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Short Communication

CHROMOSOME CHARACTERIZATION OF THREE VARIETIES OF GINGER (*Zingiber officinale* Rosc.)

Budi Setiadi Daryono[1]*, Siti Nur Azizah Fauziyati Rahma[1], Purnomo[2] dan Sudarsono[3]

[1]Genetics Laboratory, Faculty of Biology Universitas Gadjah Mada Jl. Teknika Selatan-Sekip Utara Yogyakarta 55281
[2]Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan-Sekip Utara Yogyakarta 55281
[3]Departement of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip, Utara Yogyakarta 55281, Indonesia

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*Corresponding author
Budi Setiadi Daryono
E-mail :
bs_daryono@mail.ugm.ac.id

ABSTRACT

Giant ginger (*Zingiber officinale* Rosc. var. *officinale*), red ginger (*Zingiber officinale* Rosc. var. *rubra*) and small ginger (*Zingiber officinale* Rosc. var. *amarum*) are three varieties of *Zingiber officinale* Rosc. They have a lot of benefit and often used by Indonesian as a traditional drug. Moreover, they have a big chance to be use as a flavor in world wide. Therefore, research for their quality, quantity and continuity of supplyare needed. Characterization of their chromosomes is one effort for improving ginger cultivation. The objective of this research was to study mitotic time and chromosome characters of three varieties of ginger. Squashing method was used for chromosome preparation. The results showed that mitotic time of giant ginger is 09.00-10.05 am, red ginger is 09.00-10.30 am, while small ginger is 08.45-11.00 am. Chromosome number of giant ginger and small ginger are 2n=2x=30, while red ginger is 2n=2x=22. Giant ginger has R= 3,109, Red ginger has R = 3,206 and small ginger has R = 4,065. Based on chromosome characters it is revealed that relationship between giant ginger and red ginger is closer that of compare to small ginger. This result is important as basic information for improving the gingers production through breeding program.

Key words: *Zingiber officinale* Rosc., mitotic time, chromosome characterization, *squash* method

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belongs to *Zingiberaceae* is often used in pharmaceutical industry, food and beverage. It has been using for ingredient basic in Indonesian food. In pharmaceutical industry, it has been widely used for treat different ailments (Ali *et al.*,2008). Ginger has finger rhizome shape with bulge in the middle (Hamiudin, 2007). There are three well-known varieties of ginger from Indonesia, which are both national and international interest. They are red ginger (*Zingiber officinale* Rosc. var. *rubra*), small ginger (*Zingiber officinale* Rosc. var. *amarum*) and giant ginger (*Zingiber officinale* Rosc. var. *officinale*). Therefore, advanced research is needed for increasing their quality, quantity and continuity. Characterization chromosome of three varieties of ginger is the first step for increasing quality of ginger. Chromosome has steady characteristic and form during cell cleavage, likewise gene showed similar continuity. In general, individual in one species has similar number of chromosomes, but both chromosome size and shape are different in intraspesific (subspecies, variety and forma) (Singh, 1999). Standard map made from chromosome structure based on shape, size and number is called karyotype. The function of karyotype is to know life divergence, review relationship, analysis evolution process, and detect organism genetic abnormality (Tamarin, 1999). Therefore, advanced research in these three varieties of ginger are very important on genetic characterization for breeding and cultivating ginger plant.

METHODOLOGY

Ginger Collection

Material used in this research are the roots tip of giant ginger, red ginger and small ginger. They were collected from Imogiri, Bantul D.I. Yogyakarta in August and September 2008.

Chromosome preparation

Methods used in this research are *Squash* methods (Jahier and Tanguy, 1996). The roots of ginger were grown in petridish until 0,5 cm and then the tip of root was cut between 08.00-12.00 a.m. The tip of root were fixated with glasial acetic acid 45% (45 mL glacial acetic acid in 55 mL water) at 4°C for 15 minutes and washed with aquades three times. Then, it was maserated with hidrochloride acid 1 N (5 mL concentrated HCl in 55 mL aquades) at 55°C for 15 minutes. It was washed with aquades three times. Aceto orcein 1% (1 gram orcein in 100 mL acetic glacial acid 45%) was used as staining agent at 25°C for 24 hours. After staining 24 hours, it was put into slide glass and dropped with glicerin before covered with cover glass. Footage of the root tip was than squashed with tip of wooden brush until it formed single cell layer.

Analysis data

Fixed slide was observed using light microscopy in Genetics laboratory and photographed in Animal Anatomy laboratory Faculty of Biology UGM. In each interval time of cutting, the tip of root was observed and counted 30 cells in 3 repeated with different ginger plant. Then, stage of cleavage was observed. Chromosome number of three varieties of ginger was counted through the image which taken in Animal Anatomy laboratory Faculty of Biology UGM. Long arm (p) and short arm (q) were measured using software *AutoCAD Map 2000i*. Sentromer Index was counted in determining chromosome form.

RESULTS AND DISCUSSION

Mitotic time and Cell Cycle

Chromosome preparation on Giant ginger, Red ginger and small ginger was conducted at 08.00-12.00 am. Experiment showed that giant ginger, red ginger and small ginger have different mitotic time. The mitotic time for giant ginger is 09.00-10.05 am with prometaphase time approximately at 09.35 am. Red ginger has mitotic time at 09.00-10.30 am with prometaphase at 09.45 am. While, mitotic time for small ginger occurred at 08.45-11.45 am, with prometaphase time at 10.20 am.

Chromosome number of ginger

The results showed that diploid chromosome number on giant ginger (*Zingiber officinale* Rosc. var. *officinale*) and small ginger (*Zingiber officinale* Rosc. var. *amarum*) is similar, 2n=2x=30 (Figure 1 and 2). This research results is differ from previous study which reported that all varieties of ginger (*Zingiber officinale* Roscoe) possessed chromosome number 2n=32 (Etikawati and Setyawan, 2000). On the other hand, diploid chromosome number on red ginger (*Zingiber officinale* Rosc. var. *rubra*) is different, 2n=2x=22 (Figure 3) and it is similar to Thailand ginger as reported by Eksomtramage *et al*. (2002), Saensuk and Saensouk (2004) and (Sanpote, 2004). They reported that diploid chromosome number of ginger (*Zingiber officinale*) is 2n = 22. Moreover, research by Moringa *et al*. (1929) and Sugiura (1936) showed that chromosome number of ginger was 2n = 22. Detailed study that conducted by Raghavan and Venkatasubban (1943) on cytology of the three species (*Zingiber officinale*, *Z. cassumunar*, *Z. zerumbet*) proved that they had somatic chromosome 2n = 22. Based on differences off ideogram morphology, chromosome in *Z. officinale* was different from the other two. Darlington and Ammal (1945) conclude that there was two chromosome B on particular type of ginger as additional on normal from chromosome number 2n=22. B chromosome was predicted as satellite chromosome. Generally, B chromosome have different form and didn`t have any homolog pairs. Chakravorti (1948) reported that diploid chromosome on ginger was 2n = 22. Sharma and Bhattacharya (1959) explained that unevenly spread occurred because of inconsistency chromosome number on several species *Zingiberaceae* including *Z. officinale*.

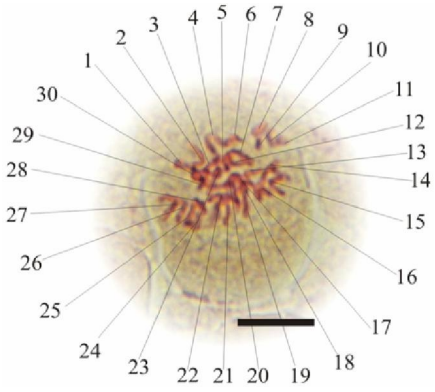

Figure 1. Chromosome number on giant ginger (2n=2x=30) Bar line : 10 μm

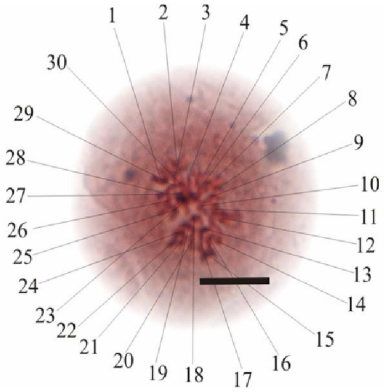

Figure 2. Chromosome number on small ginger (2n=2x=30) Bar line : 10 μm

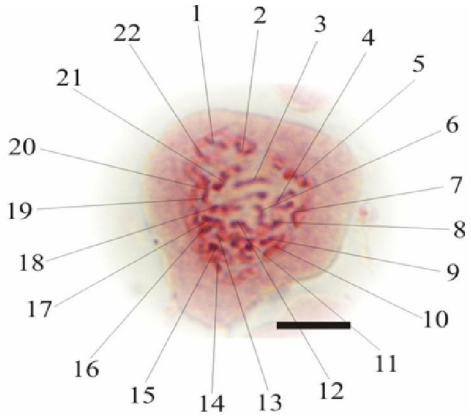

Figure 3. Chromosome number on red ginger (2n=2x=22) Bar line : 10 μm

Ramachandran (1969) study of cytology from *Zingiber macrostachyum, Z. roseum, Z. wightianum, Z. zerumbet, Z. officinale* found that diploid choromosome number for all species were 2n=22. Ratnambal (1979) had been *karyotiping* on 32 cultivar of ginger (*Zingiber officinale*) and found that all somatic chromosome were 2n = 22 (Ravindran and Babu, 2005).

Chromosome number might be used in systematic characteristic. Closest relationship between two plants can be known from similar number of chromosome among them. In the present study, Giant ginger has the closest relationship with small ginger which they posses similar number chromosome (2n=30). Species has constant number of chromosome. Nevertheless varies in number of chromosome might be happened without varies of morphology (Judd *et al*., 1999). According to Sudarnadi (1989), in species, evolution processes have relationship with differentiation on geography, climate, soil or ecological environment. Genetic altering in all native population is caused by habitat changing in genetic processing. Genetic Altering cause altering of morphological structure and physiological process. Genotype of species could be influenced by environment.

Table I. Comparison of Chromosome characters of three varieties of ginger

Characters	Giant ginger	Red ginger	Small ginger
Karyotype formula	2n = 2x = 30 m	2n = 2x = 22 m	2n = 2x = 30 m
Short Arm (μm)	0,477-1,487	0,447-1,447	0,272-1,167
Long Arm (μm)	0,594-1,843	0,574-1,826	0,357-1,391
Absolute length (μm)	1,071-3,330	1,021-3,273	0,629-2,557
Index of Centromer	44,432-46,232	39,046-47,038	38,862-45,882
Ratio of Absolute length (R)	3,109	3,206	4,065

In the present study, we are assumed that environmental altering might influence the possibility of differentiation in number of chromosome. Therefore, genotype variety might be appeared in one species.

Chromosome characterization of giant ginger, red ginger and small ginger

Giant ginger, red ginger and small ginger have un-advanced evolution hence of symmetric chromosome (Table I). High value of R indicates large varies chromosome size. In addition, Differences of R values between varieties of plants are used to describe differences of chromosome character or genetic variety on observed cultivar. According to Singh (1999), $R \leq 0,27$ meaning for reinforcement varieties position. In the present study, varieties of ginger having $R \leq 0,27$ were supposed from similar main species or subspecies. Due from those statement, it was supposed to be that giant ginger and red ginger are product from similar main species. Differences of R value between giant ginger-small ginger are $R \geq 0,27$. Nevertheless, it does not meaning that giant ginger-small ginger come not from one main species.

Pharmacological Activity of Ginger

Ginger is not only use as spice food or beverage addictive but also use in pharmacy as herbal remedy. Ginger rhizome has been long used as medicine in Ayurvedic (Indian), Chinese, Japan and Tibb-Unani (Srivastava *et al*., 1989; Ali *et al*, 2008; Singh *et al*, 2010). Ginger consist of volatile oils approximately 1%-3% of its weight. The main constituent of volatile oil is sesquiterpenoids with zingiberene. The punguency odor and flavour of ginger was caused by gingerols, with other analogues, such as zingerone, shogaols and paradol. It is found at high level in extract rhizome (Singh *et al*., 2010; Rehman *et al*., 2011). The punguency odor in fresh ginger is mainly consist of gingerols, while zingerone, shogaol and paradols (5-deoxygingerols) are the punguency of ginger during thermal degradation (dry ginger) (Jolad *et al*, 2004). Ginger has been known as medicine in the long history. The main pharmacological activity of ginger and its isolated compound includes immune-modulatory, anti-emetic, anti-hyperglycemic, anti-inflammatory, anti-apoptotic, anti-tumori-genic and anti-hyperlipidemic (Rehman *et al*., 2011). It has compound which are responsible in those certain functions. For example, 6-gingerol and 6-shogaol are known to suppress gastric contraction *in situ* (Suekawa *et al*., 1984). 6-gingerol, 8-gingerol, 10-gingerol, and 6-gingerol are reported to be partly responsible for ginger`s anti-emetic properties (Heba *et al*., 2006). Fresh gingers are reported to lower blood pressure through blockade Calcium of voltage-dependent calcium channels (Ghayur and Gilani, 2005). Ginger has been widely used related to treat different ailment in the world.

CONCLUSION

Giant ginger has mitotic time with range at 9.00-10.05 a.m. with prometaphase time at 9.35 a.m. Red ginger has range at 9.00-10.30 a.m. with prometaphase time at 9.45 a.m. While mitotic time in small ginger is at 8.45-11.00 a.m. with prometaphase time at 10.20 a.m.

Chromosome number of giant ginger and small ginger are 2n=2x=30 with karyotype formula 2n=2x=30m, while red ginger is 2n=2x=22 with karyotype formula 2n=2x=22m. R-value on giant ginger is 3,109, red ginger is 3,206, and small ginger is 4,065.

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