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CHROMOSOME NUMBERS AND KARYOTYPES OF *EURYCOMA LONGIFOLIA* JACK AND *EURYCOMA APICULATA* A.W. BENN (SIMAROUBACEAE)

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ABSTRACT

Knowledge of chromosome cytology of the *Eurycoma longifolia* and *Eurycoma apiculata* was limited. The goal of this study was to investigate number, size, karyotype, and ideogram of chromosomes of *E. longifolia* and *E. apiculata*. The total of chromosome lengths, relative lengths, the ratio of long arm to short arm, and index of centrometric of somatic cell chromosomes were measured. The basic number of chromosomes of both species was x=7, chromosome counting displayed that *E. longifolia* and *E. apiculata* were diploid. Somatic chromosome numbers that are found were 2n = 2x = 14 for both species. The total chromosome length was ranged from 5.33 to 9.22 µm for *E. longifolia* and ranged from 4.15 to 10.52 µm for *E. apiculata*. Karyotype formula of *E. longfolia* and *E. apiculata* was 2n = 2x = 14 = 6m + 1sm and 2n = 2x = 7m, of which six pairs metacentric chromosomes and one pair submetacentric chromosome in *E. longifolia* and seven pairs metacentric chromosomes in *E. apiculata* respectively. Based on Eroglu's classification, karyotype symmetric/asymmetric index (S/AI) value of the *E. apiculata* was 1.0 and was classified into perfectly symmetric karyotype that is characterized by completely metacentric chromosomes, while S/AI value of *E. longifolia* was 1.14 and was grouped as symmetric karyotype.

Key words: *Eurycoma*, chromosome, karyotype. symmetric and asymmetric index

INTRODUCTION

Eurycoma spp belongs to Simaroubaceae family. This genus consisted of three species, namely *Eurycoma longifolia* Jack, *Eurycoma apiculata* A. W. Benn and *Eurycoma harmandiana* Pierre. *E. longifoli*a was generally found in tropical forest of Southeast Asian countries, encompass Indonesia, Malaysia, Myanmar, Cambodia, and Thailand (Nooteboom, 1962), *E. apiculata* was only distributed in Sumatra-Indonesia and Malaysia peninsular (Nooteboom, 1962) while *E. harmandiana* was only reported growing in Thailand and Laos (Kanchanapoom et al., 2011). They are known as tropical medicinal plant having numerous types of important bioactive compounds which are mostly found in root part. It has been utilized as antioxidant (Varghese et al., 2013), antimicrobial (Khanam et al., 2015), antimalarial, a booster of male sexual health and etc. (Kanchanapoom et al., 2011; Thu et al., 2017; Abubakar et al., 2017).

Although they are important medicinal plants, information on chromosome number of Eurycoma spp is poorly known and no literature reported on detailed cytological data of Eurycoma spp. Therefore, the cytogenetic study of these species is important to be implemented, which one of the main aims was the identification of chromosome and composes the karyotype based on microscopic morphological characteristics of the chromosomes. Chromosomes are hereditary material present inside the plant cell for carrying genetic information through generations. Knowledge of chromosome morphology description is required to characterize genomes of plants and this information is important for plant systematic (Stace, 2000) evolutionary analysis (Baltisberger and Horandl, 2016), and adaptability of the environment (Wang et al., 2011). In addition, in plant breeding, parent plants cytogenetic information is very useful for determining suitable parent combinations and for tracking parent markers in hybrid plants.

The karyotype is a complete-chromosomes collection of the species; it is built to reflect the size, shape, and number of chromosome complement, where each chromosome that identified is related to their morphological characteristics. The number, size, and asymmetry of chromosomes were beneficial traits in cytotaxonomy of plant and were important to explain the plant origin, plant speciation, and genetic relationships among plants (Stebbins, 1971).

In this study, we conducted a cytological analysis for two species of Eurycoma, namely *Eurycoma longifolia* Jack and *Eurycoma apiculata* A.W. Benn. The goal of this study was to investigate number, size, karyotype, and ideogram of chromosomes of *E. longifolia* and *E. apiculata.*

MATERIALS AND METHODS

E. longifolia and *E. apiculata* seedlings were collected from Forest Reserve of Kenegerian Rumbio, Kampar-Riau Indonesia for sampled, and chromosome analysis was performed in Biology Research Center, Indonesia Life Science, Cibinong-Bogor, Indonesia.

Root tips meristems were collected directly from the seedling that were maintained in Laboratory. Chromosome preparation was followed (Jahier and Tanguy 1996) method with modifications. Root tips were soaked in 45% glacial acetic acid solution for 30 minutes at 4ºC. Subsequently, root tips were taken out and washed with distilled water and hydrolyzed in 1N HCl for 55 minutes at 55ºC. After hydrolyzed, they were rinsed with distilled water three times and then transferred into staining solution (2% aceto-orcein) for 20 minutes at room temperature, root tips were mounted on the slide and a drop acetic acid (45%)-glycerol mixture (9:1) and squashed with a glass cover.

The chromosome slides were observed under a Nikon AFX II microscope, and five well-scattered metaphase plates were chosen to be analyzed. The selected metaphase plates were photographed and arranged the karyotype based on their length. Chromosomes were measured and paired using Photoshop CS 5.0 software. The several parameters including total chromosome length (TCL), long arm length (LAL) and short arm length (SAL) were measured for each chromosome while arm ratios (R=LAL/SAL) and index of centromeric (IC=[SAL/TCL] x 100) were calculated. Arm ratio was used to classify the chromosome based on the system proposed by Levan et al. (1964), namely metacentric for arm ratio of 1.0–1.69, submetacentric for arm ratio of 1.7–2.99, subtelocentric for arm ratio of 3.0–7.0, telocentric for arm ratio of above 7.0. The number of chromosome assigned was based on the descending order of chromosome length.

The intra-chromosomal and inter-chromosomal karyotype asymmetries were obtained from mean centromeric asymmetry (M_{CA}) and the coefficient of variation of chromosome length (CV_{CL}) according to Peruzzi and Eroglu (2013) and Paszko (2006). M_{CA} was obtained from the mean (LAL-SAL)/LAL+SAL) x 100, where, for each chromosome, LAL is long arm length and SAL is short arm length; whereas CV_{CL} was obtained from the standard deviation of (LAL+SAL) divided by the mean (LAL+SAL) x 100. Beside that, we also determined the index of karyotype symmetric/asymmetric (S/AI) according to Eroglu (2015), this index was calculated as, S/AI = [(1 x M) + (2 x SM) + (3 x A or ST) + (4 x T)] / 2n, where M was chromosome number of metacentric; SM was chromosome number of submetacentric; ST was chromosome number of subtelocentric; A was chromosome number of acrocentric; T was chromosome number of telocentric, and 2n was chromosome number of diploid.

RESULTS AND DISCUSSIONS

In this study, the number, size, karyotype and ideogram of chromosomes of two species of Eurycoma were determined. The cytological data of *E. longifolia* and *E. apiculata* were the first reports. Mitotic metaphase chromosomes of both species are given in Figure 1.

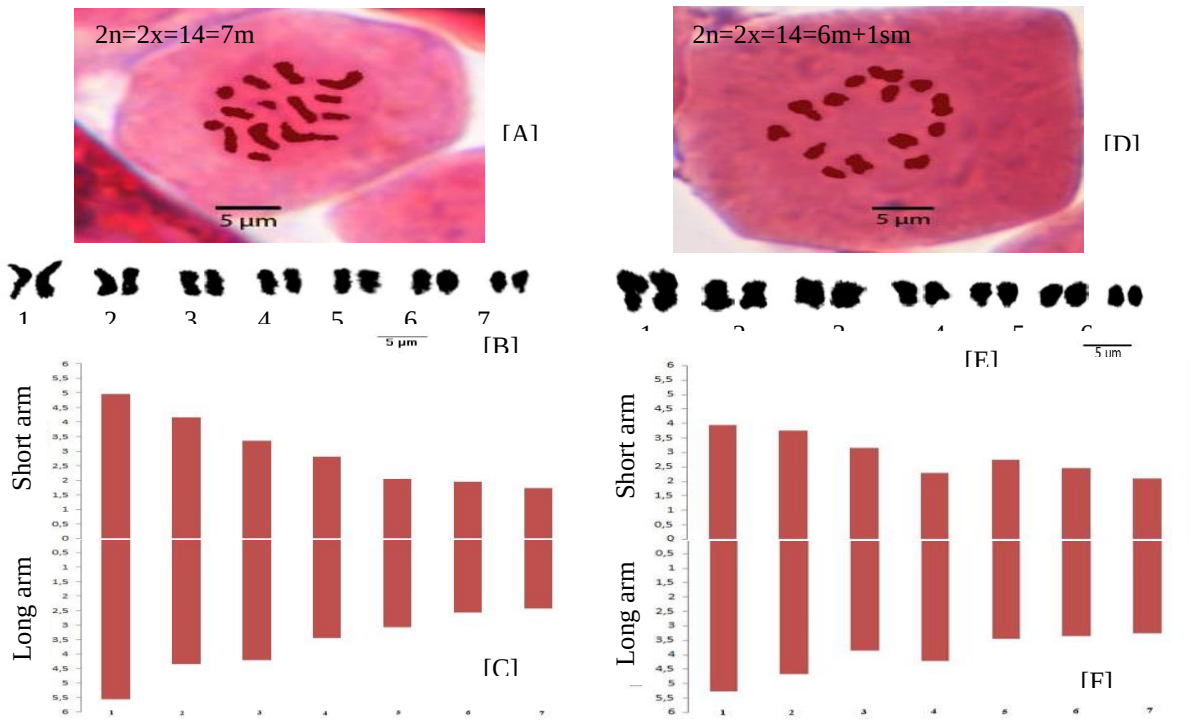

Figure 1: Metaphase, karyogram and ideogram *E. apiculata* (A-C) and *E. longifolia* (D-F)

Analysis of somatic metaphases displayed that *E. longifolia* and *E. apiculata* represented diploid species with basic number chromosome (x) of 7 and number of chromosomes of both species were similar, namely 2n = 2x = 14. The chromosomes measurement data of *E. longifolia* and *E. apiculata* are shown in Table 1. Both species exhibited low variation in chromosome sizes. The length of chromosomes of *E. longifolia* was ranged from 5.33 to 9.22 µm with the mean length of chromosomes of 6.92 µm. The length of long arm was varied from 3.25 to 5.27 µm and length of short arm was varied from 2.09 to 3.95 µm. The index of centromere (IC) varied from 35.17 to 44.95 that showed centromeres located at the median and sub-median of chromosomes. Arm ratio was ranged from 1.22 to 1.84 so that chromosome types of *E. longifolia* was metacentric and sub-metacentric. The length of chromosomes of *E. apiculata* varied from 4.15 to 10.52 µm with a mean length of chromosomes of 6.66 µm. The long arm ranged from 2.43 to 5.56 µm while short arm ranged from 1.72 to 4.97 µm. The IC varied from 39.95 to 48.78 that showed a predominantly centromeres located at the median. The arm ratio of *E. apiculata* was ranged from 1.05 to 1.54, so that chromosome types of *E. apiculata* was metacentric (Table 1).

Table 1. Morphometric characteristic of the chromosome of the *E. longifolia* and *E. apiculata*

Chromosome pairs	*Eurycoma longifolia*						*Eurycoma apiculata*					
	TCL (µm)	LAL (µm)	SAL (µm)	IC	R	CT	TCL (µm)	LAL (µm)	SAL (µm)	IC	R	CT
1	9.22	5.27	3.95	42.82	1.34	M	10.52	5.56	4.97	47.18	1.12	m
2	8.42	4.68	3.74	44.44	1.25	M	8.50	4.36	4.15	48.78	1.05	m
3	7.00	3.85	3.15	44.95	1.22	M	7.56	4.21	3.36	44.38	1.25	m
4	6.50	4.21	2.29	35.17	1.84	Sm	6.26	3.45	2.80	44.80	1.23	m
5	6.19	3.45	2.74	44.28	1.26	M	5.12	3.07	2.04	39.95	1.50	m
6	5.79	3.35	2.44	42.19	1.37	M	4.53	2.58	1.95	43.11	1.32	m
7	5.33	3.25	2.09	39.14	1.55	M	4.15	2.43	1.72	41.46	1.41	m

Note: TCL: Total chromosome length; LAL: long arm length; SAL: short arm length; IC: index of centromere; R: ratio of long arm to short arm; CT: chromosome types; m = metacentric; sm = submetacentric

Karyotype formula for *E. longifolia* was 2n = 2x = 14 = 6m + 1sm, which consisted of six pairs of metacentric chromosome and one pair of submetacentric chromosome. Karyotype formula for *E. apiculata* was 2n = 2x = 14 = 7m which all chromosome types of *E. apiculata* were metacentric (m) (Table 1). Differences of karyotype can occur within or among species and that has been reported in some of the species such as *Phlomis olivieri* (Yousefi et al., 2018); *Ocimum basilicum* (Edet and Aikpokpodion, 2014) and genus *Vernonia* (Kemka-Evans and Bosa, 2013). Kang et al., (2008) stated that karyotype divergence among species occurred due to rearrangement of the chromosome, such as the event of translocation, deletion, and inversion, but interspecific hybridization can also change karyotypes. Futhermore, Seijo and Fernandez (2003) stated that among species are found differences in the number of the chromosomes, formula of karyotype and indexes of asymmetric that may create to the distinction of genus.

Eroglu (2015) expressed that in karyotype research, karyotype asymmetry is the essential parameter to determine. CV_{CL} and M_{CA} were a reliable parameter to determine the karyotype asymmetry (Peruzzi and Eroglu, 2013). M_{CA} values of *E. longifolia* and *E. apiculata* were 15.82 and 9.99, respectively. The CV_{CL} values of *E. longifolia* and *E. apiculata* were 20.46 and 34.94, respectively. According to Peruzzi and Eroglu (2013) and Paszko (2006) that M_{CA} and CV_{CL} values vary between 0-100, where the value of 0 indicates perfectly symmetry and the value of 100 indicates perfectly asymmetric. In other words, M_{CA} and CV_{CL} values increase with increasing asymmetry. Based on M_{CA} and CV_{CL} values, the chromosome of both species tend to symmetric karyotypes, that is indicated by their karyotypes mainly consists of metacentric and submetacentric chromosomes, have similar size and no existance of chromosome subtelocentric or acrocentric. This a line with the arm ratios chromosome pairs did not exceed 2.0 (Table 1), which denoted that both species have very high intra-chromosomal symmetry. In the symmetrical karyotype, the entire chromosome is of approximately equal size and has median or sub-median centromere (Eroglu, 2015). Karyotype symmetry have been reported in many species, such as *Santalum album* (Zhang et al., 2010); *Morus* spp (Venkatesh and Munirajappa, 2014); *Rheum palmatum* species (Ye et al., 2014) and *Allium* species (Ramesh, 2015). Karyotype chro-

mosome within a taxon (genus or species) can alter from symmetry to asymmetry karyotype that reflected from increasing the ploidy level of species. Weiss-Schneeweiss and Schneweiss (2013) explained that change in karyotype symmetry often involves modification in chromosome size and morphology usually caused by DNA sequence expansion or deletions or by centric fusion/fission (accompanied by dysploidy). In addition, rearrangements chromosomes via an event of inversion of pericentric, unequal translocation, fission, and fusion centric can alter the morphology of chromosomes (Moraes et al., 2016) so that occurs accumulation of variation of relative sizes of chromosomes that tend to create the karyotype more heterogeneous and increased asymmetry.

On the other hands, we also estimated the karyotype symmetric/asymmetric index based on Eroglu (2015). He proposed new classification model to determined the level of karyotype symmetric/asymmetric, through calculating the karyotypes Symmetric/Asymmetric (S/A_I), with categorized full symmetric (S/AI =1); symmetric ($1.0 < S/AI \leq 2.0$); between symmetric and asymmetric ($2.0 < S/AI \leq 3.0$); asymmetric ($3.0 < S/AI < 4.0$); and full asymmetric (S/AI = 4). Based on Eroglu's classification S/A_I value of *E. apiculata* was 1.0 and was classified as perfectly symmetric karyotype that is characterized by completely metacentric chromosomes, while S/A_I value of *E. longifolia* was 1.14 and was grouped as symmetric karyotype. These results also explained that *E. apiculata* is more primitive than *E. longifolia* due to *E. apiculata* have full symmetric karyotype. Stebbins (1971) stated that karyotype evolution in higher plants is commonly derived from symmetry karyotype to asymmetry karyotype. In other words, species that are considered primitive have larger symmetrical chromosomal karyotype whereas non-primitive species tend to have more asymmetric karyotypes. The result of the study of Medeiros-Neto et al. (2017) also supported Stebbins' hypothesis that asymmetric karyotypes derived from a symmetric karyotype.

This study concluded that the basic number of chromosomes of *E. longfolia* and *E. apiculata* were same, with x=7, karyotype formula was 2n = 2x = 14 =6m + 1sm for *E. longfolia* and 2n = 2x = 7m for *E. apiculata*. Karyotype of the *E. longifolia* was symmetric while *E. apiculata* was full or perfectly symmetric.

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