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Ploidy levels and reproductive behaviour in natural populations of *Centotheca lappacea* from Jambi, Indonesia

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Master's thesis at the

Faculty of Forest Sciences and Forest Ecology

Georg-August-University of Göttingen

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Masterarbeit an der

Fakultät für Forstwissenschaften und Waldökologie

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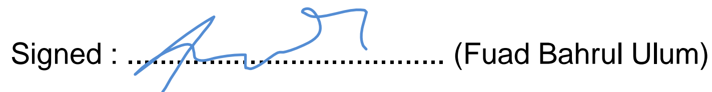
Statement of Declaration

This thesis is the result of my own work and investigations, except where otherwise stated. This work is being submitted for the degree of MSc and has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed :  .... (Fuad Bahrul Ulum)

Date : 21 July 2014

Summary

Flowering plants reproduce in three fundamentally different modes of reproduction via outcrossing sex, selfing, and asexuality, i.e. apomixis. Selfing and apomixis have often been seen as alternative evolutionary strategies of flowering plants that are advantageous for colonization. Their invasiveness may influence the conservation and management of tropical ecosystems. The Jambi province in eastern central Sumatra, Indonesia, is one of the largest regions of tropical lowland rainforest in Southeast Asia with the massive transformation of lowland rainforest into rubber and oil palm plantations.

Centotheca lappacea from subfamily Panicoideae (family Poaceae) is one of the predominant plants growing in the understory of plantations in the Bukit Duabelas and Harapan region, in Jambi Province (Sumatra, Indonesia). Many widespread Panicoid grasses reproduce via apomixis which enhances colonization of new habitats and is a factor for invasiveness.

The aims of this research were to determine the reproduction mode and the chromosomal ploidy level of *Centotheca lappacea*. Our hypothesis was that *Centotheca lappacea* in the rainforest transformation systems of Sumatra would be polyploid and mainly reproduces asexually via seed (apomixis). The mode of reproduction was determined by the microscopic study of megasporogenesis, embryo sac development, and pollen development. Flow Cytometric Seed Screen (FCSS) was applied to reconstruct the reproductive pathways of mature seeds.

Centotheca lappacea from Jambi, Indonesia, is diploid with 2n = 2x = 24 chromosomes. Female gametophytes develop through meiotic division from megaspore mother cells. The embryo sac has the Polygonum type structure (monosporic, eight nuclei and seven cells in the embryo sac). Pollen develops from pollen mother cell (pmc), meiotic division of pmc results coenocytes which develop further into mature pollen with each one vegetative and generative cell. Flow cytometry seed analysis indicated sexual reproduction and rejected the hypothesis of apomictic development in *Centotheca lappacea*.

The abundance status of the species might be due to the epizoochorous dispersal strategy by spikelets which are adapted to efficient seed dispersal. We suggest further studies on selfing and outcrossing preferences of this species.

Zusammenfassung

Blütenpflanzen reproduzieren sich auf drei grundlegend verschiedene Arten, durch Auskreuzung, Selbstbefruchtung und asexuell, wie zum Beispiel durch Apomixis. Selbstbefruchtung und Apomixis werden oft als alternative evolutionäre Strategien der Blütenpflanzen betrachtet, welche von Vorteil für eine Besiedlung sind. Ihre Invasivität könnte die Erhaltung und das Management tropischer Ökosysteme stark beeinflussen. Die Untersuchungsgebiete liegen in der Jambi Provinz (Sumatra, Indonesien), hierbei handelt es sich um eine der größten Regionen von tropischen Tieflandregenwäldern in Südost Asien, mit einer massiven Umwandlung von Tieflandregenwäldern in Kautschuk- und Ölpalmplantagen.

Centotheca lappacea aus der Unterfamilie der Panicoidae (Familie der Poaceae) ist eine der dominantesten Grasarten, welche im Unterwuchs von Plantagen in Bukit Duabelas und Harapan Gebiet (Jambi Provinz) vorkommen. Viele weitverbreitete Panicoide Gräser reproduzieren sich vorrangig durch Apomixis, welches die Kolonisierung von neuen Gebieten und somit ihre Invasivität vorantreibt.

Das Ziel dieser Studie war es, den Reproduktionsmodus und den Ploidiegrad von *Centotheca lappacea* zu bestimmen. Unsere Hypothese war, dass die Art *Centotheca lappacea*, die sehr häufig auf den untersuchten Flächen vorkommt, polyploid ist und sich überwiegend durch asexuelle Samenproduktion (Apomixis) reproduziert.

Der Reproduktionsmodus wurde durch mikroskopische Untersuchungen der Megasporogenese, Embryosackbildung und Pollenentwicklung bestimmt. Durchflusszytometrie an Samen (Flow cytometric seed screening) wurde angewendet, um den Reproduktionsverlauf der reifen Samen zu rekonstruieren.

Centotheca lappacea aus der Jambi Region, Indonesien, ist diploid mit 2n=2x=24 Chromosomen. Die weiblichen Gametophyten entwickeln sich durch meiotische Teilung aus der Megasporenmutterzelle.

Der Embryosack hat den Polygonum-Typ, d.h. monospor, mit acht Zellkernen und sieben Zellen im Embryosack. Die Pollenentwicklung verläuft von der Pollenmutterzelle durch meiotische Teilung in einen Coenozyt und entwickelt sich weiter in reifen Pollen mit einer vegetativen und generativen Zelle. Die durchflusszytometrischen Untersuchungen an reifen Samen deuten auf sexuelle Vermehrung hin und wiederlegen die Hypothese von apomiktischer Fortpflanzung bei *Centotheca lappacea*.

Das vermehrte Vorkommen der Art im Untersuchungsgebiet könnte sich durch die epizoochore Samenverbreitungsstrategie (anhaftende Ährchen), die eine effiziente Samenverbreitung der Gräser ermöglichen, erklären lassen.

Weitere Studien im Bereich Selbstbefruchtung und Auskreuzungspräferenzen sind empfehlenswert.

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List of abbreviations and acronyms

AE	adventitious embryony
MMC	megaspore mother cell
FM	functional megaspore
FCSS	flow cytometry seed screen

Chapter 1: Introduction

Flowering plants reproduce in three fundamentally different modes of reproduction via outcrossing sex, selfing, and asexuality (Richards, 2003). Sexual reproduction involves meiosis (recombination and segregation) and sexual fusion, whereas asexual reproduction, i.e. apomixis, avoids meiotic reduction and infers the parthenogenetic development of egg cells (Richards, 1997). Selfing makes the individual plant independent from the requirement of mating partners, allows rapid colonization, but it also has negative consequences such as inbreeding depression, pollen discounting, and genetically uniform populations (Barrett, 1998). On the other hand, vegetative propagation separates physically parts of the plants and results in local spreading (Klimeš et al., 1997).

Apomixis is defined as asexual reproduction through the seed (Savidan, 2007). Apomixis occurs in three major forms, i.e. adventitious embryony (AE), diplospory, and apospory (Rodriguez-Leal & Vielle-Calzada, 2012; Appendix 1). Adventitious embryony involves the formation of asexual plant embryos out of a somatic cell of the nucellus or the integument and occurs most frequently in tropical trees and shrubs. Adventitious embryony is usually facultative and runs in parallel to sexual development, produces sexual and asexual embryo within the same seed. Diplospory and apospory involve the formation of an unreduced female gametophyte (gametophytic apomixis). Diplospory starts via a restitutional meiosis and gametophytic development of the unreduced megaspore, while apospory starts from a somatic initial cell of the nucellus which forms an unreduced embryo sac. In both cases, parthenogenetic development of the unreduced egg cell preserves the maternal genotype in the offspring (Naumova, 1993). Diplospory is more obligatory and is often completely pollen-independent (autonomous), whereas apospory is often more facultative, as the same plant can form both sexual and apomictic seeds in different ovules (Asker & Jerling, 1992).

Selfing and apomixis have often been seen as alternative evolutionary strategies of flowering plants that is advantageous for colonization scenarios and in bottleneck situations (Hörandl, 2006). This colonization ability is most efficient after long distance-dispersal of seeds (Baker’s law; Baker, 1967). Plants with selfing and apomictic reproduction are capable of uniparental reproduction (Rambuda & Johnson, 2004). Unlike vegetative propagation, which restricts their dispersal and is limited by natural enemies (DeWalt et al., 2004), selfing and apomixis enable plants to release from their enemies, which make them pre-adapted to invasions (Hao et al., 2011). These advantages may explain the wide geographical distributions of plants reproducing via selfing and apomixis

(Hörandl, 2006). The main feature of selfing and apomixis is the frequent presence of polyploidy (Hörandl et al., 2008).

Polyploidy is the presence of three or more complete sets of chromosomes (Ramsey & Schemske, 1998). In flowering plants, polyploidy represents a major mechanism of adaptation and speciation (e.g., Stebbins, 1957). It is estimated that between 47% and 70% of angiosperm species are polyploid (e.g., Grant, 1981). Polyploidy also can cause a breakdown of outcrossing sex especially in gametophytic self-incompatibility systems (Hörandl et al., 2008). Polyploids often have large distribution areas, and the geographical success of apomictic plants have often been referred to side-effects of polyploidy (Bierzychudek, 1985). Strikingly, many invasive plants are polyploids (Te Beest et al. 2012).

Invasive species have influenced the conservation and management on tropical ecosystems. Displacement of native biota, environmental disturbance, change of ecosystems, and hybridization with native species are the major threats to the maintenance of tropical ecosystems (Peh, 2010). In contrast, the information on mode of reproduction of invasive flowering plants correlated to apomixis in tropical areas is in general insufficient. Because of the methodological difficulties to prove and quantify apomixis in tropical plants and the focus on model plants has so far hampered to develop a solid causal model for the correlation of apomixis and invasiveness.

Tropical rainforest transformation systems provide interesting study sites for invasive plants. The Jambi province in eastern central Sumatra, Indonesia, is one of the largest regions of tropical lowland rainforest in Southeast Asia. The lowland rainforest was cut massively in the 1970s and 1980s by concession logging, leaving only few sites of natural forest, which are predominantly located in national parks e.g. in National Park Bukit Duabelas and Harapan Rainforest (Laumonier et al., 2010). In particular, the massive transformation of lowland rainforest into oil palm plantations has been identified as a major threat for biodiversity and a potential driver of climate change (Wilcove & Koh, 2010).

Centotheca lappacea is one of the abundant plants growing in understory of plantations in Bukit Duabelas and Harapan area according to field observation by Nicole Opfermann (unpubl.). *Centotheca lappacea* from subfamily Panicoideae (family Poaceae) (Morrone et al., 2012) is a perennial grass with 30-100 cm long erect culms. It is used as a forage grass and widely native from west tropical Africa, temperate Asia, tropical Asia, Australia, and Pacific Island (USDA, 2014). In Indonesia it mainly grows invasively in clearings,

forest edges and paths, road sides, waste places, cocoa, oil palm and rubber plantations (Biotrop, 2014). In 2006, Ye shu reported the chromosome number 2n = 24, although Levy & Feldman (2002) suggested that it might be more frequently polyploid. However, the information about ploidy level and reproduction mode of this species still limited. The species was not recorded in the Apomixis database www.apomixis.uni-goettingen.de (Hojsgaard et al., 2014). Many widespread Panicoid grasses reproduce via apomixis (Hojsgaard et al., 2013) which enhances colonization of new habitats and invasiveness (Te Beest et al., 2012).

The aims of this research were to assess the reproduction mode and the chromosomal ploidy level of *Centotheca lappacea*. This information will be useful to investigate the key factors for introduced species to reach rapidly dominance in disturbed habitats. Our hypothesis was that *Centotheca lappacea* in the rainforest transformation systems of Sumatra is polyploid and mainly reproduces asexually via seed (apomixis). The mode of reproduction was determined by the microscopic study of megasporogenesis, embryo sac development, and pollen development following methods by Young et al. (1979). Flow Cytometric Seed Screen (FCSS) described by Matzk et al. (2000) and Hojsgaard et al. (2013) was followed to reconstruct the reproductive pathways of mature seeds.

Chapter 2: Materials and Methods

2.1 Plant material

Plant materials of *Centotheca lappacea* (180 dried leaves in silica gel, 180 bags of seeds, and 64 young inflorescences fixed in FAA) were collected by Nicole Opfermann from May to September 2013 from 180 individuals inside the plots in three transformation systems, i.e. oil palm plantation, rubber plantations, and jungle rubber of Bukit Duabelas National Park and Harapan Rainforest, in Jambi, Indonesia**.** The sampling scheme followed the plot design of the SFB Project 990 “Ecological and socioeconomic functions of tropical lowland rainforest transformation systems (Sumatra, Indonesia)”, (Appendix 3). Four plots with 50 x 50 m size were selected in each transformation system. To avoid duplicated sampling because of vegetative reproduction, each individual plant was taken from patch colonies with minimum distance 5 m. Herbarium specimens of *Centotheca lappacea* were collected in the field and deposited at BIOTROP Herbarium, Bogor, Indonesia (BIOT) and Göttingen Herbarium, Germany (GOET).

2.2 Chromosome counting

Ten seeds from different transformation systems were planted in soil inside climatic chambers. Three root tips in active growth from each of eight cultivated plants were pre-treated with a saturated aqueous solution of α-bromonaphthalene for 3 hours at room temperature. Selected root tips were fixed for 12–24 hours in three absolute ethanol : one glacial acetic acid and then conserved in 70% ethanol at 4°C. Most of the pre-treated materials were directly hydrolyzed with 1 N HCl at 60°C for 10 min and stained with basic *fuchsin*. Feulgen staining following methods by Hojsgaard et al. (2009) was performed for chromosome counting. Meristem cells were macerated in a drop of 2% aceto-orcein and then squashed. Cells in mitotic stages were observed under Leica DM 5500B Microscope in 1000x magnification (Leica Microsystems, Wetzlar, Germany), the total chromosomes in a cell were counted to define the number of chromosomes and the ploidy level. The photo was taken by a DFC450C camera (Leica Microsystems, Wetzlar, Germany).

2.3 Ploidy assessment

Flow cytometry was performed on 164 plants to analyse ploidy level of the whole individual sample. The ploidy level of each individual was determined using 0.5 cm^2 of dry leaves from field collections. As a reference, a fresh leaf tissue, which was previously defined by its root’s chromosomes, was used.

A single leaf was chopped with a razor blade in a petri dish containing 200 µL Nuclei extraction buffer (Otto 1). The resulting suspension was filtered through 30-µm mesh Cell Tric disposable filter (Partec GmbH, Münster, Germany), and stained with 800 µL stained buffer (Otto 2). On the next step of analysis we used together two leaves from different individuals to make the analysis faster.

Samples were analyzed using a Partec PA II Flow Cytometer (Partec GmbH, Münster, Germany) with the UV-detector operating at 355 nm. Ploidy levels of the leaf tissues were estimated by comparing the different peak configurations to the standard peak. Approximately 3000 nuclei were measured per sample. Data analysis was performed using PA II's Partec FloMax software. The mean values of DNA content (C-values) of the leaves were established to infer the ploidy level. The coefficient of variation for each sampled peak was 5% or less.

2.4 Cyto-embryological analysis

A total of 338 spikelets from 16 different individuals was analysed for the mode of reproduction by the microscopic study of megasporogenesis and embryo sac development following methods by Young et al. (1979). Inflorescences that were previously fixed in FAA and stored in ethanol 70% were dehydrated in 100% ethanol for 30 minutes. Afterward, they were incubate in 300 µl of upgrading series of methyl salicylate (Merck) diluted in ethanol (25%, 50%, 70%, 85%, and 100%) for 30 minutes in each steps. Spikelets were dissected to prepare the ovules and anthers and to remove other spikelet organs, then ovules and anthers were amounted in methyl salicylate on glass slide. The stages of ovule and anther development were analysed by using a Leica DM5500B microscope with Nomarski DIC optics in 400x magnification (Leica Microsystems, Wetzlar, Germany). Photos were taken by a DFC450C camera (Leica Microsystems, Wetzlar, Germany).

2.5 Flow cytometry seed analysis

Flow Cytometric Seed Screen (FCSS) protocols described by Matzk et al. (2000) and Hojsgaard et al. (2013) were followed to reconstruct the reproductive pathways of 310 mature seeds. A single seed was chopped with a razor blade in a petri dish containing 200 µL Nuclei extraction buffer (kit Cystain UV precise P, Partec). The resulting suspension was filtered through 30-µm mesh Cell Tric disposable filter (Partec GmbH, Münster, Germany), and stained with 800 µL stained buffer (kit Cystain UV precise P, Partec). All samples were incubated for 30 sec to 60 sec on ice before measurement. As the results did not give variation in the reproduction pathways to seed formation, on the

next step of analysis we pooled five seeds from the same individual to make the analysis faster.

The fluorescence intensity of 4',6-diamidino-2-phenylindole (DAPI)-stained nuclei was determined using a Partec PA II Flow Cytometer (Partec GmbH, Münster, Germany) with the UV-detector operating at 355 nm. Ploidy levels of the endosperm and embryo tissues were estimated by comparing the different peak configurations. Approximately 3000 nuclei were measured per sample. Data analysis was performed using PA II's Partec FloMax software. The mean values of DNA content (C-values) for embryo and endosperm of a single seed were calculated to infer the sexual or apomictic origin of each seed. The coefficient of variation for each sampled peak was 5% or less.

The rationale for using FCSS is based on the different embryo to endosperm relative DNA content among seeds as a consequence of fertilization of unreduced (apomictic) versus reduced (sexual) embryo sacs (Matzk et al., 2000). In the sexual seed, the double fertilization leads to the formation of a 2n (2C) embryo and a 3n (3C) endosperm. On the other hand, in the asexual pathway, fertilization occurs only in the central cell of the unreduced embryo sac, produces a 2n (2C) parthenogenetic embryo and 5n (5C) pseudogamous endosperm (1n from sperm plus 4n from central cell).

2.6 Data analysis

For flow cytometry data the mean, minimum and maximum of the histogram peak values, and Pearson product-moment correlation for the mean values were calculated. A regression analysis for histogram data of dried leaves was applied to confirm that the mean value of G1 and G2 peak is linear. This analysis confirms the reliability of the measurements for the ploidy level assessments. All statistical analysis was carried out with Statistica software; StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.

Chapter 3: Results

3.1 Chromosome number

Photomicrograph of chromosomes of *Centotheca lappacea* are represented in Fig. 1. The chromosome number was diploid with 2n = 2x = 24 chromosomes in all 24 investigated root tissues. The chromosomes had a small size (< 2 µm) with metacentric or submetacentric form. The metacentric chromosome has a centromere at or near the middle, whereas the submetacentric has a centromere somewhat displaced from the middle point.

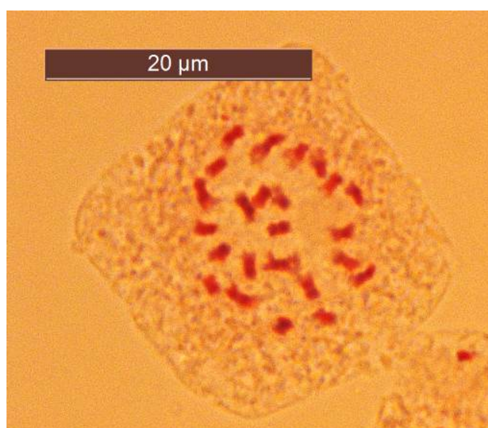

Fig. 1. Mitotic chromosomes of *Centotheca lappacea* HR4-01 in prophase stages.

3.2 Ploidy of the leaf samples

Ploidy level of *C. lappacea* was screened by flow cytometry of leaf material from field collection compared to fresh leaf material from which its chromosome number was known (Fig. 2a, b). The diploid fresh leaves of 21 samples had a mean value of the first peak fluorescence intensity (G1) 100.37 ± 8.93. The second peak (G2) had the double value of peak position and the number of nuclei was much smaller than the G1 peak, indicating that the chromosomes were in or after the S-phase of mitotic cells (mean value 199.02 ±20.77). If there would be a tetraploid individual in a pooled sample, we would expect also the G2 peak in this position, but the peak would be much larger than a G2 peak. Overall, the samples had a coefficient of variation (CV%) value less than 5 %. Pearson product-moment correlation for the mean values were $p>0.05$, indicating linear correlations between both peaks (Table 1).

Ploidy level of dried field-collected leaves of a total of 93 samples representing 123 individuals indicated the same DNA content compared to fresh leaves (e.g.Fig. 2b). The mean value of G1 was 92.01±7.24 and G2 was 186.55±15.97. Regression analyses of G1

and G2 mean value support linear correlation between the both values (Appendix 6). Based on the ratio of DNA content (2.03±0.07), all samples were diploid.

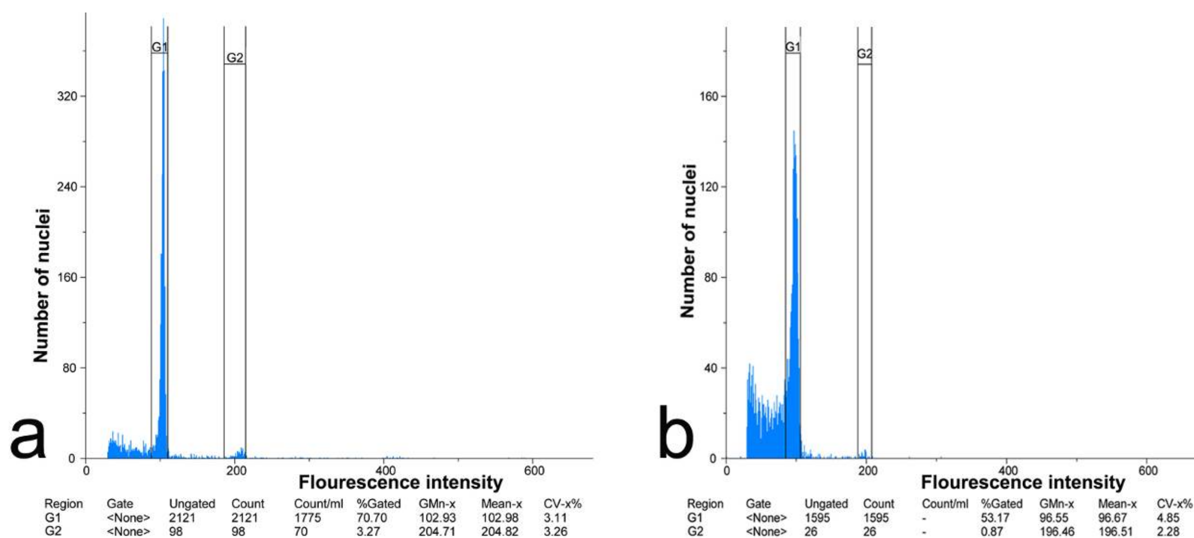

Fig. 2. Histogram of fluorescence intensity (a) in fresh leaf sample of HJ2-10; (b) dried leaf sample of HJ3-04

The higher variability of the peak value from dried leaves compared to fresh leaf samples was influenced by the quality of the samples during the storage process in silica gel. Diploid level was confirmed by the similar DNA counted of G1 peaks between dried and fresh leaf. In general, the CV value was <5%, and a linear correlation existed between mean peak values G1 and G2 by Pearson product-moment correlation for the mean values in 0.94.

Table 1. Flow cytometry statistical analysis of leaves

Sample	Variable	N	Mean	Min	Max	SD	r (p<0,05)
	G1	93	92.01	63.03	103.63	7.24	0.92
Dried leaves	G2	86	186.55	132.17	211.86	15.97	0.92
	Ratio G1:G2	86	2.03	1.84	2.31	0.07	
	CV% G1	93	4.73	3.24	8.40	0.71	
	CV% G2	86	2.94	0.61	5.48	1.02	
	G1	21	100.37	80.20	114.98	8.93	0.94
Fresh Leaves	G2	21	199.02	146.11	230.00	20.77	0.94
	Ratio G1:G2	21	1.98	1.77	2.07	0.08	
	CV% G1	21	3.64	2.55	7.22	1.29	
	CV% G2	21	2.38	1.39	4.57	0.83	

3.3 Ovule development

More than 300 ovules from 16 individuals from six transformation systems were evaluated to analyze the developmental stages of ovules. Ten steps of ovule development were recognized, i.e. Megaspore Mother Cell (MMC), Dyad, Tetrad, Functional Megaspore (FM), Post Meiotic Development (P.MD), One-Nucleate Embryo Sac (1N), Bi-nucleate Embryo Sac (2N), Four Nucleate Embyo Sac (4N), Eight Nucleate Embryo Sac (8N), and Mature Embryo Sac (Mature) were discriminated to show the whole developmental process (Appendix 4). Due to the limitation of inflorescences at the appropriate age, only individuals from two plots provided all developmental stages, whereas individuals from another six plots had only the younger stages, mainly in earlier megasporogenesis. Therefore the figures of ovule developmental stages mainly represented the individuals from two plots, Bukit Duabelas Oil Palm (BO) and Bukit Duabelas Jungle Rubber (BJ).

3.3.1 Megasporogenesis

a. Megaspore Mother Cell

Megasporogenesis started after an archesporial cell had differentiated from a single hypodermal cell of the nucellus in the ovule, the cell then continuously enlarged and became the megaspore mother cell (Fig. 3a, b). The megaspore was already surrounded by challose and had entered into the first meiotic prophase 1, which is indicated by a large nucleus and cell volume. During this stage the chromatin has already condensed around the nucleus. The ovule was approximately 150° rotated, whereas the inner integument had not yet reached the micropyle, while outer integument was very short developed. Both integuments consisted of two layer cells.

b. Dyad

The megaspore mother cell underwent meiosis. First meiotic division was observed on anaphase 1 where chromosomes in each bivalent separate and migrate toward the opposite poles as the result of the pulling action of spindle (Fig. 3c). In next stage cytokinesis occurred and formed a linear dyad (Fig. 3d). Furthermore both integuments continued growing as a ring around the nucellus with unequal length. Ovule continuously rotated through anatropous placement of ovule.

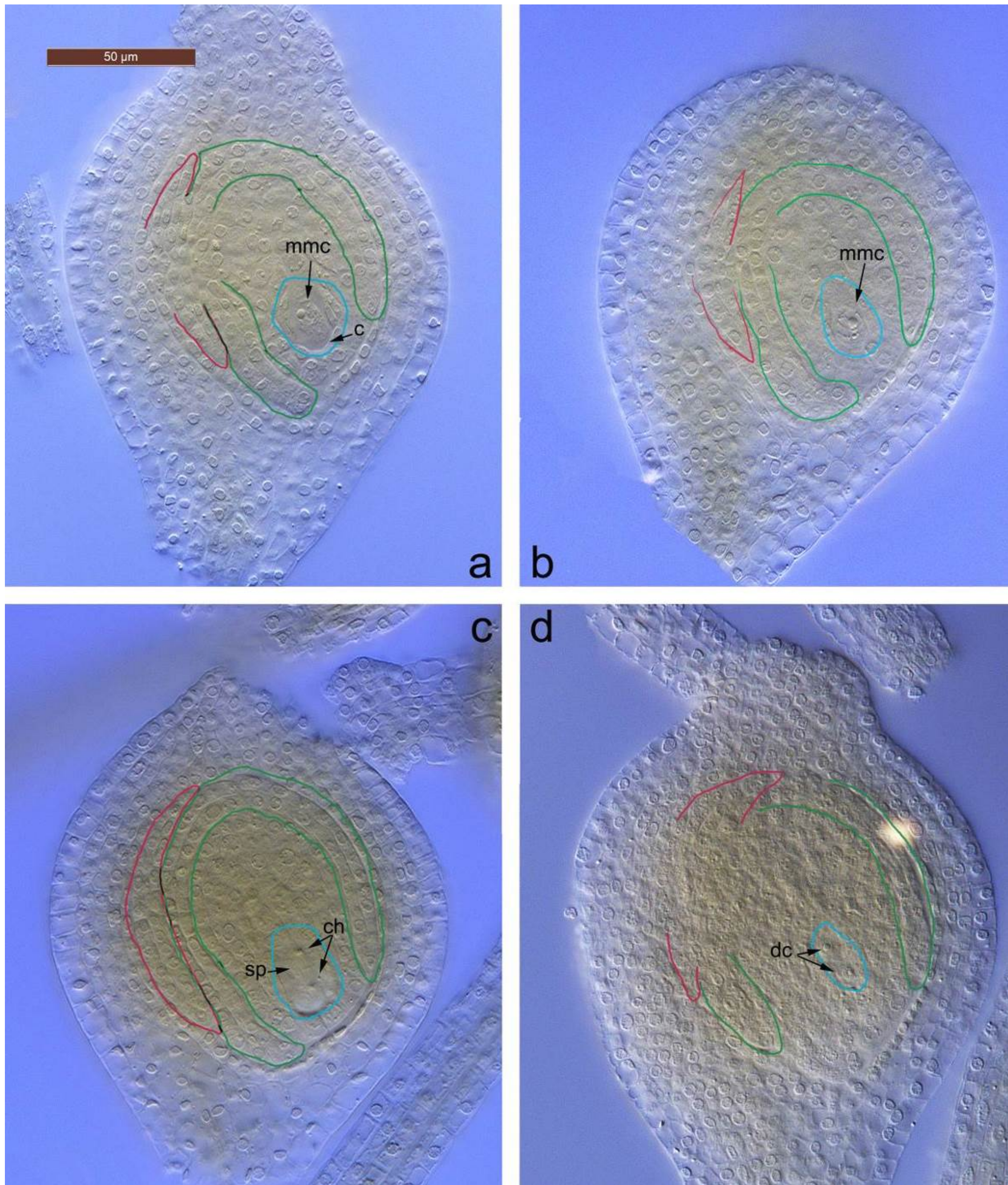

Fig. 3. Megaspore Mother cell and Dyad stage in ovules of *C. lappacea*. (a) MMC from BO4-16; (b) MMC from BJ3-17; (c) Dyad from HR2-2; (d) Dyad from BJ2-8.
Red line: outer integument; green line: inner integument; blue line: meiotic cell;
c: challose; ch: chromosome; dc: dyad cell; mmc: megaspore mother cell; sp: spindle bundle; Scale bar 50μm.

c. Tetrad

An asynchronous meiosis 2 produced a T-shaped tetrad (Fig. 4a) or a linear tetrad (Fig. 4b), with two ephemerous micropylar megaspores. The chalazal megaspore with the largest size was the functional one, but the epichalazal did not degenerate and remained

functional. The presence of callose associated with the cross wall of degenerated megaspore. The inner integument reached the tip of the nucellus. The outer integument was not detected due to the orientation of ovule to the microscope. The nucellus rotated further than in the previous stage.

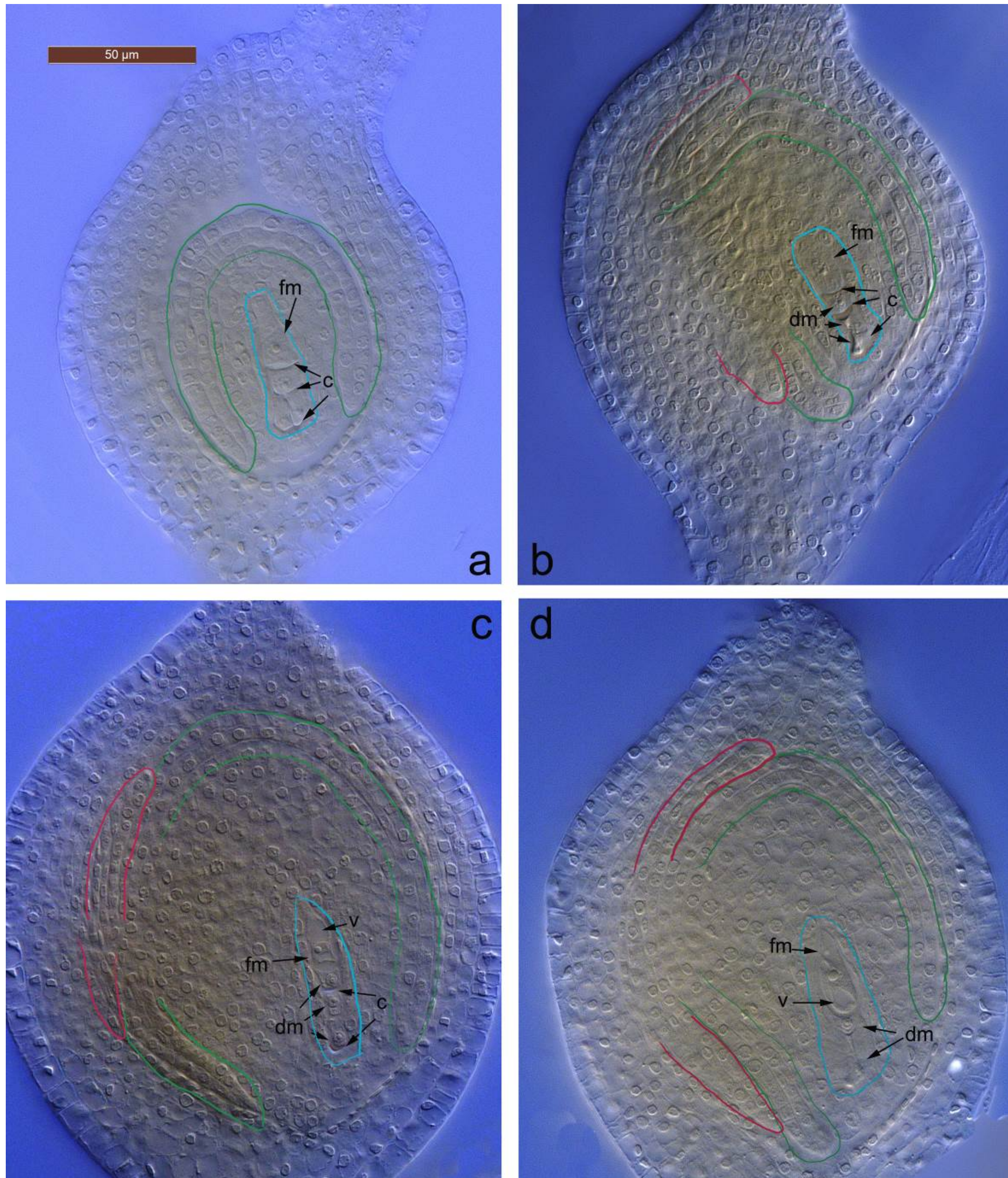

Fig. 4. Tetrad, functional megaspores, and early embryosac in ovule of *C. lappacea*. (a) Tetrad from BO1-17; (b) Functional megaspore from BJ2-16; (c) Early embryosac from BO4-14; (d) Embryosac from BJ2-22.
Red line: outer integument; green line: inner integument; blue line: meiotic cell;
c: challose; dm: degenerated megaspore; fm: functional megaspore; v: vacuole; Scale bar 50µm.

d. Functional Megaspore

Monosporic embryo sac development was observed in *C. lappacea*. Only one megaspore remained functional and subsequently three megaspores, adjacent to the micropyle aborted (Fig. 4c). The functional megaspore enlarged and developed into the mature megagametophyte. The three degenerated meiotic products were surrounded by accumulation of callose.

3.3.2 Megagametogenesis

a. Postmeiotic Development

Vacuoles appeared inside the functional megaspores. The vacuole was located in the micropylar area whereas the nucleus in the chalazal area. Vacuoles expanded, increased the cell size, and led to polarity (Fig. 4d). Initially, functional megaspore became the coenocytic embryosac and underwent mitosis without cytokinesis, resulting in a multinucleate coenocyte.

b. One-Nucleate Stage

Cell walls formed around the nuclei, resulting in a cellularized female gametophyte and a big nucleolus appeared (Fig. 5a). The female gametophyte had a teardrop-shape with a broader micropylar end. On the other side, three degenerating megaspores were still present. Throughout development, the female gametophyte exhibited a polarity along its chalazal-micropylar axis. The outer and inner integument surrounded the ovule, while the ovule still rotated.

c. Bi-Nucleate Stage

The two-nucleate female gametophyte formed after the first mitotic division (Fig. 5b). Several small vacuoles were present. During a later stage, the vacuoles began to coalesce in the center. Two-nucleate female gametophytes with a central vacuole were clearly observed in this stage. The two nuclei were located near the chalazal and micropylar poles. A large central vacuole was present between the nuclei and a second smaller vacuole was also present at the micropylar pole. Degenerate megaspores were still present.

d. Four-Nucleate Stage

Four-nucleate female gametophytes were formed after second mitotic division (Fig. 5c), with two nuclei at each pole. The two pairs of nuclei were separated by a large central vacuole. During the early stage, the one nucleus from each pole moved to the center of the embryo sac. During the late stage, the chalazal nuclei had continued third mitotic

division and had produced six nuclei (Fig. 5d). Inner integument and outer integument were more developed than in the previous stage, whereas the ovule continuously rotated.

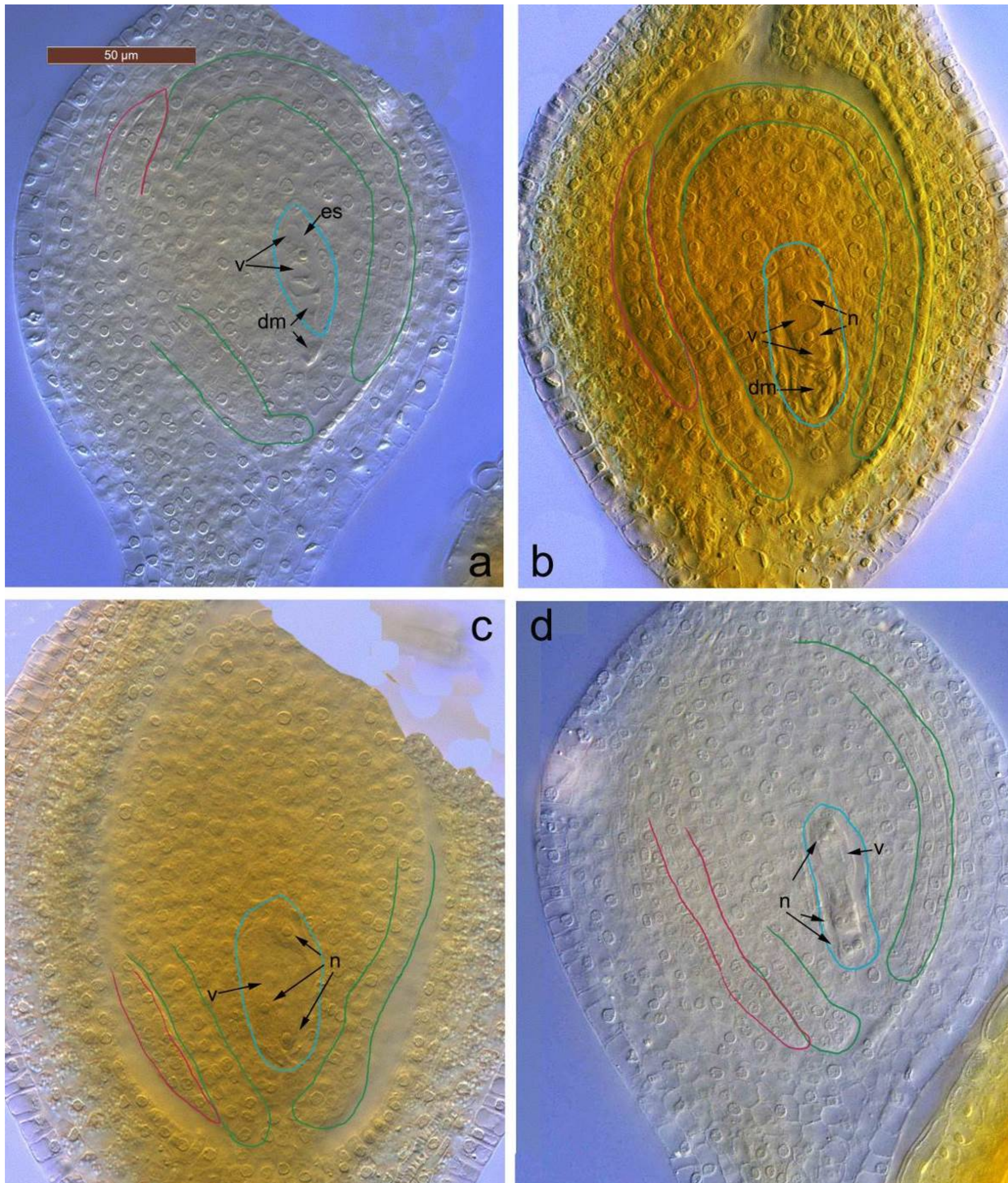

Fig. 5. Embryosac and its mitotic division in ovules of *C. lappacea*. (a) Embryosac with single nucleate from BJ2-19; (b) Bi-Nucleate from BO1-14; (c) Four-Nucleate from BO1-10; (d) Six-Nucleate from BJ2-21.
Red line: outer integument; green line: inner integument; blue line: meiotic cell;
c: challose; dm: degenerated megaspore; es: embryosac; n: nucleate; v: vacuole; Scale bar 50µm.

e. Eight-Nucleate Stage

The third mitotic division produced eight nuclei, which were separated by a big vacuole in the centre and formed a structure with four chalazal nuclei and four microphylar-end nuclei. One nucleus from each pole migrated to the centre to form polar nuclei (Fig. 6a). Both integuments already reached the tip of nucellus to form the micropyle. The ovules had rotated approximately 150 degrees.

f. Mature Embryo

The polar nuclei underwent cellularization to form a central cell without nuclear fusion. On the other hand, three cells remained in the chalazal embryo sac to form antipodals with tree nuclei whereas in the micropylar end, two nuclei positioned in the axial part to form the synergids. The egg cell also formed a cell membrane, without a cell wall in the middle of synergid. Antipodal cells remained active in mitosis to form multicelled antipodes (Fig. 6b).

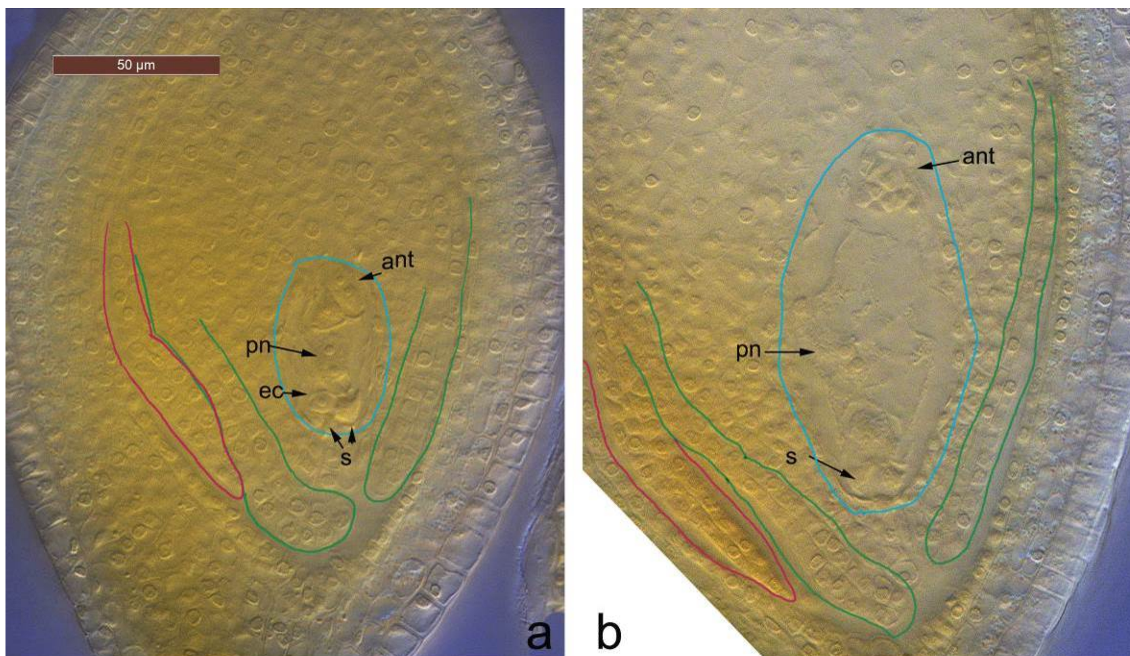

Fig. 6. Mature embryo sac in ovule of *C. lappacea*. (a) Mature embryo sac with 3 antipodals from BO1-14; (b) Mature embryo sac with multi antipodals from BO1-2.
Red line: outer integument; green line: inner integument; blue line: embryo sac;
ant: antipodal; ec: egg cell; pn: polar nuclei; s: synergid; Scale bar 50µm.

3.4 Pollen development

Observation of pollen development was examined in comparison to the stages of the ovule development. At the beginning, during the early development of archespore in the ovule (Fig. 7a), many sporogenous cells inside the anther proliferated by mitosis, and then each cell secreted an isolating callose sheath around itself. Each pollen mother cell (pmc) underwent microsporogenesis by meiotic division. At the early of first meiotic division, on leptotene stage, the nucleolus moved towards the nuclear periphery and the chromosomes were polarized and condensed (Fig. 7b). Subsequently, during diplotene, shortened and thickened homologous chromosomes separated and more than one chiasma was observed (Fig. 7c). The nucleolus disappeared and all chromosomes were arranged on the equatorial plate at metaphase I (Fig. 7d). The homologous chromosomes then equally separated and pulled to two poles during anaphase I (Fig. 7e). No cytokinesis during telophase 1 was observed.

In the next step, second meiotic and simultaneously-type cytokinesis produced haploid tetrad microspores with a tetrahedral arrangement (Fig. 7f). After meiosis, each haploid microspore or microsporocyte dissolved in callose, releasing microsporocyte into the fluid environment of the pollen sac (Fig. 8a). Microsporocytes enlarged, became vacuolated, and started forming the exine (Fig. 8b, c), and then first mitotic division occurred to produce a two-celled pollen. The vacuolate pollen then has a large vegetative cell and a small generative cell (Fig. 8d).

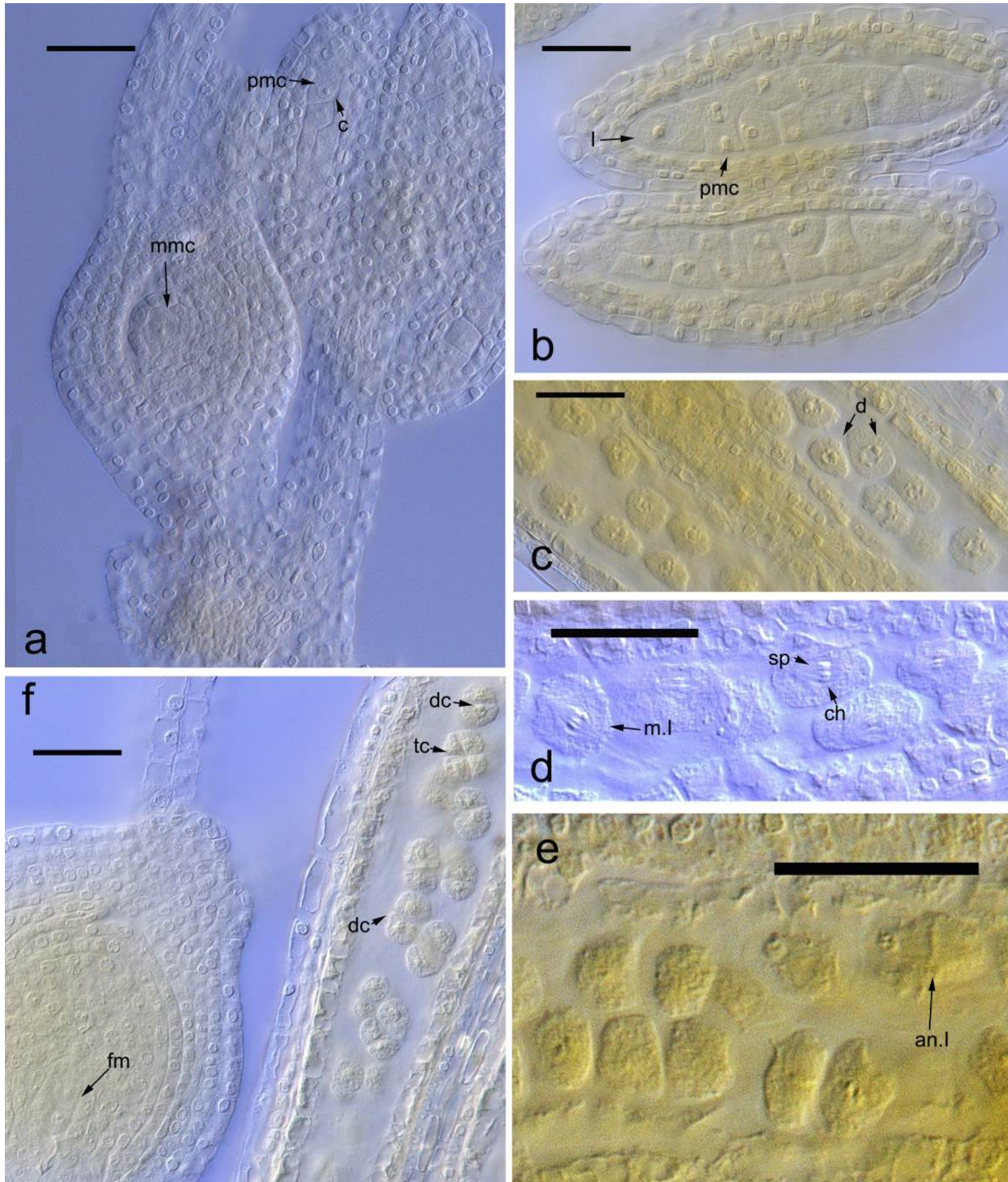

Fig. 7. Early pollen development of *C. lappacea.* (a) Archespore in early mitotic division from pollen of BR3-08; (b) PMC at early meiotic division from pollen of HR3-02; (c) PMC in diplotene from pollen of HR2-05; (d) PMC in metaphase I from pollen of HR2-01; (e) PMC in anaphase I from pollen HR2-02; (f) PMC in dyad and tetrad from pollen of HR2-06.

an1; anaphase; cr: chromatin; d: diplotene; dc: dyad cell; fm: functional megaspore; m.**I**: metaphase **I**; MMC: megaspore mother cell; pmc: pollen mother cell; sp: spindle; tc: tetrad cell; Scale bar 25µm.

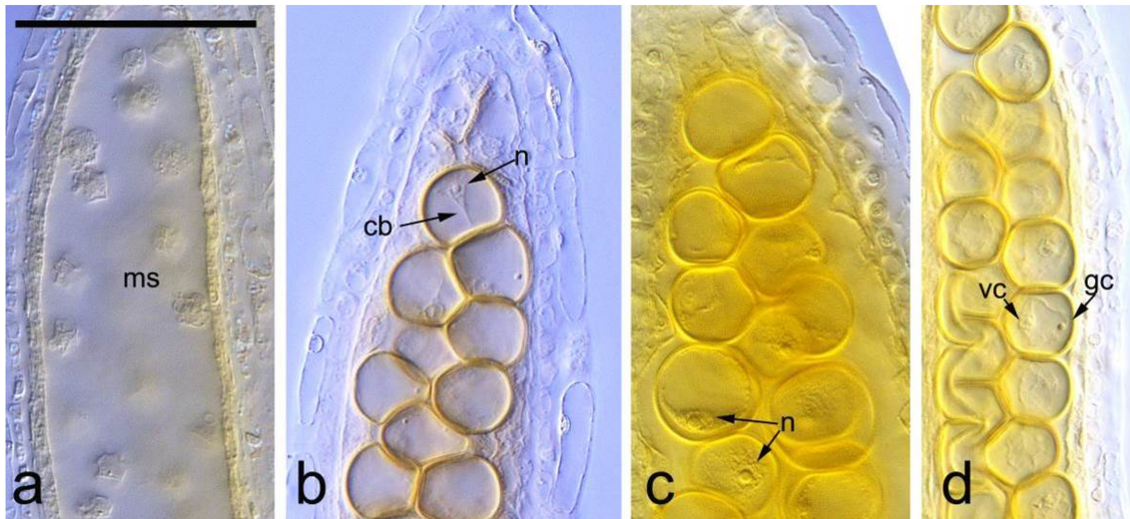

Fig. 8. Next stages of pollen development of *C. lappacea.* (a) Haploid microsporocytes in single cells from pollen of BO4-15; (b) Early pollen with a cytoplasmic bridge from pollen of BO4-04; (c) Pollen with a nuclei from pollen of BJ3-6; (d) Mature pollen with generative cells and vegetative cells from pollen of BJ2-08.
cb: cytoplasmic bridge; gc: generative cell; ms: microsporocytes; n: nuclei; vc: vegetative cell; Scale bar 25µm.

3.5 Seed Flow Cytometry

The reproduction mode of *C. lappacea* was reconstructed from a total of 107 samples representing 310 individual seeds (caryopsis) and indicated sexual reproduction mode with a ratio of nuclear DNA content between embryo and endosperm 1:1.5 or 2C:3C (Matzk et al., 2000). Embryo and endosperm mean peak values were calculated for all analyzed seeds based on an average total number of nuclei gathered for every peak. An example of flow cytometry histogram with peak value of embryo (Em) and endosperm (End) is provided in Fig. 9. The whole data set then was compiled and analyzed statistically to figure out the population character. The first peak histogram, representing embryo nuclear DNA content, had a mean value 98.93±8.27 and the second peak, representing endosperm nuclear DNA content, had a mean value 145.71±11.52. Therefore, the mean value ratio between embryo and endosperm was 1:1.47±0.03. The statistical analysis of CV values revealed <5%, and both mean values were linear correlated based on r value, 0.98 (Table 2).

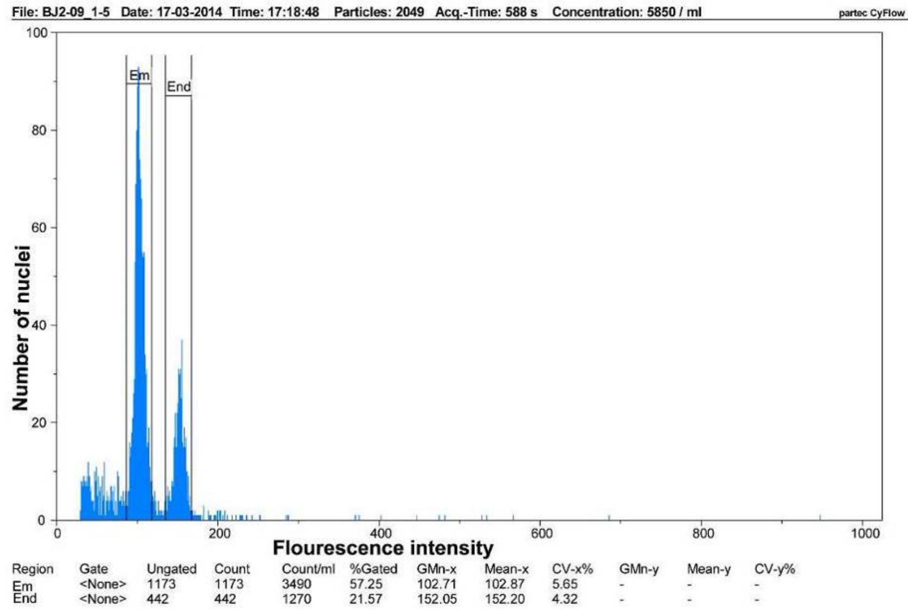

Fig. 9. Seed Flow Cytometry histogram of sample BJ2-09 with five seeds.
Em: embryo peak, End: the endosperm peak

Table 2. Statistical analysis of seed Flow cytometry.

Sample	Variable	N	Mean	Min	Max	SD	r (p<0,05)
Seeds	Embryo	107	98.93	56.63	116.28	8.27	0.98
	Endosperm	107	145.71	90.99	166.65	11.52	0.98
	Ratio Embryo:Endosperm	107	1.47	1.41	1.61	0.03	
	CV% Embryo	107	4.60	2.63	7.87	0.89	
	CV% Endosperm	107	3.35	1.98	5.70	0.71	

Chapter 4: Discussion

Flow cytometry analysis aided by chromosomes counting and cleared-pistil technique on cyto-embryological analysis confirmed that *Centotheca lappacea* from Sumatra, Indonesia is diploid and reproduces sexually. The chromosome number 2n = 2x = 24, also confirmed previous reports by Ye shu (2006). These results reject the hypothesis of polyploidy and apomictic reproduction mode of this species.

4.1 Chromosome number

Some authors had suggested that the basic chromosome number of Poaceae is x = 12 (e.g. Avdulov, 1931 in Gaut, 2002), as same as in all the primitive grass subfamilies, namely Anomochlooideae, Pharoideae, and Puelioideae (Grass Phylogeny Working Group (GPWG), http://www.ftg.fiu.edu/grass/gpwg, in Levy & Feldman, 2002). This would support that the ancestor of the grasses was itself a polyploid (Levy & Feldman 2002). According to Gaut (2002), in the grass family, Poaceae, polyploidy is more common and no single basic chromosome number occurs among grass subfamilies. Therefore, the feature of chromosome number correlated to the variation both within and among subfamilies of grasses. For instance, subfamily Panicoideae, Chloridoideae and Pooideae have four basic chromosome numbers in common (x = 7, 8, 9, 10), but different numbers exist in other groups. Current reports by Zuloaga et al. (2007) implied that the tribe of Centotheceae (subfamily Panicoideae) has a basic chromosome number of x = 12, and this supports our interpretation that *Centotheca lappacea* is diploid.

4.2 Ploidy level

Diploid plants usually reproduce sexually, only a few apomictic plants are diploid. According to Cosendai & Hörandl (2010), diploid plants maintain themselves via sexual reproduction and high seed set. Naumova et al. (1999) observed that less than 15% of diploid *Brachiaria decumbens* (a Panicoid grass) are facultative apomictic but more than 80% of the polyploids are apomictic. In angiosperms, the proportion of diploid plants are less than 30 % (Masterson, 1994 in Levy & Feldman, 2002).

The application of flow cytometry analysis in this research has various advantages. Suda & Trávníček (2006) had mentioned simplicity and convenient sample preparation even for huge number of samples, high accuracy in nuclear DNA amount, applicability for a variety of plant tissues, rapid detection of mixed samples, and low costs. During measuring DNA content values in dried leaves to determine ploidy, we got slightly lower values compared to fresh leaves, but the difference was less than 10%. The dried leaves gave higher CV values, lower peak values, wider range of values and more prominent background

fluorescence. The DNA content measurement of the fresh leaf is almost always slightly higher than the dried leaf material (Suda & Trávníček, 2006). The smaller DNA content values are due to silica gel-dried materials that were six months old. Dried leaf samples might be unreliable for absolute genome-size estimation, but are still reliable assessment of ploidy levels (e.g., Suda & Trávníček, 2006; Cosendai & Hörandl, 2010).

Contamination factors on the sample should be considered during sample preparation. For instance, the sample of BJ4-03 showed a higher value of G1 and G2 than the other samples (Appendix 8). After rechecking the sample we found epiphyllous liverworts on the surface of the leaf. Although the mass of the contaminant was lower than the leaf sample, it highly influenced the measurement result due to the better viability of the liverworts. Poikilohydry is an adaptive structure of bryophytes that makes them significantly tolerant to desiccation (Hearnshaw & Proctor, 1982), this group of plants is even able to survive under storage for more than 20 years (Makinde & Fajuke, 2009).

4.3 Mode of reproduction

Clearing techniques for cyto-embryological analysis are the common method to identify developmental stages of ovules (Young et al., 1979) which makes it also possible to detect aposporous embryo sacs (e.g. Delgado et al., 2014). The clearing technique only observes the apo-meiosis, but not the apomictic seed formation. The flow cytometry avoids this weakness of the clearing technique. It is also a fast and simple procedure (Matzk et al., 2000). Therefore I analysed the mode of reproduction of *C. lappacea* by both cyto-embryological analysis and flow cytometry analysis of seeds (caryopsis). The plants which were used in the studies represent the occurrence of *C. lappacea* in 22 population based on the sampling procedure of Collaborative Research Centre (CRC) 990: "Ecological and Socioeconomic Functions of Tropical Lowland Rainforest Transformation Systems (Sumatra, Indonesia) 2012 - 2015. Therefore, general conclusions can be made on the mode of reproduction in the Jambi area.

Megasporogenesis and megagametogenesis of *C. lappacea* followed the Polygonum type as described by e.g. Bahnwra et al. (1982). Megasporogenesis (female meiosis) occurred in megaspore mother cells (MMC), and the female gametophyte (embryo sac) normally develops from one of four meiotic products (megaspores). Most early mature embryo sacs of *C. lappacea* were eight-nucleate and contained an egg, two synergid cells, a central cell composed of two polar nuclei, and three antipodal cells (Polygonum-type embryo sac), but antipodals continuously mitotically divided to produce approximately nine cells. After pollination, the egg cell was fertilized to produce a zygote, which

developed further into an embryo. The second sperm nucleus fused with the central cell to produce endosperm.

After observation of all spikelets no asexual reproduction via adventitious embryony and gametophytic apomixis (diplospory or apospory) were observed (Appendix 1). In adventitious embryony (AE), numerous adventitious initial cells differentiate next to an embryo sac that is derived from sexual processes. After double fertilization of the sexual embryo sac, numerous developing adventitious embryos grow by accessing the nutritive endosperm. Therefore a seed contains multiple embryos. Polyembryony, however, can also arise from sexual pathways, e.g. from rare cases of functional twin megasporocytes (MMC) or by fertilization of a synergid that has assumed egg-like properties (Hojsgaard et al., 2014). Polyembryony is an evidence of AE and it is widespread among tropical plants (Naumova, 1993). Observation on germination of *Centotheca*'s seeds indicated no evidence of polyembryony. Each seed produced only a single seedling which developed from an embryo in the seed.

According to Hojsgaard et al. (2014), gametophytic apomixis develops from an unreduced embryo sac (apomeiosis) and embryo comes from an unreduced egg cell, within the embryo sac, without fertilization (parthenogenesis). The unreduced embryo sac can develop from a somatic cell of the nucellus, which replaces the megaspore (apospory). Another option is that the megaspore mother cell may continue a restitutional meiosis or mitotic-like division to produce an unreduced functional spore (diplospory). In gametophytic apomicts, endosperm formation may require fertilization of polar nuclei (pseudogamous apomixis) or it may develop independently (autonomous apomixis).

Pollen of *C. lappacea* developed in accordance with the ovules. Pollen mother cells follow a general process from meiosis to the tetrad stage. There were no multi-nucleate microspores during microgametogenesis. Multi-nucleate microspores are the result from an abnormal mitosis of microspores (Gallo et al., 2006). In *C. lappacea* meiosis was normal and successive cytokinesis led to the formation of microspore tetrads. All microspores or microsporocytes increased in size and the nucleus was pushed to a parietal position by vacuolation. The microspore divided to form a small generative cell and a larger vegetative cell. In the next developmental step, the generative cell migrated to the cytoplasm of the vegetative cell and divided to form two sperm cells. The outer structure of pollen, i.e. exine and intine, developed through mitotic division of microspores.

The FCSS proved sexual caryopsis of *C. lappacea*. Parthenogenesis of reduced egg cells was never observed. The embryo with 2C peak derived from fertilization of reduced

egg cells, indicating the complete sexual pathway. The endosperm 3C peak resulted from fusion of a male gamete and two nuclei in the central cell. There were no contaminants influencing the measurement as the caryopsis consists mainly of starch. DNA content of the endosperm cells was determined from the aleuron. The difference between embryo and endosperm DNA peaks was easily discriminated because the amount of endosperm nuclei is much lower than that of the embryo (Matzk et al., 2000).

Following Delgado et al. (2014), a histogram with 2:3 ratio is also possible derived from tetraploid plants where the fertilization of a reduced embryo sac by a reduce male gamete from a tetraploid pollen; embryo (2n♀+2n♂) : endosperm (2n2n♀+2n♂) = 4:6 or 2:3. Other possibilities of DNA content from diploid plants had been reported. Considering the case from *Paspalum rufum*, the fertilization possibly occurs between a meiotic or aposporous embryo sac with reduced pollen from a diploid or a tetraploid donor (Fig. 10). A meiotic embryo sac fertilized by a reduced pollen from a tetraploid could give rise to a triploid with a DNA embryo : endosperm ratio 3:4. On the other hand, a functional aposporous embryo sac may develop a diploid embryo by parthenogenesis (2n+0) and the endosperm by pseudogamy, generating maternal progeny. In this case, polar nuclei are fertilized with a reduced gamete from a diploid or a tetraploid, generating seeds with DNA embryo : endosperm ratios of 2:5 or 2:6, respectively. Moreover, functional aposporous embryo sacs can undergo double fertilization with reduced pollen from a diploid or a tetraploid plant, generating seeds with a DNA embryo : endosperm ratio of 3:5 or 4:6, respectively (Delgado et al., 2014); see Fig. 10.

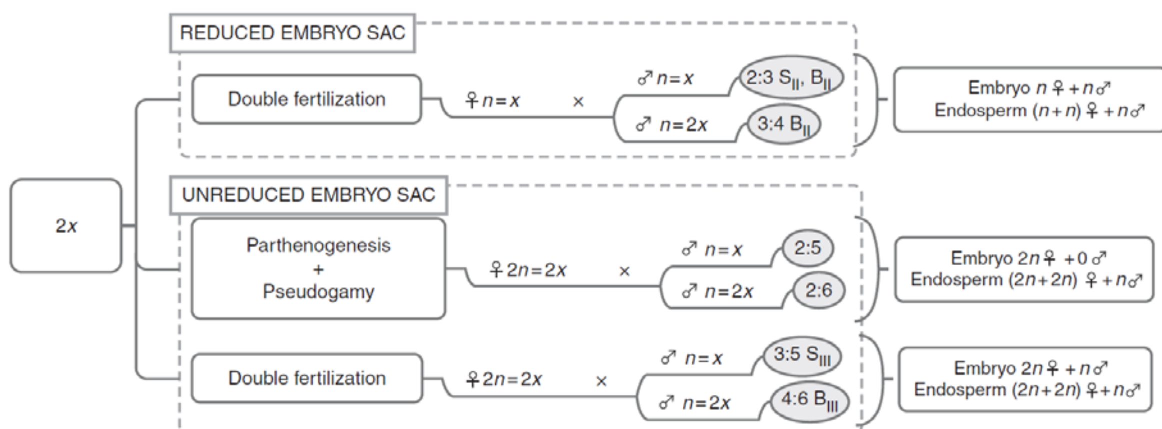

Fig. 10. Theoretical developmental pathways of seed formation in diploid (2n = 2x = 20) *Paspalum rufum* carrying functional meiotic and aposporous embryo sacs, fertilized with diploid and tetraploid pollen donors.

x: ploidy level; n: chromosome number; B_{II}: diploid hybrid derived from sexual reproduction; B_{III}: hybrid derived from the fertilization of an unreduced egg cell by a haploid gamete; S_{II}: diploid individual derived from selfing; S_{III}: triploid individual derived from the fertilization of an unreduced egg cell by selfing. Source: Delgado et al. (2014).

Our results reject the hypothesis of apomixis, but they do not inform about stigmatic or stylar self-compatibility. Baker's Law predicts that self-compatible species, and particularly those capable of autonomous seed production, should have a higher chance to become invasive than self-incompatible species. Therefore, the frequency of self-compatible species and of species capable of autonomous seed set should be higher among invasive species than among the potential source flora (Rambuda & Johnson, 2004). The advantages of self-compatible genotypes to produce seeds in isolation, suggest that the transition to self-compatibility may be favored by natural selection in all populations inhabited (Busch, 2005). According to Ye shu (2006) *Centotheca lappacea* is annual or might be perennial. Invasiveness is frequently associated with an annual life-cycle (e.g. Barrett, 1998). Because annual species are frequently prevalent among invasive species, particularly as ruderals in areas disturbed by humans (e.g., Mulligan & Findlay, 1970). Further studies on selfing and outcrossing preferences of this species are needed to test a hypothesis that mode of reproduction contributes to the abundance of this species in tropical regions.

4.4 Alternative hypotheses

Since mode of reproduction and ploidy level do not explain the abundance of the species, alternative explanations are discussed. *Centotheca lappacea* in the study area was common in palm oil and rubber plantations, in jungle rubber and in natural forests these species becomes rare (Nicole Opfermann, pers. obs.). According to Soerjani et al. (1975), it is frequent to dominant as long as the understory is not completely shaded. Wind-pollination and wind-dispersal in grasses infer low impact on pollinator spectra, but a high impact on ecosystem function is expected by rapid cover of ground, thus suppressing seedling recruitment of native species, competition for light and nutrients, and effects on soil ecology (e.g., Tjitrosoedirdjo & Tjitrosoedirdjo, 1994). Chemical control often affects just above-ground parts of the plant, while recruitment happens easily from rhizomes or from dormant seeds (Tjitrosoedirdjo & Tjitrosoedirdjo, 1994). Seed dispersal of this species is not well studied yet, but epizoochory and seed dispersal by ants and other herbivores such as wild pig and monkey is likely (Nicole Opfermann, pers. obs.).

Dispersal in flowering plants could be defined as the removal of the seed from the parent plant and its transport to a new site. The size of a population of a single species depends, however, not only on the number of seeds available and the efficiency of dispersal but also upon the availability of suitable 'safe sites' for germination and establishment of the seed (Harper et al., 1961). Schulze et al. (2005) discriminate three types of seed

dispersals, i.e. autochory (by itself), allochory (plant exploit different vectors), and atelochory (dispersal is inhibited). In zoochory (animal vector), epizoochory and endozoochory are more common. Epizoochory differs from other mechanisms of animal mediated dispersal, such as endozoochory (dispersal by ingestion and later defecation), in that the resulting seed deposition is not clumped in space and time, but evenly distributed (Sorensen, 1986).

Following Sorensen (1986), dispersal of *Centotheca*'s spikelets might be enhanced by adhesion and epizoochory. Hooked or barbed outgrowths are the most common means of adhesion. The stiff bristles on the spikelets (Appendix 9), which are reflexed at maturity, aid dispersal by catching in the fur of catching animal (Ye shu, 2006). Adhesive dispersal differs from other types of animal dispersal because adhesive fruits do not possess nutritional or energetic attractants. This means that animals usually pick up adhesive fruits by chance and may be unaware that they are carrying the fruits. As a result, adhesive fruits have the potential to travel long distances because they remain attached to the dispersal agent until they are groomed off or the animal molts or dies (Sorensen, 1986).

Centotheca lappacea is a fast growing grass. Germination took place within a week inside the climate chamber, and plants developed flowers after five months. This beneficial character might support their dominance over their competitors in their habitats. According to Peart (1979), it may be enough for the plant to produce and disperse the seeds, but it could be a great advantage if the seeds are dispersed to microsites suitable for germination and establishment. Moreover, reseach in tropical areas by Peters (2006) found out that the population of invasive plants is confined almost exclusively to high light environments. The location of plants in gaps of vegetation was significantly correlated with past disturbance by wild pigs, suggesting that soil disturbance and light availability are essential for their establishment.

The information about native habitat and the origin of *C. lappacea* is still unclear, many publications report the invasive and distribution status of this species (e.g. USDA, 1996; Biotrop, 2014; Ye shu, 2006; Ramana et al., 2013). Accurate data for the year of introduction are not available for most of those reports. Further study on wider scope to observe global invasion plants might be applied to get more comprehensive views. Theotharides & Dukes (2007) suggested that transport, colonization, establishment, and landscape spread are key factors influencing invasiveness.

Chapter 5: Conclusion

Based on the results of our study, *Centotheca lappacea* from Sumatra, Indonesia was diploid and reproduced sexually. There was no evidence of polyploidy in its chromosome number and DNA content nor apomictic reproduction in the ovule samples. Flow Cytometric analysis on the seeds also show that they originated from sexual fertilization. Observation on the pollen development demonstrated fertility and developmental stages. The abundance of this species might be due to an epizoochorous dispersal strategy by spikelet structures which are adapted to efficient seed dispersal.

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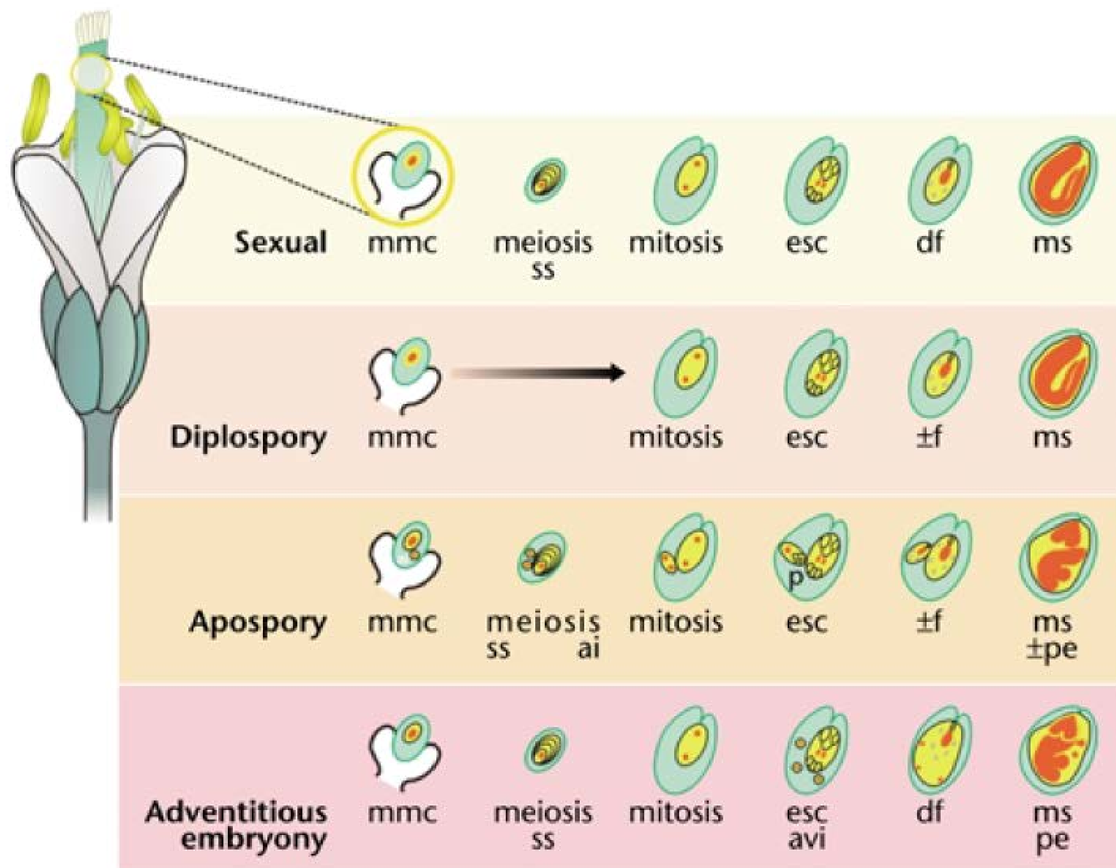
Appendices

Appendix 1. Apomictic reproduction compared to sexual reproduction in flowering plants.

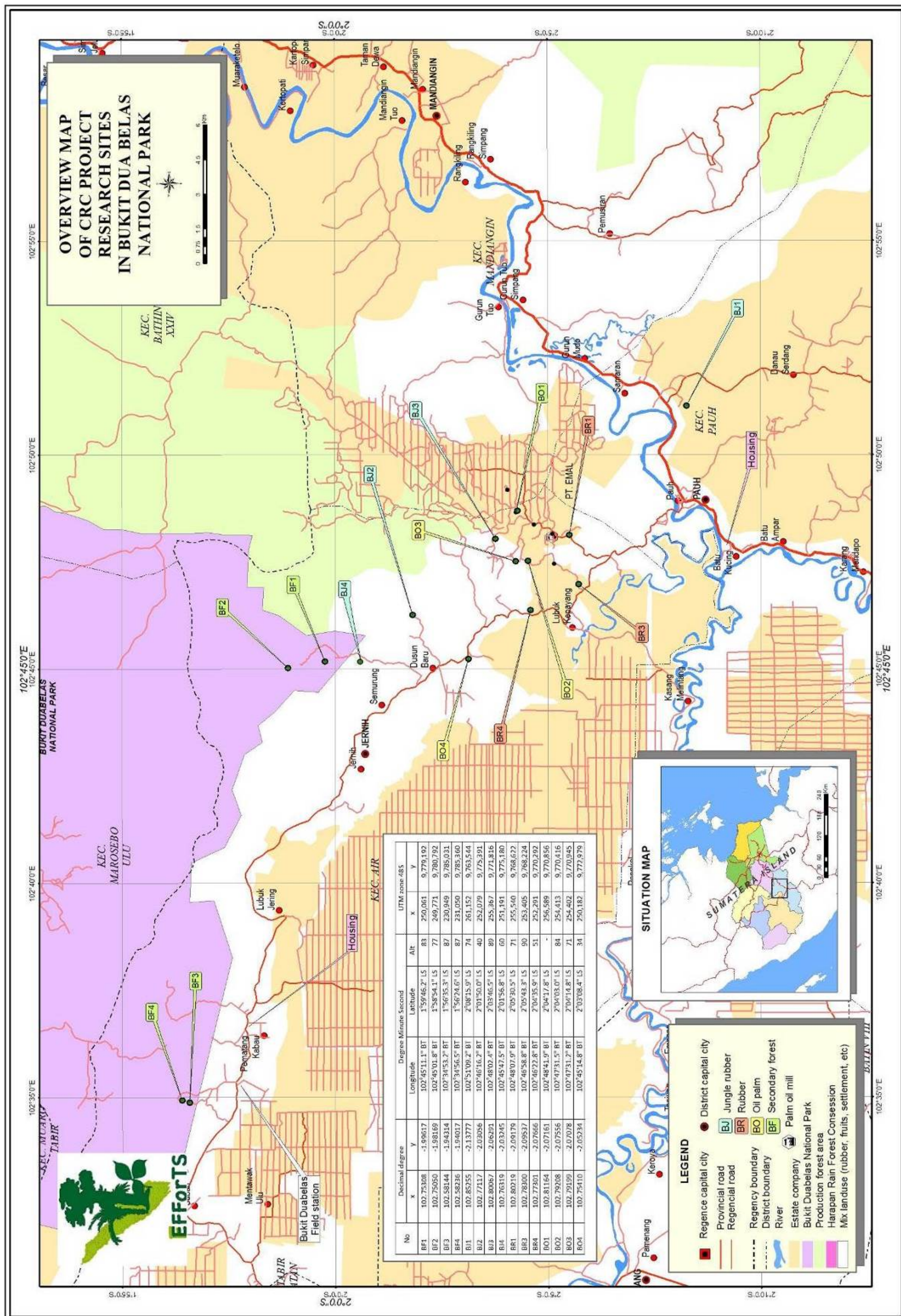


ai: aposporous initial; avi: adventitious initial cell; df: double fertilization; esc: eight cell embryo sac; mmc: megaspore mother cell; ms: mature seed; pe: poly embryony; ss: spore section. Source: Chaudhury et al., 2001.

Appendix 2. Photo of *Centotheca lappacea*'s herbarium specimen.

Appendix 3. Photo of *Centotheca lappacea* in the field by Nicole Opfermann.

Appendix 4. Plot positions.



No	Decimal degree		Degree Minute Second			UTM zone 48S	
	x	y	Longitude	Latitude	Alt	x	y
BF1	102.75308	-1.99617	102°45'11.1" BT	1°59'46.2" LS	83	250,061	9,779,192
BF2	102.75050	-1.98169	102°45'01.8" BT	1°58'54.1" LS	77	249,771	9,780,792
BF3	102.58144	-1.94314	102°34'53.2" BT	1°56'35.3" LS	87	230,949	9,785,031
BF4	102.58236	-1.94017	102°34'56.5" BT	1°56'24.6" LS	87	231,050	9,785,360
BJ1	102.85255	-2.13777	102°51'09.2" BT	2°08'15.9" LS	74	261,152	9,763,544
BJ2	102.77117	-2.03056	102°46'16.2" BT	2°01'50.0" LS	40	252,079	9,775,391
BJ3	102.80067	-2.06291	102°48'02.4" BT	2°03'46.5" LS	89	255,367	9,771,816
BJ4	102.76319	-2.03245	102°45'47.5" BT	2°01'56.8" LS	60	251,191	9,775,180
BR1	102.80219	-2.09179	102°48'07.9" BT	2°05'30.5" LS	71	255,540	9,768,622
BR3	102.78300	-2.09537	102°46'58.8" BT	2°05'43.3" LS	90	253,405	9,768,224
BR4	102.77301	-2.07666	102°46'22.8" BT	2°04'35.9" LS	51	252,291	9,770,292
BO1	102.81164	-2.07161	102°48'41.9" BT	2°04'17.8" LS	-	256,589	9,770,856
BO2	102.79208	-2.07556	102°47'31.5" BT	2°04'03.0" LS	84	254,413	9,770,416
BO3	102.79199	-2.07078	102°47'31.2" BT	2°04'14.8" LS	71	254,402	9,770,945
BO4	102.75410	-2.05234	102°45'14.8" BT	2°03'08.4" LS	34	250,182	9,772,979

OVERVIEW MAP OF CRC PROJECT RESEARCH SITES IN HARAPAN RAIN FOREST

SITUATION MAP

LEGEND

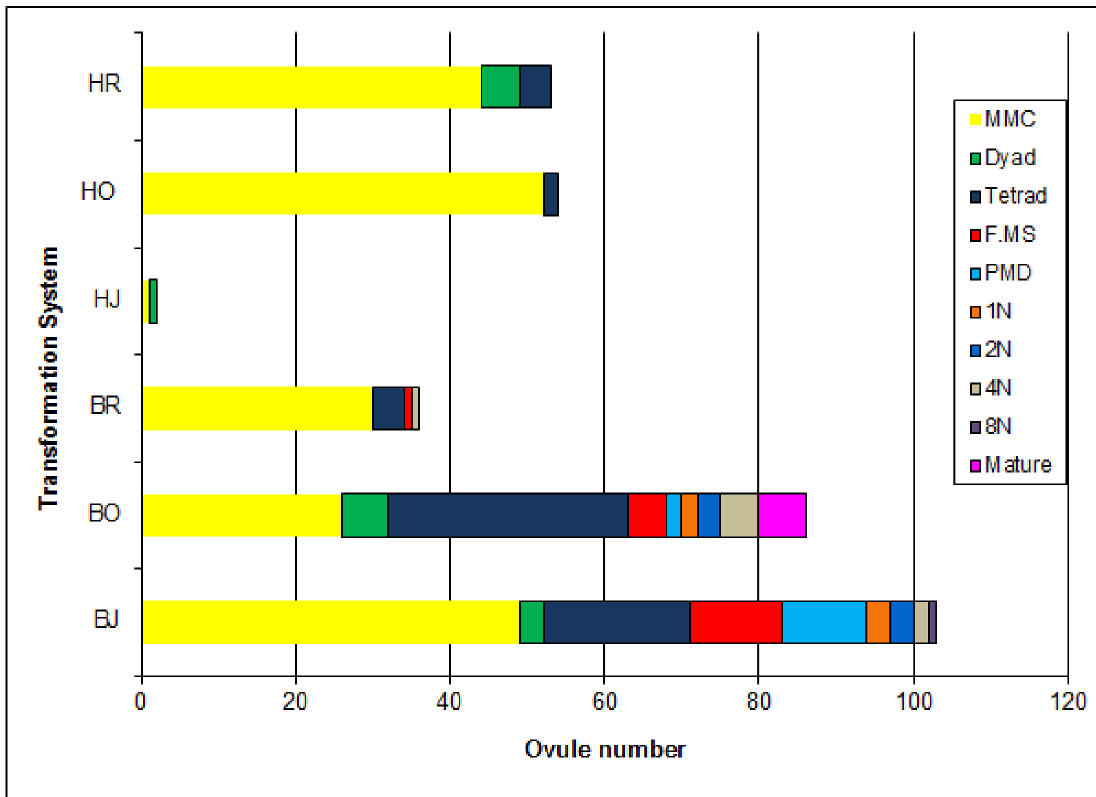
- Regence capital city
- District capital city
- Provincial road
- Regencial road
- Regency boundary
- District boundary
- River
- HJ Jungle rubber
- HR Rubber
- HO Oil palm
- HF Secondary forest
- Palm oil mill
- Estate company
- Bukit Duabelas National Park
- Production forest area
- Harapan Rain Forest Consession
- Mix landuse (rubber, fruits, settlement, etc)

No	Decimal degree		Degree Minute Second			UTM zone 48S	
	x	y	Longitude	Latitude	Alt	x	y
HF1	103.36161	-2.15264	103°21'41.8" BT	2°09'09.5" LS	65	317,791	9,761,970
HF2	103.33428	-2.16344	103°20'03.4" BT	2°09'48.4" LS	74	314,752	9,760,772
HF3	103.33336	-2.17861	103°20'00.1" BT	2°10'43.0" LS	65	314,652	9,759,094
HF4	103.34258	-2.18828	103°20'33.3" BT	2°11'17.8" LS	62	315,679	9,758,027
HJ1	103.25985	-1.92756	103°15'35.5" BT	1°55'39.2" LS	51	306,444	9,786,847
HJ2	103.27727	-1.87824	103°16'38.2" BT	1°52'41.7" LS	84	308,376	9,792,302
HJ3	103.29942	-1.84972	103°17'57.9" BT	1°50'59.0" LS	95	310,838	9,795,459
HI4	103.27690	-1.79873	103°16'36.8" BT	1°47'55.4" LS	51	308,327	9,801,096
HR1	103.26647	-1.91145	103°15'59.3" BT	1°54'41.2" LS	77	307,178	9,788,629
HR2	103.26547	-1.88014	103°15'55.7" BT	1°52'48.5" LS	59	307,064	9,792,092
HR3	103.30050	-1.85971	103°18'01.8" BT	1°51'34.9" LS	90	310,959	9,794,355
HR4	103.26491	-1.80560	103°15'53.7" BT	1°48'20.2" LS	71	306,993	9,800,334
HO1	103.26578	-1.90996	103°15'56.8" BT	1°54'35.9" LS	81	307,102	9,788,793
HO2	103.26715	-1.88269	103°16'01.7" BT	1°52'57.7" LS	55	307,251	9,791,809
HO3	103.30790	-1.85771	103°18'28.4" BT	1°51'27.8" LS	64	311,782	9,794,576
HO4	103.27060	-1.78700	103°16'14.2" BT	1°47'13.2" LS	48	307,624	9,802,392

EFForTS

Created by : Mohd. Zuhdi
08127419283 zuhdi67@yahoo.com

Appendix 5. Proportion of reproductive pathways of *Centotheca lappacea* at different stages collected from six plots.



BJ: Jungle rubber, BO: Oil Palm plantation, BR: Rubber plantation, in Bukit Duabelas National park; whereas HJ: Junggle rubber, HO: Oil palm plantation, and HR: Rubber plantation, in Harapan Forest.

Appendix 6. Flow cytometric data.

Flow cytometry seed analysis result.

No	Sample code	Gain	Embryo			Endosperm			Ratio
			Number of nuclei	DNA content	CV%	Number of nuclei	DNA content	CV%	
1	BJ2-09_1	450	208	108.29	3.03	117.00	163.71	3.03	1.51
2	BJ2-09_1-5	450	1173	102.87	4.65	442.00	152.20	4.32	1.48
3	BJ2-09_16-20	450	1017	97.96	4.96	306.00	145.43	3.54	1.48
4	BJ2-09_2	450	353	103.45	2.86	144.00	153.94	3.11	1.49
5	BJ2-09_21	449	208	108.19	3.02	117.00	163.65	2.33	1.51
6	BJ2-09_22	449	353	103.34	2.86	340.00	153.84	3.12	1.49
7	BJ2-09_23	449	311	103.62	2.64	118.00	156.41	2.23	1.51
8	BJ2-09_24-28	448	976	98.46	4.42	478.00	148.25	4.10	1.51
9	BJ2-09_29-33	448	1119	90.37	4.21	467.00	135.41	3.44	1.50
10	BJ2-09_3	450	311	103.76	2.63	117.00	156.30	2.21	1.51
11	BJ2-09_34-38	448	829	98.76	4.87	406.00	146.71	3.55	1.49
12	BJ2-09_39-43	448	1573	86.04	5.39	486.00	128.28	4.83	1.49
13	BJ2-09_44-48	448	1215	85.99	4.62	483.00	129.33	3.83	1.50
14	BJ2-09_6-10	450	1055	88.84	4.39	430.00	135.06	3.33	1.52
15	BJ3-04_1-5	450	697	97.59	4.96	200.00	145.20	3.02	1.49
16	BJ3-04_6-10	450	601	99.97	4.17	219.00	149.72	3.16	1.50
17	BJ3-05_1-5	450	658	105.97	5.81	215.00	157.00	3.54	1.48
18	BJ3-05_6-10	450	814	103.86	5.02	301.00	155.74	3.57	1.50
19	BJ4-01_1	450	215	102.47	2.90	95.00	152.28	2.47	1.49
20	BJ4-01_1-5	450	405	109.01	4.26	164.00	157.58	2.14	1.45
21	BJ4-01_6-10	450	538	108.01	4.79	216.00	155.86	3.16	1.44
22	BO1-05_1-5	450	1290	100.51	5.84	453.00	147.70	4.32	1.47
23	BO1-07_11-15	448	465	94.88	5.26	237.00	136.85	4.20	1.44
24	BO1-07_1-5	448	459	96.70	5.06	225.00	140.09	3.05	1.45
25	BO1-07_6-10	448	725	94.99	5.82	332.00	138.35	4.43	1.46

26	BO1-08_11-15	448	951	94.36	5.38	352.00	137.42	4.03	1.46
27	BO1-08_1-5	448	641	87.78	5.28	241.00	131.10	4.04	1.49
28	BO1-08_6-10	448	628	87.61	5.88	251.00	126.70	4.19	1.45
29	BO3-06_11-15	448	955	102.57	5.13	318.00	150.69	3.74	1.47
30	BO3-06_1-5	448	1049	106.85	3.88	417.00	154.96	4.11	1.45
31	BO3-06_6-10	448	803	105.66	4.68	246.00	153.04	3.33	1.45
32	BO3-08_1-5	488	512	89.82	4.48	275.00	130.02	4.11	1.45
33	BO3-08_6-10	488	1288	96.19	4.10	370.00	142.37	3.19	1.48
34	BR1-01_1-5	448	667	79.64	5.29	177.00	120.24	3.69	1.51
35	BR1-03_1-5	450	1599	102.49	5.14	512.00	151.79	3.75	1.48
36	BR1-12_11-15	448	596	96.53	4.97	184.00	140.85	2.44	1.46
37	BR1-12_1-5	448	331	96.41	4.48	169.00	144.21	3.54	1.50
38	BR1-12_6-10	448	660	94.16	5.24	175.00	139.78	3.36	1.48
39	BR2-01_1	450	295	99.36	3.75	159.00	146.61	3.07	1.48
40	BR2-01_16	448	112	75.98	4.31	97.00	115.22	3.55	1.52
41	BR2-01_17	448	164	84.14	5.51	168.00	125.54	5.34	1.49
42	BR2-01_18	448	138	93.67	4.74	66.00	137.64	2.65	1.47
43	BR2-01_2	450	302	101.02	3.36	102.00	151.47	2.26	1.50
44	BR2-01_3	450	96	100.20	3.16	56.00	149.46	2.41	1.49
45	BR2-01_4-8	448	805	92.05	5.11	459.00	133.49	3.87	1.45
46	BR2-01_9-13	448	579	56.63	4.67	250.00	90.99	3.54	1.61
47	BR2-02_1	450	67	100.10	4.41	61.00	148.16	2.30	1.48
48	BR3-02_1	450	239	103.14	5.22	39.00	145.77	3.00	1.41
49	BR3-02_13-15	449	799	106.56	7.28	204.00	153.74	5.70	1.44
50	BR3-02_2	450	133	102.07	3.59	33.00	146.17	3.03	1.43
51	BR3-02_3-7	449	549	104.84	4.42	206.00	150.97	4.55	1.44
52	BR3-02_8-12	449	886	105.45	4.53	218.00	150.75	3.57	1.43
53	BR4-06_1	450	186	105.12	5.17	39.00	155.83	3.39	1.48
54	BR4-06_2	450	461	105.00	4.93	114.00	157.98	4.09	1.50
55	BR4-06_3-7	448	1139	100.99	4.70	327.00	150.34	3.85	1.49

56	BR4-06_8-9	448	361	90.75	5.13	152.00	138.88	3.68	1.53
57	HJ2-06_11-15	448	514	91.19	4.04	310.00	136.36	3.04	1.50
58	HJ2-06_1-5	448	1264	96.84	5.69	522.00	141.61	4.14	1.46
59	HJ2-06_16-20	448	716	96.40	7.45	363.00	138.62	3.90	1.44
60	HJ2-06_6-10	448	1336	92.82	4.82	384.00	137.39	3.87	1.48
61	HJ2-10_1	454	197	99.33	4.99	65.00	145.72	2.92	1.47
62	HJ2-10_12	449	288	102.66	5.08	102.00	147.27	3.15	1.43
63	HJ2-10_12	449	474	110.83	5.08	64.00	160.89	4.13	1.45
64	HJ2-10_2	454	337	100.23	7.87	106.00	142.78	2.89	1.42
65	HJ2-10_3	454	227	100.09	5.32	98.00	147.36	3.53	1.47
66	HJ2-10_4-5	454	538	104.44	4.55	173.00	152.58	2.92	1.46
67	HJ2-10_6-10	454	864	116.28	3.93	312.00	166.12	3.23	1.43
68	HJ3-01_11-15	448	1361	99.75	4.26	430.00	146.33	2.18	1.47
69	HJ3-01_1-5	449	304	104.82	4.47	500.00	153.34	3.25	1.46
70	HJ3-01_16-20	448	744	91.19	5.96	360.00	134.40	4.32	1.47
71	HJ3-01_6-10	448	797	101.19	4.44	412.00	148.88	3.23	1.47
72	HJ3-09_1-3	448	394	101.51	4.08	213.00	147.19	2.74	1.45
73	HJ4-01_1-5	449	1297	106.08	4.57	418.00	152.55	2.58	1.44
74	HO1-02_1	450	631	109.45	4.41	97.00	162.63	3.97	1.49
75	HO1-02_2	450	682	106.88	3.88	73.00	160.33	3.40	1.50
76	HO1-02_3	449	344	105.10	4.52	83.00	156.72	3.71	1.49
77	HO1-10_1-5	454	1000	104.93	5.28	291.00	158.80	4.35	1.51
78	HO1-10_6-10	454	1352	109.02	3.95	337.00	166.65	4.10	1.53
79	HO2-05_1	449	409	103.82	4.67	99.00	152.19	2.66	1.47
80	HO2-05_14-18	448	1222	103.47	4.70	505.00	147.98	3.05	1.43
81	HO2-05_19-23	448	913	103.75	5.09	490.00	149.15	2.89	1.44
82	HO2-05_2	449	296	100.37	3.86	106.00	149.88	2.93	1.49
83	HO2-05_24-28	448	843	100.40	4.82	397.00	142.59	2.68	1.42
84	HO2-05_3	449	226	101.46	3.28	128.00	151.78	2.35	1.50
85	HO2-05_4-8	448	1120	96.03	4.12	394.00	139.99	2.60	1.46

86	HO2-05_9-13	448	787	102.07	4.03	453.00	148.31	3.37	1.45
87	HO3-06_1	449	417	103.09	3.63	131.00	152.33	2.73	1.48
88	HO3-06_2	449	796	101.61	4.08	179.00	149.33	2.41	1.47
89	HO4-04_1	449	165	99.08	4.10	95.00	146.88	3.10	1.48
90	HO4-04_11-15	448	339	95.85	4.77	241.00	140.73	4.16	1.47
91	HO4-04_16-20	448	676	91.11	5.01	360.00	132.64	3.00	1.46
92	HO4-04_2	449	104	99.78	3.29	76.00	147.81	1.98	1.48
93	HO4-04_21-25	448	265	88.74	3.80	240.00	130.99	3.17	1.48
94	HO4-04_3-4	449	519	101.04	4.36	247.00	146.12	2.54	1.45
95	HO4-04_5-10	448	871	99.13	5.54	427.00	143.88	3.74	1.45
96	HR1-01_11-16	448	446	80.57	5.01	143.00	119.15	2.47	1.48
97	HR1-01_1-5	448	870	89.62	4.43	257.00	133.44	2.51	1.49
98	HR1-01_6-10	448	508	97.95	4.14	225.00	145.06	3.53	1.48
99	HR1-07_1-5	449	1052	104.18	4.91	416.00	154.16	2.75	1.48
100	HR2-06_1-5	449	1026	94.22	4.31	430.00	137.96	2.55	1.46
101	HR3-09_1-5	449	730	103.00	4.31	404.00	148.23	3.57	1.44
102	HR4-07_11-15	449	947	103.44	4.19	357.00	151.80	2.90	1.47
103	HR4-07_1-5	449	875	106.01	4.61	416.00	152.98	3.41	1.44
104	HR4-07_6-10	449	890	105.15	4.64	717.00	152.52	4.21	1.45
105	HR4-08_1-5	449	915	104.39	5.06	371.00	152.16	3.08	1.46
106	HR4-08_3-4	454	428	85.46	4.67	179.00	129.42	3.66	1.51
107	HR4-08_5-6	454	362	108.79	3.93	173.00	159.64	3.09	1.47

Flow cytometry of fresh leaf.

No	Sample code	Gain	G1			G2			Ratio
			Number of nuclei	DNA content	CV%	Number of nuclei	DNA content	CV%	
1	BJ2-01	448	116	80.20	3.64	11	165.70	1.52	2.07
2	BJ2-02	448	97	80.52	6.14	10	159.26	2.10	1.98
3	BJ2-01_2	448	49	82.69	5.13	11	146.11	1.39	1.77
4	BJ2-03	448	10	97.55	3.01	5	172.19	2.22	1.77
5	BJ2-03_2	448	92	105.39	5.54	21	202.61	4.18	1.92
6	BJ3-4	448	78	106.32	7.22	21	203.39	2.89	1.91
7	BJ2-01	453.5	738	98.48	2.75	17	199.43	1.49	2.03
8	HJ2-10_24chr+BJ2	452.5	1044	99.60	2.66	46	199.91	3.07	2.01
9	HJ2-10_24chr+BJ3	460	550	109.95	2.66	19	222.83	2.01	2.03
10	HJ2-10_leaf_24chr	460	664	114.98	2.86	12	230.00	2.29	2.00
11	HJ2-10_leaf_24chr	452.5	1371	102.42	3.12	29	205.12	4.57	2.00
12	HJ2-10_1	453	2099	103.34	2.75	92	206.18	2.64	2.00
13	HJ2-10_2	453	1931	100.15	2.84	49	203.60	1.51	2.03
14	HJ2-10_3	453	2006	104.50	3.20	203	211.02	2.13	2.02
15	HJ2-10_4	453	1714	102.62	2.62	178	207.41	1.51	2.02
16	HJ2-10_5	453	1378	105.50	3.69	71	212.89	2.54	2.02
17	HJ2-10_6	453	1435	105.43	2.55	86	212.98	2.15	2.02
18	HJ2-10_7	453	1867	103.22	2.87	97	208.45	2.52	2.02
19	HJ2-10_8	453	1199	102.49	3.83	82	203.62	2.98	1.99
20	HJ2-10_10	453	1451	101.90	3.72	59	204.06	2.24	2.00
21	HJ2-10_11	453	433	100.57	3.56	15	202.71	1.98	2.02
22	Leaf_BJ2-01	448	116	80.20	3.64	11	165.70	1.52	2.07

Flow cytometry of dried leaves.

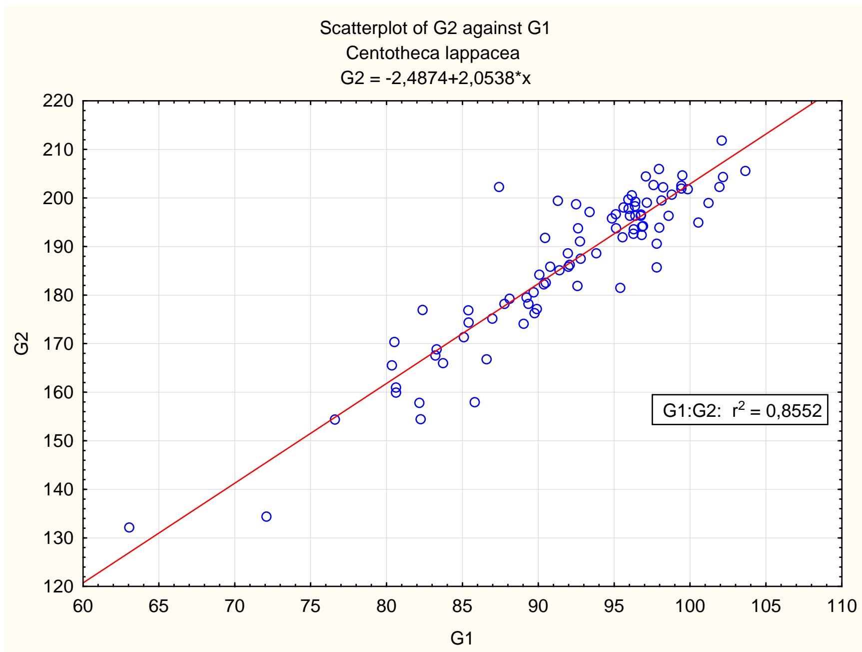
No	Sample code	Gain	G1			G2			Ratio
			Number of nuclei	DNA content	CV%	Number of nuclei	DNA content	CV%	
1	Leaf_BJ2-05	450	517	100.30	4.09				
2	Leaf_BJ2-06	450	717	94.81	5.36				
3	Leaf_BJ2-07	450	694	99.08	5.37				
4	Leaf_BJ2-08	450	987	89.34	4.79	7.00	178.21	1.20	1.99
5	Leaf_BJ3-09	449	900	97.97	4.06	22.00	193.93	2.81	1.98
6	Leaf_BJ3-10	449	1040	94.86	4.98	49.00	195.79	3.32	2.06
7	Leaf_BJ3-11	449	1113	98.13	5.33	29.00	199.50	3.44	2.03
8	Leaf_BJ3-12	448	1224	89.75	5.32	28.00	176.29	1.75	1.96
9	Leaf_BJ4-01	448	1040	96.29	4.07	16.00	193.55	3.51	2.01
10	Leaf_BJ4-03	450	976	154.04	4.07	78.00	307.13	1.40	1.99
11	Leaf_BO1-09	448	710	103.63	3.24	32.00	205.58	1.97	1.98
12	Leaf_BO1-10	448	1185	98.23	4.29	11.00	202.22	0.61	2.06
13	Leaf_BO1-11	448	906	99.42	4.37	73.00	202.54	2.92	2.04
14	Leaf_BO1-12	450	878	97.08	4.02	22.00	204.47	1.82	2.11
15	Leaf_BO2-01	450	897	100.53	4.48	23.00	194.95	3.56	1.94
16	Leaf_BO2-02	450	1022	102.16	4.45	55.00	204.35	3.96	2.00
17	Leaf_BO2-03	450	1196	95.10	5.42	34.00	196.69	3.05	2.07
18	Leaf_BO3-05	450	743	90.79	5.69	10.00	185.82	1.68	2.05
19	Leaf_BO3-06	450	722	93.62	5.36				
20	Leaf_BR2-08	450	951	93.83	5.25	54.00	188.60	3.21	2.01
21	Leaf_BR1-06	450	750	88.11	5.04	11.00	179.26	2.72	2.03
22	Leaf_BR1-07	450	1298	89.90	5.80	16.00	177.15	3.12	1.97
23	Leaf_BR1-08	448	1127	80.63	6.04	32.00	159.91	4.19	1.98
24	Leaf_BR1-09	448	1327	95.08	5.11				
25	Leaf_BR2-05	448	720	96.04	3.78	43.00	196.31	2.87	2.04
26	Leaf_BR2-06	448	894	91.98	4.96	59.00	185.84	3.75	2.02

27	Leaf_BR2-07	448	862	97.81	4.00	28.00	190.60	2.88	1.95
28	Leaf_BR3-01	448	775	99.49	4.54	49.00	204.64	2.06	2.06
29	Leaf_BR3-02	448	878	97.15	4.41	16.00	199.07	3.98	2.05
30	Leaf_BR4-03	448	830	95.95	5.16	58.00	197.77	2.89	2.06
31	Leaf_BR4-04	448	931	96.80	3.93	7.00	192.36	1.07	1.99
32	Leaf_BR4-05	488	845	96.40	4.67	7.00	196.34	1.01	2.04
33	Leaf_BR4-06	488	707	98.58	3.94	66.00	196.36	4.66	1.99
34	Leaf_HJ2-07	448	742	90.44	4.77	19.00	191.75	1.89	2.12
35	Leaf_HJ2-08	450	652	99.86	3.88	11.00	201.83	2.97	2.02
36	Leaf_HJ2-09	448	778	95.13	4.23	49.00	193.76	4.46	2.04
37	Leaf_HJ2-10	448	1397	95.61	5.76	14.00	198.05	2.16	2.07
38	Leaf_HJ3-03	448	1531	92.74	5.82	12.00	191.03	2.81	2.06
39	Leaf_HJ3-04	450	1596	96.77	4.86	26.00	196.62	2.30	2.03
40	Leaf_HJ3-05	448	1243	96.84	5.14	10.00	194.12	3.48	2.00
41	Leaf_HO2-07	448	693	102.09	5.03	45.00	211.86	2.95	2.08
42	Leaf_HO2-08	448	615	97.60	4.17	32.00	202.69	2.98	2.08
43	Leaf_HO2-10	450	690	96.16	5.00	29.00	200.55	2.67	2.09
44	Leaf_HO3-01	450	733	101.23	4.31	6.00	198.98	1.04	1.97
45	Leaf_HO3-02	448	553	96.76	4.63	31.00	196.35	2.18	2.03
46	Leaf_HO4-02	448	513	97.94	4.52	13.00	205.95	3.36	2.10
47	Leaf_HO4-03	450	805	90.37	4.91	19.00	182.24	4.14	2.02
48	Leaf_HO4-04	450	1063	87.76	5.34	31.00	178.20	3.59	2.03
49	Leaf_HO4-05	449	914	92.57	5.08	19.00	181.85	4.05	1.96
50	Leaf_HR1-06	450	599	101.94	4.05	37.00	202.29	2.97	1.98
51	Leaf_HR1-07	449	719	95.56	4.14	124.00	191.89	3.71	2.01
52	Leaf_HR1-08	449	408	91.28	4.20	31.00	199.42	2.74	2.18
53	Leaf_HR2-10	450	567	98.79	4.11	29.00	200.71	2.69	2.03
54	Leaf_HR2-01	450	534	85.41	3.67	48.00	174.39	2.75	2.04
55	Leaf_HR2-02	448	953	95.92	4.93	57.00	199.68	2.33	2.08
56	Leaf_HR2-03	448	749	97.79	3.62	62.00	185.73	4.58	1.90

57	Leaf_HO1-02	448	854	93.37	4.91	36.00	197.14	2.58	2.11
58	Leaf_HO1-03	448	646	96.36	4.88	40.00	198.29	3.07	2.06
59	Leaf_HO1-04	448	632	85.81	5.08	21.00	157.93	1.26	1.84
60	Leaf_HO1-05	448	659	91.40	4.65	14.00	185.13	1.31	2.03
61	Leaf_HR4-09	454	698	96.89	4.15	11.00	194.25	1.97	2.00
62	Leaf_HR4-10	449	923	96.27	3.43	11.00	192.64	1.91	2.00
63	Leaf_HR4-01	449	1450	75.79	8.40				
64	Leaf_BJ2-1,2	454	546	95.41	4.95	57.00	181.47	3.00	1.90
65	Leaf_BJ2-3,4	454	360	96.40	3.80	64.00	199.26	3.32	2.07
66	Leaf_BJ3-5,6	454	525	91.94	4.60	96.00	188.61	4.08	2.05
67	Leaf_BJ3-7,8	454	652	90.07	5.68	24.00	184.17	2.27	2.04
68	Leaf_BJ4-3_Few mosses	448	409	99.43	3.52	35.00	201.95	1.80	2.03
69	BJ4-5,6	449	531	92.50	4.74	17.00	198.75	2.66	2.15
70	BO1-3,4	448	843	80.63	5.20	60.00	160.98	4.41	2.00
71	BO1-7,8	448	874	89.23	4.99	25.00	179.52	3.28	2.01
72	BO3-9,10	448	963	87.13	5.04				
73	BR1-2,3	449	1130	86.96	4.43	53.00	175.17	4.50	2.01
74	BR1-4,5	450	979	82.24	4.74	46.00	154.45	4.93	1.88
75	BR2-1,3	450	845	63.03	4.74	23.00	132.17	4.05	2.10
76	BR4-8,9	449	797	89.68	5.02	78.00	180.54	3.60	2.01
77	BR4-10,1	454	11.09	85.10	4.38	26.00	171.36	3.26	2.01
78	HR4-01_b	454	1246	83.71	5.42	51.00	165.99	4.90	1.98
79	HJ2-5,6	449	892	72.08	5.14	37.00	134.40	5.48	1.86
80	HJ3-1,2	448	529	83.22	4.94	44.00	167.60	3.77	2.01
81	HJ4-2,3	448	769	90.49	4.41	30.00	182.58	2.67	2.02
82	HO2-5,6	449	810	82.38	4.84	39.00	176.97	2.65	2.15
83	HO3-7,8	448	837	87.41	4.94	14.00	202.25	2.26	2.31
84	HO3-9,10	449	1180	80.34	5.02	22.00	165.52	2.51	2.06
85	HO4-01	448	1009	92.79	4.38	32.00	187.47	2.36	2.02
86	HR1-1,2	448	709	86.59	4.64	48.00	166.78	2.71	1.93

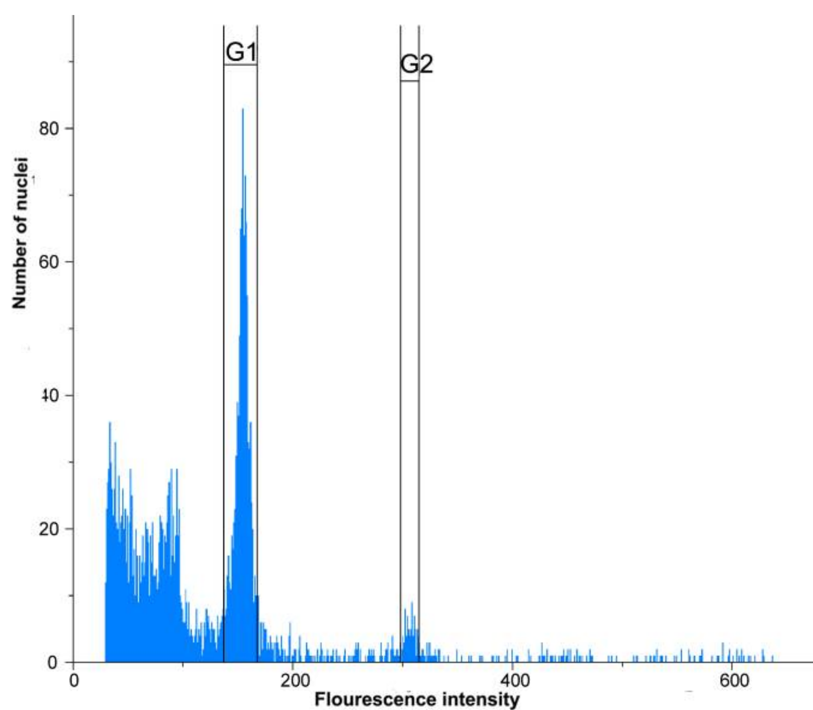
87	HR1-3,4	449	1077	82.15	5.05	44.00	157.84	3.54	1.92
88	HR2-5,6	449	751	89.04	4.84	69.00	174.11	4.64	1.96
89	HR2-7,8	449	1089	80.53	5.02	9.00	170.31	1.83	2.11
90	HR4-1,2	448	1120	76.60	5.35	38.00	154.36	2.69	2.02
91	HR4-4,5	448	1052	92.06	3.63	28.00	186.25	2.27	2.02
92	HR4-7,8	449	243	85.39	4.46	11.00	176.86	1.52	2.07
93	HO1-7,8	448	1133	83.30	5.39	79.00	168.82	3.96	2.03
94	HO1-9,10	453	954	92,62	4.25	21.00	193.77	3.18	2.09

Appendix 7. Statistical analysis of DNA content values.



Linear regression of G1 and G2 of dry leaves

Appendix 8. Histogram of fluorescence intensity of dried leaf sample of BJ3-04.



Region	Gate	Ungated	Count	Count/ml	%Gated	GMn-x	Mean-x	CV-x%
G 1	<None>	976	976	-	32.53	153.82	153.95	4.07
G 2	<None>	79	79	-	2.63	307.11	307.15	1.42

Appendix 9. *Centotheca lappacea* (Linnaeus) Desvaux.

1. Habit; 2. Spikelet; 3. Lower glume; 4. Upper glume; 5. Lower lemma; 6. Upper lemma; 7. Palea; 8. Palea with stamen; 9. Pistil. Source: http://foc.eflora.cn/illast/Centotheca%20lappacea.jpg