ISSR and SSR markers reveal sex-specific DNA sequences in three *Calamus* species from India. Agroforest Syst 91: 509–513

Sarmah P, Sarma RN (2011) Identification of a DNA marker linked to sex determination in *Calamus tenuis* Roxb., an economically important rattan species in northeast India. Molecular Breeding 27: 115-118

Sulasmi IS, Nisyawati, Purwanto Y, Fatimah S (2012a) The population of Jernang rattan (*Calamus draco*) in Jebak Village, Batanghari District, Jambi Province, Indonesia. Biodiversitas 13: 205-213

Sulasmi IS, Nisyawati, Purwanto Y, Fatimah S (2012b) Jernang rattan (*Calamus draco*) management by Anak Dalam Tribe in Jebak Village, Batanghari, Jambi Province. Biodiversitas 13: 2085-4722

Younis RA, Ismail OM, Soliman SS (2008) Identification of sex-specific DNA markers for date palm (*Phoenix dactylifera* L.) using RAPD and ISSR techniques. Research Journal of Agriculture and Biological Sciences 4: 278-284