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Research Article

IDENTIFICATION OF BAWANG SABRANG (*Eleutherine americana* Merr. ex K. Heyne) IN INDONESIA BASED ON CHROMOSOME CHARACTERS

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ABSTRACT

Bawang Sabrang (*Eleutherine americana* Merr. ex K. Heyne) is a plant belongs to Iris family (Iridaceae). Genetic study of the Eleutherine species should be investigated to yield valuable information for breeding program. The aim of this research was to determine chromosome characters as a preliminary research on the genetic characterization of Bawang Sabrang. Squash method on the root tips was used for chromosome preparation of this plant. The results showed that the time of cell division and prometaphase stages of Bawang Sabrang were occurred at about 08.00-08.30 a.m. and 08.20 a.m., respectively. Chromosome number of Bawang Sabrang was 2n=12 and the karyotype consisted of 8 (4 pairs) of metacentric chromosomes, 2 (1 pair) submetacentric chromosomes and 2 (1 pair) subtelocentric chromosomes which have the longest of total length chromosomes. Therefore, the karyotype formula of Bawang Sabrang was $2n=12=8m+2sm+2st^{SAT}$. Besides that, on the pair of subtelocentric chromosomes there was a satellite at each of the chromosome. Analysis of chromosome characters exhibited that the long of total length chromosomes was about 1.687 ± 0.111 µm to 5.320 ± 0.716 µm. Based on the R value (3,65 ± 0,41), it revealed that there was variation of chromosome size on this Eleutherine species in Indonesia. Moreover, data of the chromosome characters is important to complete the database of *Bawang Sabrang* as a potential medicinal herb in Indonesia

Keywords: Bawang Sabrang, *Eleutherine americana*, chromosome, karyotype

INTRODUCTION

Iridaceae is one of family which most of the members are perennial herbs. One of its member, which is neither well known nor beneficial understand yet but has been examined is from the genus *Eleutherine*. According to Govaerts (2006), this genus consists of 13 species. Most researchers examine more Eleutherine species in the southern America and southern Africa. This is because they are scattered in these areas. However, Goldblatt (1991) reported that the origin habitat of this plant is still unknown. One of its members growing in Indonesia, especially in Borneo and Java is *Eleutherine americana* Merr. ex K. Heyne. Currently, it is said that *Eleutherine bulbosa* is the revised name of *Eleutherine americana* Merr. ex K. Heyne (personal discussion with Peter Goldblatt, Ph.D.). This plant has a quite a lot of local names; for example in Kalimantan it is known as *bawang Sabrang* or *bawang Tiwai* (Heyne, 1988).

Morphology of this plant has bright red underground storage organs of bulb like onion. This plant also has a pseudo-trunked, green sword-shaped leaves that overlap each other transversely at the base which the apical and the base are pointed. Its inflorescence is definite composite; rhipidia shaped, white color, and have 6 petaloid tepals which are arranged in a double circle.

Bawang Sabrang is a wild plant, but in Java it is being kept as ornamental plants, cultivated, and naturalized. *Bawang Sabrang* can grow well in the altitude of 600 to 1500m above sea level, in the cool and cold areas as in mountains. This plant is vegetative reproductive using bulbs. Although it can grow

well in Indonesia, however cultivation of *Bawang Sabrang* is still low. Cultivation is generally done in the household scale, meanwhile it has many benefits. Therefore, to improve the cultivation and also to preserve the germ plasma of the plant in both quantity and quality, conservation efforts are required. One of the initial steps that can be done to realized it by knowing its genetic characterization, because it can be further known its genetic identity.

Research on the molecular and cytology of this plant has been done, but not yet in Indonesia. One of the research on the cytology *Bawang Sabrang* which was done by Goldblatt and Snow (1991), states that the number of chromosome *Eleutherine americana* (syn. *Eleutherine bulbosa*) is 2n = 12. Moreover, research on the chemical constituents from *Eleutherine Americana* in China has resulted 9 compounds (Liu *et al*., 2009). It was reported that naphthoquinones, anthraquinones and naphthalene was isolated from the Bulbs of the plant (Mahabusarakam, 2010). Whereas, an active compound from the bulb of *Eleutherine americana* L. Merr. collected from East Kalimantan, Indonesia, was tested for its antidermatophyte and antimelanogenesis activity (Kusuma *et al.,* 2010). Therefore, the object used of this research were to study the mitotic time, the number of chromosome of *Bawang Sabrang*, and the chromosome characters that includes the shape and size of the chromosome. Data of the chromosome characters is expected to study a potential of *Bawang Sabrang* as a medicinal herb in Indonesia.

METHODOLOGY

Materials

Plant used in this research was *Bawang Sabrang* plants (*Eleutherine americana* Merr. ex K. Heyne) collected from Lambungmangkurat University, South Kalimantan and herbal nursery in Yogyakarta, Indonesia.

Method

Preparation of chromosome was using squash method on the plant root-tip (Jahier *et al*., 1996). It was conducted at 08.00-14.00 WIB, started with 30 minutes time interval to determine the active mitotic time. Stages preparation chromosome is as follows:
Sample Preparation: *Bawang Sabrang* obtained from Pontianak, West Kalimantan. First, the bulb of *bawang Sabrang* soaked in a Petri dish fill with aquadest to germinate the root. Roots will grow ± 3 days after soaking and will be able to use in sample preparation; Root cutting: Pieces are taken from the root tip of the meristem region, which is ± 3 mm from the root at the estimated mitotic time. At this research, pretreatment were not been done because it didn't show much different; Fixation Root: tip is inserted to the bottle flacon that has been filled with glacial acetic acid 45% (45ml glacial acetic acid added with 55 ml aquadest). Then the root tip of the snippet is stored at a temperature of 4°C for 15 minutes. After that, the root-tips washed by aquadest; Hydrolysis by HCl : After fixation, the root-tips hydrolyzed by chloride acid (HCL) 1N (1 ml chloride acid plus 11 ml aquadest) and then than inserted in the incubator at a temperature of 55 ° C for 5 minutes. After that, the root-tips washed by aquadest.; Tint : After fixation and hydrolysis, the root-tips cleaned and colored using aceto-orcein 1% (1gr orcein synthetic acid in 100 ml acetate 45%) and stored at room temperature for 24 hours.; Squashing: Root- tips were placed in a colored glass objects. Aceto-orcein the excess around the ends of the root-tips cleaned with clean tissue paper to avoid the preparation for the dirty. Then the root-tips dropped by glycerol and covered with glass cover: Labeling: To seal the glass cover, use paint nail clear glass cover on the sidewalk. The sample is stored in the box preparation and placed in the refrigerator at a temperature of 4º C until observed; Observation by Light Microscope : Observation of mitotic phases and chromosomes number of *bawang Sabrang* are using a light microscope. After getting a good preparation, picture were taken by digital camera and microphotograph camera.

Data Analyze

Chromosome is calculated at the time of observation and the results of the images have formatted in digital. The number of chromosome is calculated from the root of three different plants of *Bawang Sabrang*, each with two or three recycling cell that is on a

prometaphase. Measurement the length of both chromosomes short arm (p) and long arm (q) using a computer program, AutoCAD Map 2000i. Measurement of Centromere Index was obtained to determine chromosomes form. The making of Ideogram is done by Corel Draw X3 based on the short arm length (p) and of long arm length (q) chromosome. To create a karyotype is done by cropping chromosome images with Magnetic lasso tool in the application program, Adobe Photoshop CS2.

RESULTS AND DISCUSSION

Bawang Sabrang usually forms a large clump. It is originally wild plants but can also be kept as ornamental plants and medicinal plants in the house yard. Beside it can grow well in cool and cold region, *Bawang Sabrang* also has capability to grow in hot areas such as in urban areas.

Mitotic Time and Cell Cycle

On this research, chromosome preparation and cell cycle observations were done between 08.00am-14.00pm with 30 minutes interval. Based on table I, it can be seen that between 08.00am-14.00pm all mitotic phases are clearly seen. Therefore, the chromosome preparation next step is being conducted at prometaphase time (08.00-08.30am) within a 5-minutes interval (Table II).

Based on table II, the highest percentage of prometaphase occurrence was at 08.20am. Cell cycle consists of two phases; they are interphase and mitotic phase. At the interphase, the cell nucleus appears as a large orb in the middle of a red and nucleolus located in it. Chromosome is not clearly seen because it is still in the form of fine-thread chromatins that can only absorb a bit dye.

After passing through interphase, cells enter mitotic cleavage. This phase consists of prophase, prometaphase, metaphase, anaphase and telophase. In the prophase, the chromatin was started to form a chromosome mass, but it is still unpacked well and isn't tight. After entering the late prophase, chromosomes are started to form a sister chromatids and look more solid. The core membrane and nucleolus in this phase is already being disappeared.

The next phase is prometaphase where the chromosomes movement occurs. The chromosomes move toward the metaphase plate or division area. This phase takes place quickly so that it is become the most difficult phase found during the observation. At this phase the form of the chromosomes are most excellent, in the form of sister chromatid and scattered in the cells, so this is most appropriate time to observe the chromosomes morphology, amount and size.

The further phase is the metaphase. At this phase the chromosomes has been on metaphase plate in equator region, so that the chromosomes appears in a line and gather in the middle of the cell. Position of the homolog chromosome in metaphase plate is random. At the end of this phase, the spindle thread made on prophase will be attached to the centromere.

At the next stage, chromosomes centromere that has been attached by the spindle thread will split along the chromosome arms and vertically toward the threads spindle, so that each similar chromatid will be separated. Then both of the similar chromatids will be drawn towards the pole by the opposite thread spindle. At this phase, the quantitative (number of chromosomes) and qualitative (genetic information) are equally divided. This phase is called the anaphase.

Then the cells entered the last phase of mitosis, namely telophase. At this phase each separated chromatids will put themselves in cells pole. After that there will be a sister chromatids fusion, spindle thread disappears, and the core membrane of nucleolus will be formed again. This phase ended with the new cell wall in the middle of the cell made from the middle to the edge cells. This process is called cytokines which will produce two new cells that has the same chromosome amount and genetic information with the same parent cell. Cells will then enter the next interphase to conduct the next cell division.

Chromosome Characters

Number of chromosome: Based on the observation results and calculation of the number of chromosomes in prometaphase, it is known that the amount of *Bawang Sabrang* chromosome is 2n = 12 (Table III and Figure 1). This is consistent with the results of the research conducted by Goldblatt and Snow (1991). Besides the information that *Bawang*

Table I. The percentage of cells in each phase of cell division of *Bawang Sabrang* root-tip within the 30 minutes intervals

Time	Number of cells observed	Number of cells in mitotic phase and interphase (%)				
		prophase	metaphase	anaphase	telophase	interphase
8:00	90	43.33	6.67	6.67	13.33	30.00
8:30	90	42.22	11.11	11.11	12.22	23.33
9:00	90	25.56	7.78	8.89	7.78	50.00
9:30	90	38.89	2.22	6.67	12.22	40.00
10:00	90	31.11	8.89	8.89	13.33	37.78
10:30	90	23.33	8.89	17.78	12.22	38.89
11:00	90	23.33	14.44	3.33	13.33	45.56
11:30	90	42.22	4.44	2.22	14.44	36.67
12:00	90	33.33	6.67	5.56	15.56	38.89
12:30	90	27.78	2.22	10.00	6.67	53.33
13:00	90	38.89	5.56	6.67	11.11	37.78
13:30	90	25.56	4.44	3.33	12.22	32.22
14:00	90	30.00	6.67	2.22	8.89	52.22

Table II. The percentage of cells in each phase of cell division of *bawang Sabrang* root-tip within the 5 minutes intervals

Time	Number of cells observed	Number of cells in mitotic phase (%)					
		Pro-phase	Prometa-phase	Meta-phase	Ana-phase	Telo-phase	Inter-phase
8:05	90	43.33	10.00	5.56	4.44	5.56	31.11
8:10	90	44.44	4.44	6.67	7.78	11.11	25.56
8:15	90	50.00	8.89	2.22	7.78	3.33	24.44
8:20	90	45.56	**12.22**	8.89	3.33	7.78	22.22
8:25	90	38.89	10.00	6.67	6.67	10.00	27.78

Sabrang has 2n = 12 chromosomes, it is also known that the chromosome *bawang Sabrang* has typically a pair of longest chromosome (number 3 and 8), which in each of the telomere of the short arm has long satellites (Figure 1 and 2). Both satellites have a different length one another.

Chromosome size: Measurement of the size of *bawang Sabrang* chromosome are including the length of chromosome arm (p), chromosome long arm (q), absolute length of chromosome (p + q) and the value of chromosome Centromere Index (CI) can be seen in Appendix 1. Bawang Sabrang chromosome size obtained from the average size of chromosome five recycling plants from three different *bawang Sabrang*. It can be known that *bawang Sabrang* chromosome have varied sizes. The length of short arm of *bawang Sabrang* chromosome ranged from 0,780 ± 0,051μm to 1,345 ± 0,254μm. While the long arm chromosome length ranged from 0,907 ± 0,060μm to 4,320 ± 0,668μm. Absolute length of *bawang Sabrang* chromosome ranged between 1,687 ± 0,111μm to 5,320 ± 0,716μm (Table III). Data from the size of chromosome

Table III. Average size of chromosome, centromere value index and the form of chromosome *bawang Sabrang*

Number of chromosome pair	Chromosome arm lenght (µm)			Centromere Index (CI)	Chromosome Shape
	Short arm	Long arm	Absolute length		
1	1.000 ± 0.048	4.320 ± 0.668	5.320 ± 0.716	18.910 ± 2.088	Subtelocentric
2	1.145 ± 0.131	2.585 ± 0.472	3.730 ± 0.603	30.863 ± 1.794	Submetacentric
3	1.345 ± 0.254	1.593 ± 0.107	2.938 ± 0.360	45.674 ± 2.906	Metacentric
4	1.025 ± 0.123	1.241 ± 0.141	2.265 ± 0.264	45.242 ± 0.008	Metacentric
5	0.909 ± 0.077	1.029 ± 0.060	1.938 ± 0.137	46.838 ± 0.783	Metacentric
6	0.780 ± 0.051	0.907 ± 0.060	1.687 ± 0.111	46.417 ± 0.187	Metacentric

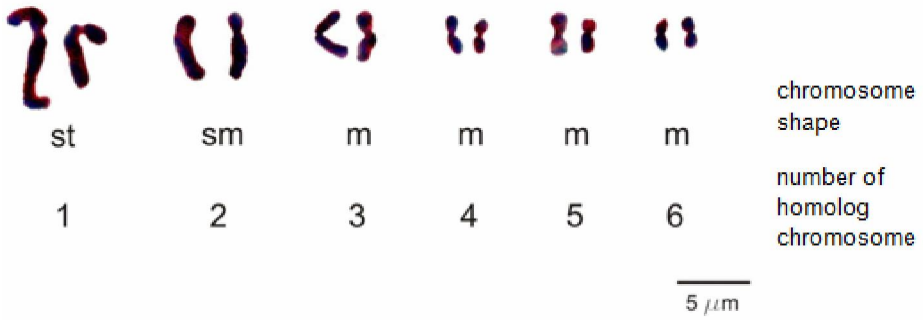

Figure 1. Karyogram of *bawang Sabrang* chromosome
Description:
karyotype formula: 2n = 12 = 8m +2 sm +2 st^{SAT} (m: metasentris, sm: submetasentris, st: subtelosentris, SAT: satellite chromosome)

pair in accordance with the longest chromosome obtained by Goldblatt & Snow (1991), which states that the size were ranged from 3.5-6µm.

Centromere index (CI) value and the form of centromere : Centromere Index (CI) Value is the value of centromere position on the chromosome arm that were obtain by comparing the length of chromosome long arm with the absolute length of the chromosome. Centromere Index (CI) Value can be used to determine the shape of a chromosome which can be categorized to metacentric, submeta-centric, subtelocentric and telocentric (Levan *et al.*, 1964).

Based on the results of the research shown in table III, it is known that the value of Centromere Index (CI) of *bawang Sabrang* were ranged between 18,910 ± 2088µm with up to 46,838 ± 0783µm. Based on the CI value it can be determined the form of each *bawang Sabrang* chromosome which were 4 pair chromosomes have metacentric shape, 1 pair chromosome have submetacentric shape and 1 pair chromosome have subtelocentric shape (Figure 1). Those metacentric chromosomes were chromosome pair number 3, 4, 5 and 6. Chromosome pair with submetacentric shape was chromosome pair number 2, while the chromosome pair with subtelocentric shape

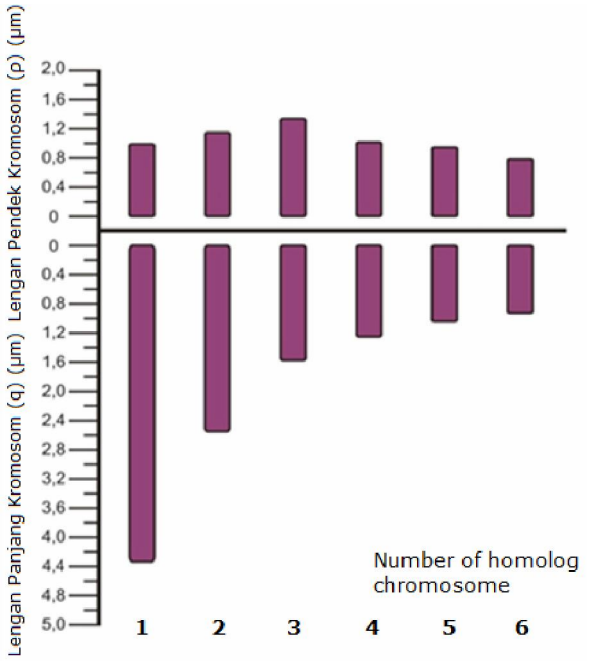

Figure 2. Idiogram of *Bawang Sabrang*

was chromosome pair number 1 which was also as the longest chromosome (Table III). This result is consistent with Goldblatt and Snow (1991) research that states that the longest chromosome pair was subtelocentric. Special to the chromosome pair number 1, each satellite of this chromosome was long enough at the end of the short arm (Figure 1). Satellite is a segment at the end of a telomere which has varying size, separated from the body or the chromosome arm and connected by a filament that does not contain DNA. The connective filament will then form a secondary constriction. Both satellites have a different length one another, so that the length between the homolog chromosomes became unequal. Probability of pericentric inversion together with small tandem duplication on the chromosome that has the longest satellite has been stated by Goldblatt & Snow (1991) and Guerra (1988). Results of Goldblatt & Snow (1991) research further explained that the difference in length between the pair on the longest chromosome of *Eleutherine americana* due to the occurrence of pericentric inversion on the chromosome that has the longest satellite. This statement was also supported by Guerra (1988) research that gave additional information that there were a small tandem duplication occurred on the short arm of the chromosome. Basically this chromosome pair is a heteromorphy chromosome; the chromosome that determines male and female sex. Thus the occurrence of pericentric inversion on one of the spouses cause reproductive plants became sterile. The occurrence of pericentric inversion made the reproductive of *bawang Sabrang* become sterile as the aberration occurred on sex determinate chromosome (heteromorphy chromosome). The tandem duplication leads to genetic variation of a species, thus it varied the chromosome size of *Bawang Sabrang*. Based on those data, karyotype formula for *bawang Sabrang* is 2n = 12 = 8m +2 sm +2 st^{SAT}. Karyotype was arranged by sorting the absolute chromosome length from the longest one to the shortest. Each chromosome pair has nearly the same size and form. Based on figure 1, it is known that *bawang Sabrang* chromosome has a special character which is the occurrence of the longest chromosome with its long satellite on its short arm telomere. Based on that karyotype data, we can then make the ideogram. Through the ideogram we can compare each chromosome length from the longest chromo-

some to the shortest one (Figure 2). It is known that *bawang Sabrang* have variation on chromosome absolute length. The shortest chromosome is the chromosome pair number 6 with 1,687μm long, while the longest one is the chromosome pair number 1 with 5,320μm long (Table III).

The ratio between the absolute lengths of the longest chromosome with the shortest chromosome (R): R value is a comparison between the absolute lengths of the longest chromosome with the shortest chromosome. From the R value it can be known the variation on chromosome size within a species; the greater its value, the larger the variation be. Besides, the R value can also indicate interspecies relationship. Previous research postulated that if the R value is greater than 0,25 so it can be used as a basic data for separating plants into two different species. Noguiera *et al*., (1995) separate *Serjania communis* from *S. gracilis* based on its R value which was greater than 0,25. On this research, the R value of *bawang Sabrang* chromosome is 3.65 ± 0.41.

Characters Comparison between *bawang Sabrang* (*Eleutherine americana* Merr. ex K. Heyne) and red onion (*Allium ascalonicum* L.) chromosomes: In Indonesia, *Eleutherine americana* is known as *bawang Sabrang*. The name indicates that the local community considers that bawang Sabrang have close relationship with the other onion family such as the red onion (*Allium ascalonicum*). This is because they have a nearly same bulb shape. However, if we compare their genetic characters, we will consider that both of two plans have a far relationship. It can be seen from their different number and morphology of the chromosomes. From the previous study we know that *A. ascalonicum* has 2n = 16 while this study resulted that *E. americana* has 2n=12 chromosomes. In taxonomical study if the number of chromosome is different, it will then indicate that those plants are come from different families. *Bawang Sabrang* family is Iridaceae, while the onion family is Alliaceae. As additional information, between *A. ascalonicum* and *E. americana* share different karyotype formula; *E. americana* or onion Sabrang karyotype formula is 2n = 12 = 8m +2 sm +2 st^{SAT}, while the *A. ascalonicum* or red onion karyotype formula is 2n = 2x = 16m. Based on the genetic data described before, basically *bawang Sabrang* cannot be referred as the red onion. Therefore, it can be used to revise the local name of *E. americana* since it is not an onion or *bawang*.

Furthermore, in Thailand, the ethanolic extract of *E. americana* demonstrated antibacterial activity against all *Campylobacter* spp. from both human and chicken (Sirirak and Voravuthikunchai, 2010). While, the anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity and the possible mechanism of action of a crude extract from red bulbs of *Eleutherine americana* Merr. was investigated (Ifesan *et al*., 2009a). The antistaphylococcal activity of *Eleutherine americana* Merr. both in vitro and in a food system was also studied (Voravuthikunchai et al., 2008; Ifesan *et al*., 2009b). Crude ethanolic extract from bulbs of *Eleutherine americana* Merr. was subjected to antimicrobial screening including six Gram-positive, seven Gram-negative bacteria, six fungal species and two yeasts (Ifesan *et al*., 2010). Therefore, to improve potential use of Bawang Sabrang for pharmacy in Indonesia, genetic characterization efforts are required.

CONCLUSION

Chromosome number of Bawang Sabrang was 2n=12 and the karyotype consisted of 8 (4 pairs) of metacentric chromosomes, 2 (1 pair) submetacentric chromosomes and 2 (1 pair) subtelocentric chromosomes which have the longest of total length chromosomes. Based on the R value (3,65 ± 0,41), it revealed that there was variation of chromosome size on this Eleutherine species in Indonesia. Moreover, data of the chromosome characters is important to complete the database of *Bawang Sabrang* as a potential medicinal herb in Indonesia

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