Chromosome analysis of *Hibiscus rosa-sinensis* using CHIAS IV software

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Abstract. *Hibiscus rosa-sinensis* is a plant that has flowers with many variations in size, petal shape, petal colors, and chromosome number. Chromosome number and morphology information is the key to study its flower morphology and physiology. Chromosome Image Analyzing System IV (CHIAS IV) is software that used for mapping and identifying chromosome automatically. This report presents the results of chromosome analysis using CHIAS IV from four *H. rosa-sinensis* flower variations (single small red, single small pale red, single big red, and double red). Chromosome number and condensation patterns of individual chromosomes analysis suggested that these flower variations of *H. rosa-sinensis* exhibited polyploidy, with the smallest number found in single small pale red and the biggest number in single big red flower. CHIAS IV software is an effective tool to analyze chromosome image, especially to predict chromosome number, chromosome type, relative length, and condensation patterns of individual chromosome.

1. Introduction

*Hibiscus rosa-sinensis* is a shrub that belongs to the family Malvaceae. It is native to China and widely distributed in tropical areas. *H. rosa-sinensis* is commonly cultivated for ornamental plant because of its beautiful flowers. Some parts of this plant have been used to treat various diseases. The root is used to treat cough. The leaves are used to treat constipation, skin disease, cardiac disease, nerve disease, and diarrhea. The flowers are used to treat bronchial catarrh, menorrhagia, and ischemic disease. It has well documented pharmacological effects such as anticancer, anti-diabetic, anti-inflammation, and antimicrobial activities. Phytochemical analysis showed that this plant contains flavonoids, flavonoid glycosides, hibiscetin, cyanidine, cyanidin glucosides, taraxeryl acetate, β-sitosterol, campesterol, stigmasterol, ergosterol, citric acids, tartaric acids, oxalic acids, cyclopropenoids, and anthocyanin pigments [1, 2].

The flower of *H. rosa-sinensis* has many variations in size, petal shape, and petal colors. *H. rosa-sinensis* has several lines with different flower colors, ranged from white, beige, yellow, orange, pink, and red [3]. There are three flower types based on petal shape: single, crested, and double flower. The single flower type has five detached petals and 60-70 stamens, while the double flower type has five
detached petals with additional petal in form of stamen petaloid and 10-40 stamens. Transitional form between single and double flower types is the crested flower type [4, 5].

Chromosome number and morphology information is the key to study its flower morphology and physiology. \textit{H. rosa-sinensis} has many relatively small-sized chromosome in every cell. Chromosome number of \textit{H. rosa-sinensis} has been reported vary from 46, 54, 63, 68, 72, 77, 84, 90, 96, 112, 132, to 144. According to those report, it is difficult to determine basic number of its chromosome and also the ploidy level [6, 7].

Chromosome Image Analyzing System IV (CHIAS IV) is software that used for mapping and identifying chromosome automatically. This software is an imaging tool associated with Image J software (http://imagej.nih.gov) developed by the NIH [8]. It is an improved version of CHIAS to minimize difficulties in analyzing small-sized chromosomes. This software has been used to determine chromosome characteristics in some plants, such as red clover [9], tea plant, ornamental camellia [10], and switchgrass [11]. This report presents the results of chromosome analysis using CHIAS IV from four \textit{H. rosa-sinensis} flower variations (single small red, single small pale red, single big red, and double red) (Figure 1).

**Figure 1.** Morphology of the flowers of four \textit{H. rosa-sinensis} variations (a) Single small red (SSR), (b) Single small pale red (SSPR), (c) Single big red (SBR), (d) Double red (DR).

### 2. Materials and Method

#### 2.1. Plant materials

Young flower buds from four flower variations of \textit{H. rosa-sinensis} (single small red, single small pale red, single big red, and double red) with the diameter less than 0.5 cm were collected at 6 a.m.-1 p.m. Each flower bud was measured by millimeter block paper. The samples were taken in a bottle containing water to prevent drying.

#### 2.2. Chromosome preparation

Chromosome spread was prepared based on modified Darlington and LaCour method [12]. Young flower buds were sampled and the anther was separated. Anther then was fixed in Farmer’s solution (ethanol: acetic acid = 3:1) and was stored at 1-4°C for 24 hours. Fixed flower buds were hydrolyzed in 5N hydrochloric acid solution for 20 minutes. Softened samples then were rinsed in water and were stained using aceto-orcein for 24 hours. The stained samples were squashed with a coverslip to generate chromosome spreads.

#### 2.3. Microscopy and chromosome analysis

Digital images were taken using AxioCam on trinocular microscope with 1,000x magnification. Chromosome number, chromosome length, arm ratio, and condensation patterns of each flower variations were determined automatically with CHIAS IV [13]. Analysis of chromosome number and morphology performed by the CHIAS IV system using the program developed by Seiji Kato, Nobuko
Ohmido, and Kiichi Fukui. The program file and manual of CHIAS IV software can be downloaded from http://www2.kobe-u.ac.jp/~ohmido/index03.htm. Analysis of Variance (ANOVA) test with significance of the differences was concluded at the 95% confidence level (P < 0.05) were conducted to ensure that the mean of chromosomes length from each flower variation were not significantly different one from another.

3. Results and Discussion

3.1. Chromosome number and morphology

Chromosome morphology data of *H. rosa-sinensis* include chromosome length and chromosome type determined by arm ratio based on data from Levan et al. [14]. Arm ratio is the length of the long arm divided by the length of the short arm. ANOVA test results indicates that the mean of chromosome length from each flower variation were significantly different one from another. Table 1 shows the chromosome number and morphological data from four flower variations of *H. rosa-sinensis*. Based on CHIAS IV analysis that summarized in Table 1, the biggest chromosome number was found in single big red (SBR) flower, followed by single small red (SSR), double red (DR) and the smallest number in the single small pale red (SSPR).

The chromosome number of the single small red flower type was 2n = 48 consist of 18 chromosomes that were classified as metacentric, 27 were as submetacentric, and 3 were as subtelocentric. The chromosomes length of single small red flower type ranged from 3.066 μm to 0.799 μm. The biggest and smallest chromosome was classified as submetacentric.

The chromosome number was 2n = 32 in single small pale red flower type with 21 chromosomes were classified as metacentric, 10 were as submetacentric, and 1 was as subtelocentric. The chromosomes length ranged from 1.566 μm to 0.399 μm. The biggest chromosome was classified as submetacentric, while the smallest one was classified as metacentric.

Chromosome number for single big red flower type were shown to be 2n = 72, consist of 28 chromosomes that were classified as metacentric, 41 were as submetacentric, and 3 were as subtelocentric. The chromosomes length of single big red flower type ranged from 1.866 μm to 0.466 μm. The biggest chromosome was classified as submetacentric and smallest chromosome was classified as metacentric.

Chromosome number for double red flower type were shown to be 2n = 42, consists of 24 chromosomes that were classified as metacentric, 17 were as submetacentric, and 1 was as subtelocentric. The chromosomes length ranged from 1.466 μm to 0.166 μm. The biggest chromosome was classified as submetacentric and smallest chromosome was classified as subtelocentric.

Song and Zhuang [15] reported that chromosome number of *H. rosa-sinensis* vary from 84, 105, 138, to 147. This report shows chromosome polymorphism in case of *H. rosa-sinensis*. Our observation also indicated that *H. rosa-sinensis* exhibited polyploidy. Moreover, there is a correlation between the size of the flower with the number of chromosome. The single big red variation showed the biggest number of chromosomes, which also has the biggest flower among the four variations studied.

| Flower variation | Chromosome number | Biggest chromosome length (μm) | Biggest chromosome type | Smallest chromosome length (μm) | Smallest chromosome type |
|------------------|-------------------|-------------------------------|------------------------|--------------------------------|-------------------------|
| SSR<sup>a</sup>  | 48                | 3.066                         | Sm                     | 0.799                          | Sm                      |
| SSPR<sup>b</sup> | 32                | 1.566                         | Sm                     | 0.399                          | M                       |
| SBR<sup>c</sup>  | 72                | 1.866                         | Sm                     | 0.466                          | M                       |
| DR<sup>d</sup>   | 42                | 1.466                         | Sm                     | 0.166                          | St                      |

<sup>a</sup>Single small red. <sup>b</sup>Single small pale red. <sup>c</sup>Single big red. <sup>d</sup>Double red. <sup>e</sup>Levan et al. [14].
3.2. Condensation patterns

Condensation patterns of each chromosome were measured using CHIAS IV software. Uneven staining along the chromosome was shown because the variation of condensation pattern (CP) from each chromosome. CP information was very useful for identifying individual chromosome. CP profile generated from each chromosome and chromosome length data were used by CHIAS IV software to generate ideogram. Together with the chromosome length and arm ratio, CP profile makes a chromosome to be distinguished from other chromosomes in a karyotype. SSR flower type quantitative ideogram generated by CHIAS IV is presented in Figure 2. SSPR flower type quantitative ideogram is presented in Figure 3. SBR flower type quantitative ideogram is presented in Figure 4. DR flower type quantitative ideogram is presented in Figure 5.

Ideogram constructed by CHIAS IV software is shown in gray scale consists of three regions. The black regions represented heterochromatic areas, which are heavily condensed chromatin regions. The gray regions represented intermediate chromatin condensation. The white regions represented euchromatic areas, which are lightly condensed chromatin regions. For examples, chromosome 29 of SSPR flower type was heavily condensed on both arms (Figure 3). Both short and long arm on chromosome 47 of SSR flower type was an intermediate condensed chromatin region (Figure 2). Euchromatic area of chromosome 27 and 29 of SBR flower type indicated by large white region on both short and long arm (Figure 4). Our data suggested that condensation level of all chromosomes from four flower variations were varied from heavy, intermediate, and lightly condensed by presence of black, gray, and white regions along the chromosomes respectively.

Figure 2. Quantitative ideogram of *H. rosa-sinensis* single small red (SSR) flower variation constructed by CHIAS IV. Heterochromatic areas are shown by black regions, intermediate condensation shown by gray regions, and euchromatic areas are shown by white regions.

Figure 3. Quantitative ideogram of *H. rosa-sinensis* single small pale red (SSPR) flower variation constructed by CHIAS IV. Heterochromatic areas are shown by black regions, intermediate condensation shown by gray regions, and euchromatic areas are shown by white regions.

Figure 4. Quantitative ideogram of *H. rosa-sinensis* single big red (SBR) flower variation constructed by CHIAS IV. Heterochromatic areas are shown by black regions, intermediate condensation shown by gray regions, and euchromatic areas are shown by white regions.
Figure 5. Quantitative ideogram of *H. rosa-sinensis* double red (DR) flower variation constructed by CHIAS IV. Heterochromatic areas are shown by black regions, intermediate condensation shown by gray regions, and euchromatic areas are shown by white regions.

4. Conclusion

The chromosome analysis using CHIAS IV software can be used to predict the chromosome number, chromosome type, relative length, and condensation patterns of four flower variations of *H. rosa-sinensis* (single small red, single small pale red, single big red, and double red). We generate and describe quantitative karyotypes for four variations of *H. rosa-sinensis* using CHIAS IV. Our data support the evidence that these flower variations exhibited polyploidy. The smallest chromosome number is found in single small pale red flower type, while the biggest chromosome number is found in single big red flower type. Chromosome type of *H. rosa-sinensis* vary from submetacentric, metacentric, to subtelocentric. We also revealed condensation patterns of each chromosome from these flower variations. Condensation level in all chromosomes varies from light, intermediate, to heavily condensed.

References

[1] Khristi V, Patel V H 2016 Therapeutic potential of *Hibiscus rosa-sinensis*: A review *Int. J. Nutr. Dietetics* 4 2 105-123.

[2] Maganha E G, Halmenschlager R C, Rosa R M, Henriques J A P, Ramos A L L P, Saffi J 2010 Pharmacological evidences for the extracts and secondary metabolites from plants of the genus *Hibiscus* *Food Chem.* 118 1-10.

[3] Slamet A 2018 The diversity of *Hibiscus rosa-sinensis* based on morphological approach *Scientiae Educatiae: Jurnal Pendidikan Sains* 7 1 33-41.

[4] Beers L, Howie J 1990 *Growing Hibiscus* (Hong Kong: G.T Setters Pty Limited) pp. 67.

[5] MacIntyre J P, Lacroix C R 1996 Comparative development of perianth and androecial primordia of the single flower and homeotic double-flowered mutant in *Hibiscus rosa-sinensis* (Malvaceae) *Can. J. Bot.* 74 1 1871-1882.

[6] Kachecheba J L 1972 The cytotaxonomy of some species of *Hibiscus* *Kew Bull.* 27 3 425-433.

[7] Singh F, Khoshoo T N 1970 Chromosomal polymorphism within the *Hibiscus rosa-sinensis* complex *Caryologia* 23 1 19-27.

[8] Abramoff M D, Magalhaes P J, Ram S J 2004 Image processing with Image *J Biophotonics Int.* 11 7 36-42.

[9] Kato S, Ohmido N, Hara M, Kataoka R, Fukui K 2009 Image analysis of small plant chromosomes by using an improved system, CHIAS IV *Chromosome Sci.* 12 43-50.

[10] Furukawa K, Sugiyama S, Ohta T, Ohmido N 2017 Chromosome analysis of tea plant (*Camellia sinensis*) and ornamental camellia (*Camellia japonica*) *Chromosome Sci.* 20 9-15.

[11] Young H A, Sarath G, Tobias C M 2012 Karyotype variation in indicative of subgenomic and ecotypic differentiation in switchgrass *BMC Plant Biol.* 12 117 1-12.

[12] Darlington C D, LaCour L F 1966 *The Handling of Chromosomes* (London: George and Unwin) pp. 166.

[13] Kato S, Fukui K 1998 Condensation pattern (CP) analysis of plant chromosomes by an improved
chromosome image analysing system, CHIAS III *Chromosome Res.* **6** 473-479.

[14] Levan A, Fredga K, Sandberg A A 1964 Nomenclature for centromeric position on chromosomes *Hereditas* **52** 201-220.

[15] Song J, Zhuang D 2001 Chromosome number and ploidy of several plants in *Hibiscus* *L.* *J. Trop. Subtrop. Bot.* **9** 3 213-216.

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