

Research Article

Chromosome numbers and karyotypes of four Thai banana varieties from Ratchaburi Province, Thailand

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Abstract - This research aims to study the chromosome numbers and karyotypes of four banana varieties from Ratchaburi Province using the root tip squashing technique. The varieties are: Kluai Leb Mu Nang (*Musa sapientum* Linn.), Kluai Tani (*Musa balbisiana* Colla), Kluai Namwa Tanao Sri (*Musa* × *Paradisiaca* L.), and Kluai Hom Khiew (*Musa* sp., AAA group “Cavendish”) at the Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University from October, 2023 to September, 2024. Chromosomes were obtained from root tips and fixed roots; meristem cells were stained with acetocarmine dye and photographed under a light microscope during the metaphase stage. Data analysis and idiogram display were conducted using a ready-made program. The results revealed that the chromosome number was $2n = 22$ for both Kluai Leb Mu Nang and Kluai Tani. For Kluai Namwa Tanao Sri and Kluai Hom Khiew, the chromosome number was $3n = 33$. Chromosome sizes ranged from 1.30 to 3.40 μm . The karyotypes consisted of metacentric and submetacentric chromosomes, except for Kluai Tani and Kluai Namwa Tanao Sri, which exhibited subtelocentric chromosomes. The complex origin of cultivated bananas and the limited clarity of their morpho-taxonomic classification markers highlight the need for more in-depth research on the *Musa* genome at the chromosomal and DNA levels. Additionally, understanding chromosome structural changes could assist banana breeders in choosing suitable parent plants for breeding. Traditional cytogenetic techniques,

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including genome size estimation, ploidy determination, and chromosome counting, offer valuable insights into the genome.

Keywords: Banana, chromosome, karyotype, idiogram

1. Introduction

Bananas (*Musa* spp.) are herbaceous plants cultivated largely in tropical and subtropical regions of Southeast Asia, Africa, and South America (Häkkinen, 2013; Janssen et al., 2016). There are over 1,000 banana varieties, each providing essential nutrients. Bananas are among the most widely produced, traded, and consumed fruits worldwide, with an annual output of around 150 million tonnes (FAOSTAT, 2024). Cooking bananas are a staple food for millions, and bananas are also a major export commodity for several developing countries, making them a crucial element of national trade with considerable socioeconomic impact. Currently, most residents of Ratchaburi Province are farmers, and the primary banana varieties grown there include Kluai Khai, Kluai Hom, Kluai Leb Mu Nang, Kluai Tani, and Kluai Namwa. Many of these banana varieties are traditional breeds used for cooking and as dessert bananas. Additionally, these bananas are noted for their nutritional value, particularly their higher protein content compared to other common fruit cultivars.

Banana classification has long posed challenges for taxonomists (Chessman & Larter, 1935). Linnaeus initially classified bananas into two types based on their culinary uses: *Musa sapientum* for bananas and *Musa × Paradisiaca* for plantains. Over time, additional names were introduced. Modern cultivated bananas are derived from natural inter- and intra-specific crosses between two wild diploid species in the *Eumusa* section: *M. acuminata* (A genome) and *M. balbisiana* (B genome). However, most edible bananas are vegetatively propagated and classified as diploid (AA, AB), triploid (AAA, AAB, or ABB), or tetraploid clones. These are categorized based on the proportion

of *M. acuminata* and *M. balbisiana* genomes in their genotypes (Simmonds & Shepherd, 1955). The currently accepted scientific names are *Musa acuminata* Colla and *Musa balbisiana* Colla for the ancestral species, and *Musa × Paradisiaca* L. for the hybrid. However, the classification of bananas remains a subject of debate, highlighting the need for further cytogenetic research to clarify the taxonomy (Phengchang et al., 2009).

We studied the chromosome numbers and karyotypes of four banana varieties from Ratchaburi Province. The aim was to investigate the chromosome numbers of Kluai Leb Mu Nang, Kluai Tani, Kluai Namwa Tanao Sri, and Kluai Hom Khiew, which are economically important species in the region. The information obtained from this study is valuable for improving banana breeding. Accurate identification of banana species is enhanced by understanding their chromosomal traits and precise chromosome counts.

2. Materials and methods

2.1 Plant materials

Specimens were collected from the natural habitats in Chombueng Sub-district, Chombueng District, and Tanaosri Sub-district, Suan Phueng District, Ratchaburi Province. The four banana varieties are: Kluai Leb Mu Nang (*Musa sapientum* Linn.), Kluai Tani (*Musa balbisiana* Colla.), Kluai Namwa Tanao Sri (*Musa × Paradisiaca* L.), and Kluai Hom Khiew (*Musa* sp., AAA group “Cavendish”) were identified using the Flora of China (Wu & Raven, 2000).

2.2 Cytogenetic investigation

The chromosome study utilized actively growing root tips from stem cuttings.

Metaphase chromosome preparations were made following the method described by Pitaktharm et al. (2024), with some modifications. Young root tips were pretreated with a 0.2% colchicine solution at $5\pm 2^{\circ}\text{C}$ for 24 hours to arrest metaphase chromosomes, then fixed in Carnoy's solution (a 3:1 mixture of absolute methanol and glacial acetic acid) at $5\pm 2^{\circ}\text{C}$ for 24 hours. For the Feulgen squash method, fixed root tips were washed in distilled water, hydrolyzed in 1N hydrochloric acid at 60°C for 10-15 minutes, stained with 2% acetocarmine for 10-20 minutes, and then squashed onto microscopic slides. Well-spread metaphase chromosomes were examined at $1,000\times$ magnification using a CX23 Olympus light microscope, and images were captured with a DSLR digital camera. Chromosome measurements, including chromosome numbers and chromosome length (CL) ranges, were analyzed using Adobe Photoshop CS5. For each banana accession, the position of the centromere was observed, which is essential for defining chromosome type. The arm ratio (AR), calculated as the length of the long arm divided by the length of the short arm, was used to determine chromosome morphology according to Levan et al. (1964). Chromosomes were classified as follows: metacentric (m) with an AR of 1.0-1.7; submetacentric (sm) with an AR of 1.7-3.0; subtelocentric (st) with an AR of 3.0-7.0; and telocentric (t) with an AR over 7.0.

3. Results and discussion

Chromosome number

The study of chromosome numbers for four banana varieties from Ratchaburi Province—Kluai Leb Mu Nang, Kluai Tani, Kluai Namwa Tanao Sri, and Kluai Hom Khiew—revealed two distinct groups. The

first group consists of diploid bananas ($2n$), with varieties Kluai Leb Mu Nang and Kluai Tani each having 22 chromosomes. The second group includes triploid bananas ($3n$), with Kluai Namwa Tanao Sri and Kluai Hom Khiew each having 33 chromosomes.

In this study, Kluai Leb Mu Nang and Kluai Tani were categorized as having a chromosome number of $2n = 22$, identifying them as diploid. Previous research has also identified several diploid varieties with the same chromosome count, including Kluai Pa Number 1, Kluai Pa Number 3, Kluai Ang Kang, Kluai Khae, Kluai Khai, Kluai Tani, Kluai Khai Boran, Kluai Thong Khi Meaw, Kluai Pa Number 22, Kluai Lai, and Kluai Mak (Silayoi & Sompen, 1991). Additional diploid varieties reported include Kluai Pa (Phrae), Kluai Pa Abyssinia, Kluai Pa Hybrid (Rayong), Kluai Pa Pli Leuang, Kluai Dang Lek, Kluai Hom Champa, Kluai Homchan, Kluai Hom Son, Kluai Khai Thong Ngoei, and Kluai Sa (Phengchang et al., 2009).

In this study, Kluai Namwa Tanao Sri and Kluai Hom Khiew were identified as having a chromosome number of $3n = 33$, classifying them as triploid. Previous research has documented several triploid varieties with the same chromosome count, including Kluai Khrao, Kluai Neu Mu Nang, Kluai Nam Kap Dum, Kluai Kung Khieo, Kluai Nam, Kluai Tip, Kluai Leb Chang Kut, Kluai Tip Khum, Kluai Khom Bao, Kluai Khom Nak, Kluai Namwa Luang, Kluai Kung, Kluai Klong Chang, Kluai Phama Haek Kuk, Kluai Nang Klai, Kluai Hom Tia, Kluai Namwa Khom, and Kluai Khai Bong (Silayoi & Sompen, 1991). Additionally, triploid varieties such as Kluai Hom Grand Nain, Kluai Hom Thong Taiwan, Kluai Hom Thong Thai, and Hom Thong Nak have also been reported (Phengchang et al., 2009).

Table 1. Number, type and size of chromosome in bananas.

Banana variety	Chromosome numbers	Chromosome pairs				Chromosome size (micron)
		Metacentric	Submetacentric	Subtelocentric	Telocentric	
Diploid group (2n)						
Kluai Leb Mu Nang	22	1, 2, 3, 4, 5, 7, 9, 11	6, 8, 10	-	-	1.56-2.68
Kluai Tani	22	3, 4, 5, 8	1, 2, 6, 7, 9, 10	11	-	1.42-2.40
Triploid group (3n)						
Kluai Namwa Tanao Sri	33	4, 7, 10	2, 3, 5, 6, 8, 9, 11	1	-	1.48-3.40
Kluai Hom Khiew	33	1, 2, 3, 4, 5, 7, 8, 10	6, 9, 11	-	-	1.30-2.26

Size and type of chromosome

The study revealed that the four banana varieties examined had chromosome lengths ranging from 1.30 to 3.40 micrometers (Table 1), consistent with findings by Silayoi and Sompen (1991) and Phengchang et al. (2009). The karyotypes of these bananas included metacentric, submetacentric, and subtelocentric chromosomes (Figures 1 and 2). Specifically, Kluai Leb Mu Nang and Kluai Hom Khiew exhibited metacentric and submetacentric chromosomes, which align with the chromosome shapes reported for Kluai Khai and Kluai Khrao (Silayoi & Sompen, 1991). These varieties were noted to have AA and AAA genomes,

respectively, reflecting their wild banana (*Musa acuminata* Colla) ancestry (Silayoi & Babpraserth, 1983).

Additionally, Kluai Tani and Kluai Namwa Tanao Sri displayed all three chromosome shapes as metacentric, submetacentric, and subtelocentric. This finding agrees with the reported chromosome shapes of Kluai Tip and Kluai Khom Nak (Silayoi & Sompen, 1991). These varieties were identified as having ABB and AAB genomes, respectively, indicating their hybrid origins from wild banana species (*Musa acuminata* Colla) (Silayoi & Babpraserth, 1983).

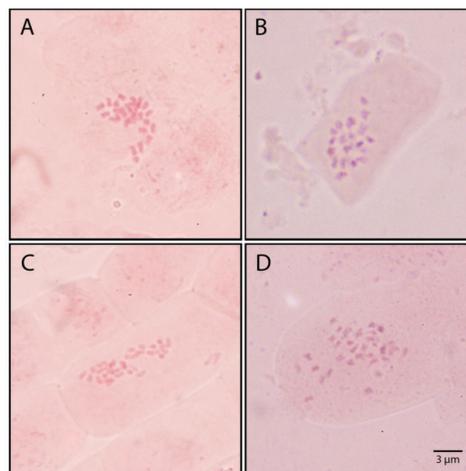


Figure 1. Somatic metaphase chromosomes of representative banana accessions from Ratchaburi Province are illustrated as follows: Kluai Leb Mu Nang (2n = 22) (A), Kluai Tani (2n = 22) (B), Kluai Namwa Tanao Sri (3n = 33) (C), and Kluai Hom Khiew (3n = 33) (D).

Figures 1, 2, and 3 depict the somatic metaphase chromosomes, karyotypes, and idiograms of four banana varieties: Kluai Leb Mu Nang (*Musa sapientum* Linn.),

Kluai Tani (*Musa balbisiana* Colla), Kluai Namwa Tanao Sri (*Musa* × *Paradisiaca* L.), and Kluai Hom Khiew (*Musa* sp., AAA group “Cavendish”).

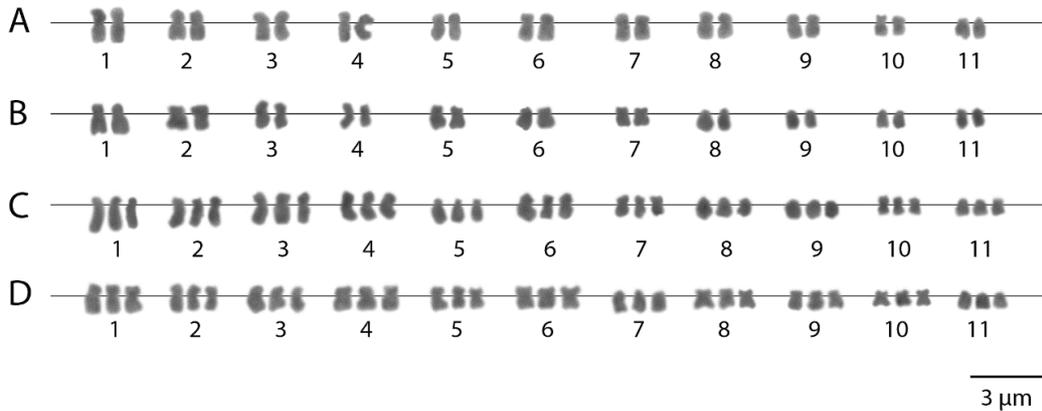


Figure 2. Karyotypes of bananas from Ratchaburi Province are presented for the following varieties: Kluai Leb Mu Nang (2n = 22) (A), Kluai Tani (2n = 22) (B), Kluai Namwa Tanao Sri (3n = 33) (C), and Kluai Hom Khiew (3n = 33) (D).

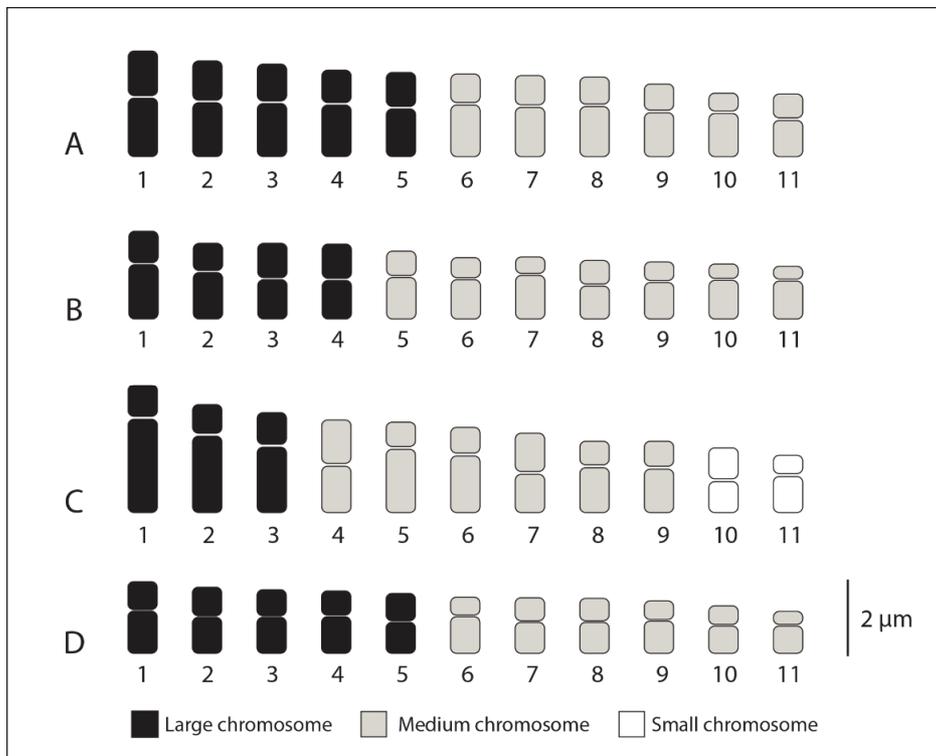


Figure 3. Standard idiograms of bananas from Ratchaburi Province are shown for the following varieties: Kluai Leb Mu Nang (2n = 22) (A), Kluai Tani (2n = 22) (B), Kluai Namwa Tanao Sri (3n = 33) (C), and Kluai Hom Khiew (3n = 33) (D).

Genome of bananas

Bananas belong to the genus *Musa*, which is divided into four sections: Eumusa, Rhodochlamys, Australisa, and Callimusa. Bananas in the Eumusa and Rhodochlamys sections typically have a chromosome number of $2n = 22$, while those in the Australisa and Callimusa sections have $2n = 20$ (Chapman, 1970; Cheesman & Later, 1935; Hutchingson, 1966; Pursglove, 1978). Bananas from the Eumusa section are particularly important as they are widely consumed and economically significant. This group exhibits a complex genetic heritage, reflecting the diverse origins of each banana variety. This can be achieved by using a scoring approach to categorize bananas based on 15 morphological characteristics (Simmonds & Shepherd, 1955). This method helps identify bananas with the following genomic classifications: AA ($2n = 22$), AAA ($2n = 33$), AAB ($2n = 33$), ABB ($2n = 33$), ABBB ($2n = 44$), and BB ($2n = 22$). Each group provides insight into its parental origin. Edible bananas typically derive from two ancestral species: *Musa acuminata* Colla (genome A) and *Musa balbisiana* Colla (genome B) (Hribova et al., 2008). The genome of the four examined samples is Kluai Leb Mu Nang (*Musa sapientum* Linn.: AA), Kluai Tani (*Musa balbisiana* Colla: BB), Kluai Namwa Tanao Sri (*Musa* × *Paradisiaca* L.: ABB), and Kluai Hom Khiew (*Musa* sp., AAA group “Cavendish”: AAA)

In Thailand, bananas exhibit various chromosomal numbers, including $2n = 22$, 33, or 44, with $x = 11$ (Oselebe et al., 2006). Consequently, cultivated bananas often have 2X, 3X, and 4X sets of chromosomes. This results in a broad range of genetic variations. Many hybrids have originated from the two wild banana species, *Musa acuminata* Colla and *Musa balbisiana* Colla (Hribova et al., 2008; Ferreira et al., 2004; Heslop-Harrison & Schwarzacher, 2007). Distinguishing between banana species can be challenging due to the multiple genome copies, which include AA, AAA, BB, BBB,

AAB, ABB, and ABBB. Additionally, local names for bananas vary, even for the same type, leading to confusion. Different local names may refer to the same banana type or even different varieties under the same name (Phengchang et al., 2009).

Furthermore, bananas with the same genome can exhibit variations in chromosomal sizes and shapes. These differences contribute to slightly distinct chromosome structures (Silayoi & Sompen, 1991), which are crucial for accurately categorizing each banana species and identifying their ancestors.

4. Conclusion

The chromosome numbers of four banana types from Ratchaburi Province were determined using the squashing root tip technique and acetocarmine staining. The banana varieties studied included Kluai Leb Mu Nang (*Musa sapientum* Linn.), Kluai Tani (*Musa balbisiana* Colla), Kluai Namwa Tanao Sri (*Musa* × *Paradisiaca* L.), and Kluai Hom Khiew (*Musa* sp., AAA group “Cavendish”). The results revealed that Kluai Leb Mu Nang and Kluai Tani are diploid ($2n = 22$), while Kluai Namwa Tanao Sri and Kluai Hom Khiew are triploid ($3n = 33$). Chromosome lengths for all four banana types ranged from 1.30 to 3.40 μm . The karyotypes of Kluai Leb Mu Nang and Kluai Hom Khiew consisted solely of metacentric and submetacentric chromosomes. In contrast, Kluai Tani and Kluai Namwa Tanao Sri displayed three chromosome shapes: metacentric, submetacentric, and subtolocentric. No telocentric chromosomes were observed in this study.

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