Meiotic characters and yield components of *Plukentia volubilis* L. in Madaya Township, Mandalay Region

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Abstract

This research aims to determine the relation of meiotic cell division with the yield traits. *Plukentia volubilis* L. is an oleaginous plant belonging to the Euphorbiaceae family. The inflorescences of *Plukentia volubilis* L. were collected in Madaya Township, Mandalay Region. The meiotic behavior of *Plukentia volubilis* L. was determined by studying the meiotic chromosome constitution of 30 pollen mother cells (PMC's). The mean chromosome pairing of *Plukentia volubilis* L. 2n = 56 at metaphase I, 2.33 I univalent + 18.87 II bivalent (ring or close and rod or open) + 1.17 III trivalent +3.13 IV quadrivalent per cell were observed. The yield related traits were observed that plant height, days to flowering, days to maturity, number of primary branch per plant, number of capsule per plant, dry weight per capsule, capsule diameter, seed weight per capsule and thousand seeds weight. Meiosis cell division processes were the meiotic stages of chromosome segregation in regular cell division, presenting the frequency of normal tetrad formation of pollen fertility analyzed plant.

Introduction

The study area is located in Madaya township, Mandalay Region. Madaya is located between North latitude 22° 12' and 22° 15' and East longitude 96° 06' and 96° 10'. *Plukenetia*, a poorly defined classification genus, belongs to the tribe Plukenetieae of subfamily Acalyphoideae in the family Euphorbiaceae. *Plukenetia* is a pantropical genus of over 20 species of twining vines and lianas, which are distributed throughout Africa and Latin America, and as far southeast as Malesia (Semino *et al.* 2008). Sacha inchi is an oleaginous perennial plant native to the Amazon region of Peru. The plant has been cultivated by indigenous people for centuries. Sacha inchi can grow to a size of two meters. The leaves have a length of 10-12 cm, and a width of 8-10 cm. Five months after the sacha inchi has been planted, it flowers. The fruits of the sacha inchi plant are capsules consisting of four (up to seven) lobes, which contain the seeds. The fruits are green, but get blackish brown when they ripe. The seeds are 1.5-2 cm in diameter, and weight around 45-100 grams. They are oval-shaped, and have a dark brown colour (Dostert, Nicolas *et al.* 2009).

Meiotic is a device of increasing the number cells. During sexual reproduction, organisms of two gametes unit give rise to a zygote and produce as a result of meiosis. Thus, meiosis is one of the important divisions for evolution. In meiosis, the process of genomic reconstruction is more complicated, as the segregation of homologous chromosomes and sister chromatics need to be implemented accurately in 2 consecutive divisions. In the early combinations, lagging univalent appeared at anaphase I or II, and their behavior are likely to cause meiotic disorders (Friebe *et al.* 2005; Tiwari *et al.* 2010). Centromere orientation and the relative strength of the spindle fibers to the sister chromatid cohesion are known to be responsible for the behavior of retarded univalent (Darlington 1939; Lukaszewski 2010). As a result, breakage may occur across the centromere region in a lengthwise or transverse manner or across the pericentric chromatin (Darlington 1939; Lukaszewski 2010; Tiwari *et al.* 2010).

The chromosome number of different cells in the metaphase of *Plukenetia volubilis* L. was so non-uniform that the single basic chromosome number of this promising oilseed crop could not been easily and correctly postulated. Analyzed a large number of species in the Euphorbiaceae and observed that the basic

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chromosome numbers of unrealted species formed a primary series of x = 7, 8 and 9 and a secondary series of x = 6, and 11 Perry (1943). The chromosome numbers were detected in the subfamily and an appropriate cytotaxonomic interpretation. In addition, chromosome counts performed in the subfamily Acalyphoideae have revealed that the tribe Plukenetieae consisted exclusively of polyploid species (Vanzela *et al.* 1997). Thus, *Plukenetia volubilis* L. is a polyploid plant, like 41.38% of other species in Acalyphoideae (Vanzela *et al.* 1997) and 50% of other species in the Euphorbiaceae family (Perry 1943; Aarestrup *et al.* 2008).

Aims and Objectives

To determine the meiotic chromosome behavior, and pollen fertility of *Plukenetia volubilis* L. To determine the relation of meiotic cell division with the yield traits of *Plukenetia volubilis* L.

Materials and Methods

Materials

The cultivar of *Plukenetia volubilis* L. that grown in Madaya township, Mandalay Region were used in this research work. The research was conducted in laboratory of Department of Botany, Yadanabon University.

Methods

Meiosis

Collection of pollen mother cells (PMCs)

The pollen mother cells (PMCs) of *Plukenetia volubilis* L. were collected at young spike and immediately fixed with Carnoy's solution (i.e, 3 ethanol: 1 glacial acetic acid) for 24 hrs. The pollen grains were stained with aceto-carmine solution. The yield components were recorded by statistical analysis and photograph.

Slide preparation

After fixing for 24 hrs in the fixative solution, one or two anthers were dissected out from young spike. These anthers were placed on the slide. After that the undesired materials were removed from the slide. They were smeared in 1% aceto-carmine on the slide. Then, the sample was covered with cover slip. In this way the slide was ready to observe the PMC's characters under the microscope. Among the 30 PMC's observed in *Plukenetia volubilis* L.

Identification of bivalent at Metaphase I, laggard and bridge of Anaphase I and Telophase I, and micronuclei at Spore tetrad

At metaphase I of meiotic, the two homologous chromosomes that paired with each other were recorded as bivalent chromosome. The paired chromosome that synapse at both end of the chromosome was recorded as closed or ring bivalent and the one that synapse only at one end and left open at the other end was recorded as open or rod bivalent.

Chromosomes that left behind during the other chromosomes moved to their destination pole (Anaphase I) and those pole reached chromosomes without enclosed in newly formed nuclear envelope (Telophase I), were recorded as laggard chromosomes at Anaphase I and Telophase I. Paired chromosomes that do not segregate and stretched along the centre of the diving cell in the form of bridge were named as bridged chromosome.

The tetrad cells were observed in with micronuclei per tetrad cell. Laggard chromosomes that left behind formed by themselves as small nuclei were micronuclei besides the large normal nucleus which were considered as abnormal sporetetrad. The normal spore tetrad has only one larger nucleus and it’s have no any small nuclei.

Pollen which formed from maturation of spore tetrad usually has one generative nucleus and two tube nucleus in normal pollen. Abnormal spore tetrad with

many micronuclei usually give rise the pollen lacking the generative nucleus or one or two tube nucleus which is abnormal or sterile pollen. A pollen tube contains a vegetative nucleus (tube nucleus) which lies at the tip of the pollen tube and a generative nucleus which divided to form the two male gametes.

Photomicrography

Morphological characters and chromosome pairing at metaphase I stage, chromosome movements at anaphase I, telophase I stages, metaphase II, anaphase II; telophase II stages, normal spore tetrad, abnormal spore tetrad, normal and abnormal pollen with the clear out image of every stages were carefully recorded.

Meiotic index

Male flower buds of *Plukenetia volubilis* L. genotypes were collected in tetrad stages during the microsporogenesis, for analysis of meiotic index.

The meiotic index (MI) as percentage of normal tetrads, is calculate according to Love (1951).

MI% = (Number of normal tetrads/Number of total tetrads)×100

Statistical analysis

The data of chromosome pairing of open and closed bivalent chromosomes in metaphase I, cells with laggard chromosomes and bridge chromosomes at anaphase I and telophase I stage, spore tetrad with micronuclei and yield components were calculated byJim Fowler, Lou Cohen and Phil Jarvis (1990) and normal and abnormal pollen were also calculated.

Results

Cytogenetical characters of *Plukenetia volubilis* L.

Metaphase I

The pollen mother cells (PMCs) from the *Plukenetia volubilis* L. was examined at metaphase I. cells exhibited stickness and clumping of chromosomes. Metaphase I shows mean number of univalent 2.33±0.95 were observed. The bivalent characters of *Plukenetia volubilis* L. 18.86±0.90 mean number were also observed. The mean value of trivalent and quadrivalent character that exhibited 1.17±0.37 and 3.13±0.67 were observed respectively. The species was show variation in chromosome number such as observed in this study. The number of laggard chromosomes in *Plukenetia volubilis* L. at anaphase I and telophase I were 0.36±0.66 and 0.46±0.73 mean number per cell (Table 1, Figure 2). The laggard frequency of anaphase I cells possess 26.67% and at telophase I cells 33.33% were observed in this species (Table 1, Figure 2).

Metaphase II

The chromosomes line up at the equator of each of the newly formed cells. Spindle fibers attached to the centromere of each sister chromatid. In metaphase , a synchronous chromosome segregation was observed in this species (Table 1, Figure 2).

Anaphase II

The sister chromatids divide and move apart. Because each sister chromatid has formed a separate centromere, the two sister chromatids were to be separate chromosomes. The single-stranded chromosomes then move to opposite ends of each cell. In anaphase II, a synchronous chromosome segregation was observed (Table 1, Figure 2).

Telophase II

The spindle fibers disappear and a nuclear membrane forms around each group of chromosomes. The cytoplasm divides around each newly formed nucleus, resulting in four new haploid cells were observed (Table 1, Figure 2).

Spore tetrad stage

The number of micronuclei per spore tetrad were observed in *Plukenetia volubilis* L. had 1.20±1.84 mean number per cell (Table 1, Figure 2). The meiotic index of *Plukenetia volubilis* L. was 69%. The meiotic index measures the degree of meiotic stability of a species through of their meiotic products. This species of meiotic index was lower than 90% to 100% (Table 1, Figure 2).

Normal and abnormal pollen

The number of normal and abnormal pollen were observed in *Plukenetia volubilis* L. was 67.20 and 32.80 mean number per 100% pollen (Table 1, Figure 2). The size of normal pollen were showed that 55.00 μm to 65.00 μm in diameter. The size of abnormal pollen was observed 37.5 μm to 42.50 μm in diameter. The number of normal pollen was shown that 69% and abnormal pollen 31% in this species. The size of normal pollen were showed that 55.00 μm to 65.00 μm in diameter. The size of abnormal pollen was observed 37.5 μm to 42.50 μm in diameter. The more number of normal pollen was, the more increases in number of pollen fertility there was (Table 1, Fig 2). Therefore, the pollen fertility depended upon the number of normal pollen and regular meiosis cell division.

Yield and yield component of *Plukenetia volubilis* L.

The plant height of *Plukenetia volubilis* L. was 2.03 (m) mean number per plant. Days to flowering of *Plukenetia volubilis* L. was 212.16 mean number per plant. Days to maturity of *Plukenetia volubilis* L. was observed that 245.00 mean number per plant. Number of primary branch of *Plukenetia volubilis* L. was observed that 2.43 mean number per pant (Table 2, Fig 1). Number of capsule of *Plukenetia volubilis* L. was observed that 82.60 mean number per plant. The capsule diameter of *Plukenetia volubilis* L. was observed that 4.54 mean number per capsule. Dry capsule weight of *Plukenetia volubilis* L. was observed that 10.11 mean number per capsule. Dry seed weight of *Plukenetia volubilis* L. was observed that 1.36 mean number per seed. Thousand seed weight of *Plukenetia volubilis* L. was 1380.00 mean number of seeds weight (Table 2, Fig 1).

Table (1) Meiotic characters of *Plukenetia volubilis* L.

Characters	Total no. of Cells Observed	Mean ± SD	Average frequency (%)	Meiotic index (%)
Metaphase I	30			
Univalent		2.33±0.95		
Bivalent		18.87±0.90		
Trivalent		1.17±0.37		
Quadrivalent		3.13±0.67		
Anaphase I	30			
No. of Laggard		0.36±0.67	26.67	
Telophase I	30			
No. of Laggard		0.46±0.73		
Tetrad	100			
No. of micronuclei		1.20±1.84		69
Pollen	100			
Normal Pollen		67.20±1.12		
Abnormal Pollen		32.80±11.13		

Table (2) Yield components of *Plukenetia volubilis* L.

Characters	Mean ± SD
Plant height (m)	2.03 ± 7.27
Days to flowering	212.16 ± 23.25
Days to maturity	245.00 ± 18.95
Number of branch per plant	2.43 ± 0.77
Number of capsule per plant	82.60 ± 18.84
Dry weight per capsule (g)	10.11 ± 1.48
Capsule Diameter (cm)	4.54 ± 0.39
Seed weight per capsule (g)	1.36 ± 0.18
1000 seed weight (g)	138.00 ± 0.22

SD = standard deviation

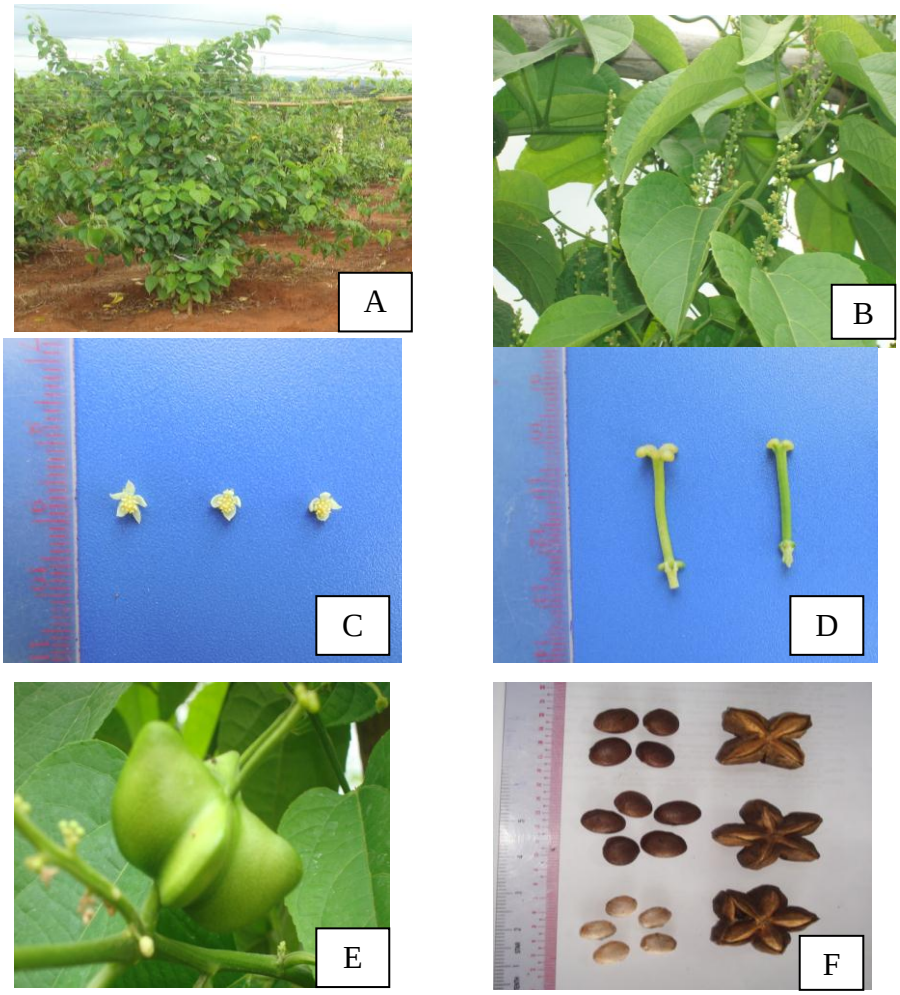

Figure (1) Morphology of *Plukenetia volubilis* L.

A. Habit

B. Inflorescences

C. Male flowers

D. Female flowers

E. Fruit

F. Seed and capsule with four or five lobes

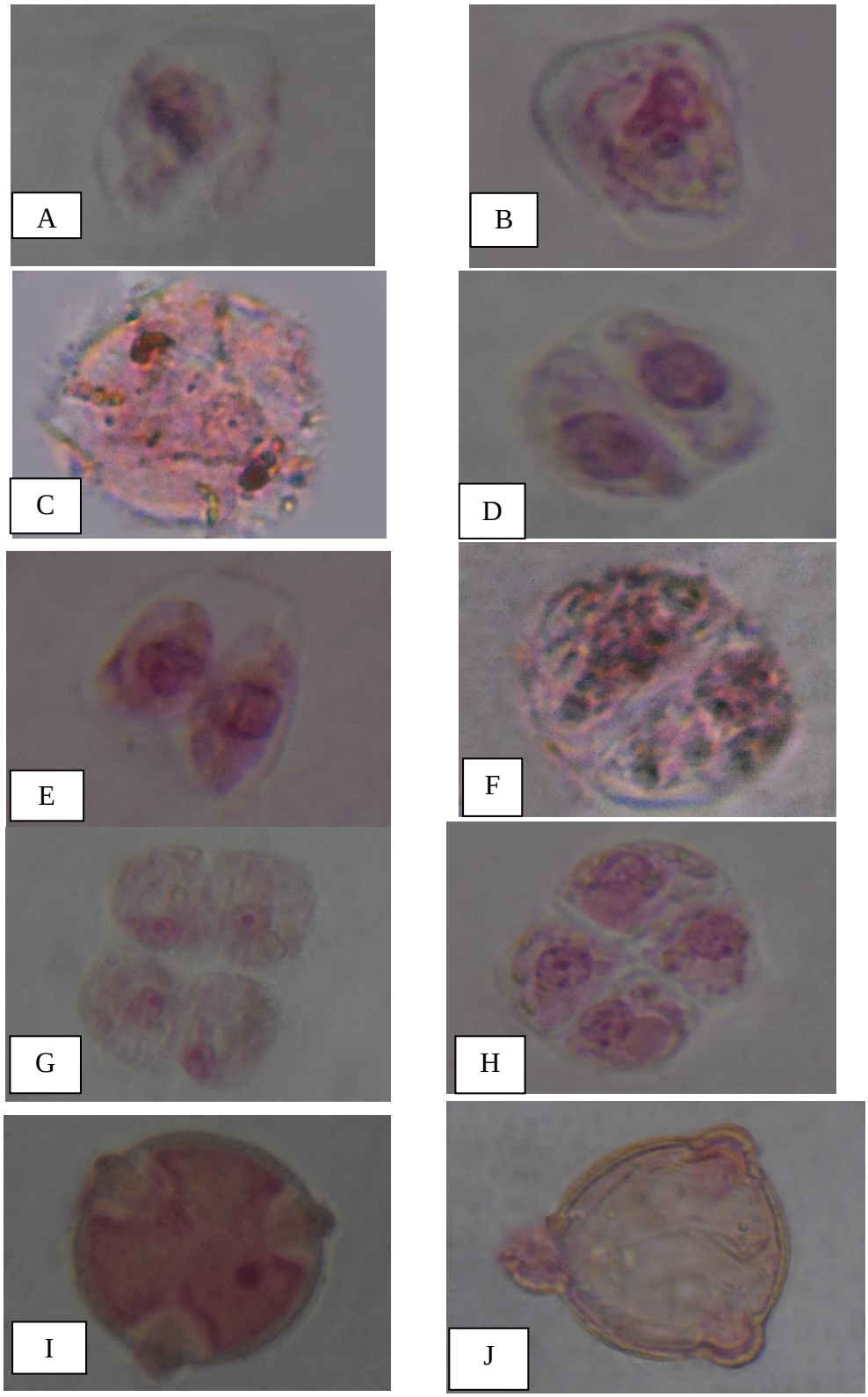

Figure (2) Meiosis division in *Plukenetia volubilis* L.

A. Metaphase I B. Anaphase I C. Telophase I
D. Metaphase II E. Anaphase II F. Telophase II
G. Normal tetrad H. Abnormal tetrad I. Normal pollen
J. Abnormal pollen

Discussion and Conclusion

Chromosome counting during microsporogenesis showed that analyzed plant of *Plukenetia volubilis* L. ranged from 2n = 58 to 2n = 86 chromosomes. In the meiotic behavior of the genus to which *Plukenetia volubilis* L. species belongs to *Euphorbiaceae* family and the few cytogenetic studies that were found are restrict to the counting of chromosome number.

Study of PMCs at the metaphase I as univalent, bivalent, trivalent and quadrivalent were observed. Separation and disjunction of chromosomes at meiosis I varied from normal to nearly normal (Han *et. al.,* 1973). Nassar *et al.,* (1994). The pollen mother cells (PMCs) from the *Plukenetia volubilis* L. was examined at metaphase I. cells exhibited stickness and clumping of chromosomes. Metaphase I shows mean number of univalent 2.33±0.95 were observed. The bivalent characters of *Plukenetia volubilis* L. 18.86±0.90 mean number were also observed. The chromosome behavior in metaphases and anaphases in the *Euphorbiaceae* family was observed by Nassar *et al.,* (1994). The laggard chromosomes were also observed in anaphase I and anaphase II. The number of laggard chromosomes in *Plukenetia volubilis* L. at anaphase I and telophase I were 0.36±0.66 and 0.46±0.73 mean number per cell (Table 1, Figure 2). The laggard frequency of anaphase I cells possess 26.67% and at telophase I cells 33.33% were observed in this species (Table 1, Figure 2). Nassar *et al.,* (1994) reported chromosomal laggards during anaphase I, this chromosomal laggards eventually form scattered univalents on cytoplasm and then are liminated as micronuclei of different sizes Nassar *et al*. (1994).

Chromosome grouping during metaphase I and anaphase I led to the formation of chromosome stickness bridges in telophase I. The presence of chromosome stickness is a factor that competes for the formation of chromosome bridges which may persist until telophase and forming micronuclei. The number of micronuclei per spore tetrad were observed in *Plukenetia volubilis* L. had 1.20±1.84 mean number per cell. The meiotic index of *Plukenetia volubilis* L. was 69%. The meiotic index measures the degree of meiotic stability of a species through of their meiotic products. This species of meiotic index was lower than 90% to 100%.

Abnormal microspores give rise pollen grains with different sizes and amount of genetic material are unbalanced. Therefore, pollen dimorphism is lead to abnormal meiosis or mitosis and the percentage of viability was higher in the normal and lower in the abnormal. The number of normal pollen was shown that 69% and abnormal pollen 31% in this species. The more number of normal pollen had, the increases in number of pollen fertility there was. The number of capsule of *Plukentia volubilis* L. was depended on pollen fertility. Therefore, the yield traits of *Plukentia volubilis* L. was related to regular meiosis cell division. Although the meiotic index is 69% and the abnormalities occurred in the meiotic process, these did not interfere in reproduction and development of *Plukenetia volubilis* L. because the frequency of viable pollen grain is high.

Polyploidy is a major force in plant evolution and occurs frequently across a number of taxa (Soltis, Soltis & Tate, 2004) estimated that 35% of extant vascular plant genera are polyoid, and that 15% of speciation events in angiosperms were facilitated by a duplication of the chromosome set. The multiplication of entire genomes is usually regarded as being associated with higher tolerance to environmental stress because polyploid taxa are known to be relatively more abundant than diploids in arctic and alpine environments (Stebbins, 1985; Hijmans *et al.,* 2007). Polyploidization therefore plays a pivotal role in the diversification of plants,

especially in mountain habitats (Soltis, Soltis & Tate, 2004). The variation in chromosome number which may led to genetic instability. In addition to diverse chromosome number of *Plukenetia volubilis* L. has rich genetic diversity and play a marked evolutionary role.

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