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Chromosome Characterization of Festival Strawberry (*Fragaria* x *ananassa* D. var. *Festival*) Result of Polyploidization

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ABSTRACT

Strawberry is fruit commodities which originating from the member of *Rosaceae* and has been widely cultivated in several countries of the world including Indonesia. One of the strawberry cultivars which consumed and cultivate in Indonesia is the strawberry Festival (*Fragaria* x *ananassa* D. var. *Festival*), which was developed in the area of Banyuroto, Magelang. In order to improve the quality and quantity of strawberry plants, polyploidization using colchicine has been employed in previous studies. The objectives of this research were study the chromosome character which include number, size, shape, and karyotype of normal strawberries and treatment strawberries with colchicine (0.05% 36 hours of root and leaf induction, 0.05% 24 hours of root and leaf induction, 0.05% 36 hours of leaf induction, 0.01% 36 hours of root and leaf induction). The results of this research shown that chromosome number difference and degree of ploidy between *Fragaria* x *ananassa* var Chandler in America with *Fragaria* x *ananassa var. Festival* in Indonesia due to natural mutation. While Festival strawberry control and treatment colchicine have same number chromosome is 2n=4x=28M and there are a difference long sleeve and short sleeve chromosome that may affect the content of chromosome expression and phenotype character are produced. The analysis of flow cytometry showed that treatment strawberry with colchicine has two peaks with intensity fluorescence equal to strawberry control so that it can be interpreted that the ploidy number of strawberries treatment with colchicine is tetraploid.

Keywords: chromosomes; colchicine; flow cytometry; karyotype; polyploidization; strawberry Festival

INTRODUCTION

Strawberry is fruit commodities which originating from the member of *Rosaceae* and has been widely cultivated in several countries of the world include Indonesia. One of the strawberry cultivar which consumed and cultivate in Indonesia is the strawberry Festival (*Fragaria* x *ananassa* D. var. *Festival*), which was developed in the area of Banyuroto, Magelang. This cultivar is derived from *Fragaria* x *ananassa* D. species which spontaneous cross between two types of octoploid, *Fragaria chiloensis* L. var. *Duschesne* dan *Fragaria virginiana* var. *Duschesne* (Hummer *et al.*, 2011).

The strawberry production in Indonesia has lower than from America. The first factor, the big threats of pest and disease attacking strawberry plants which can decrease production until 80%. The second factor, seed development is done conventional using stolon. Duplication of stolon resulted seed infected endogenous pathogen which transmitted from parent plant and affected decrease in quality and quantity of fruit production after three periods planting while duplication using seeds require a long time and large fee (Hanif & Ashari, 2012).

One of cultivation technique for repair genetic of strawberry plant is developing and producing polyploidy strawberry plants. The advantages of polyploidy plant are the size of the flower and fruit are larger, the size of leaves are wider, more green color the leaves, and increase resistance against disease (Aversano *et al*., 2012). In field, strawberry plants are polyploidy species with ploidy degree from diploid to decaploid (Nathewet *et al.*, 2009).

Polyploidization techniques are done with colchicine treatment. Colchicine is a white alkaloid derived from *Colchicum autumnale* L. Extract (Suminah *et al.*, 2002). The function of colchicine is duplication the number of chromosomes that used depolymerization microtubules in mitotic division so the spindle is

not formed and inhibited cell division (Dhooghe *et al.*, 2009).

The research prospect of polyploidization using colchicine on strawberries plant through genotype and phenotype characterization is very important. Because it can directly find out genes that encode superior properties and then serve as the protocol to make genetically modified plant of strawberries which have high competitiveness and strengthen the national innovation system. Application of polyploidization technique using colchicine on strawberry plant has yet been studied more depth in Indonesia. Initial research to applying this technique has been reported by Aristya *et al.*, (2015) that chromosome number of Festival strawberry is 2n=4x=28. Optimal concentration polyploidization using colchicine of Festival strawberry in Indonesia is 0.05% with long submergence 36 hours that produces the chromosome number 2n=8x=56 (Laboratorium scale)(Aristya, 2014). This research was used as reference in application of the scale of the field that produces Festival strawberry induction has phenotype character difference with Festival strawberry control on 0.05% and 0.01% concentration with long submergence 36 hours on induction of root, leaf, and induction of root and leaf (Aristya & Daryono, 2014).

The objectives of this research were to study the chromosome character (size, shape, karyotype and flow cytometry) of strawberry Festival of control and result which inducted with colchicine treatment (0.05% 36 hours of root and leaf induction; 0.05% 24 hours of root and leaf induction; 0.05% 36 hours of leaf induction; 0.01% 36 hours of root and leaf induction) in the application of field scale.

MATERIALS AND METHODS

Plant material. Festival strawberry was taken from "Inggit Stroberi" at Magelang on strawberry control and result of induction of concentration 0.01% and 0.05% 36 hours of root and leaf induction, 0.05% 24 hours of root and leaf induction, and 0.05% 36 hours of leaf induction. The Festival strawberry plant of colchicine induction had been planted 6 months before this experiment was conducted. While chromosome preparation experiment had been conducted in Genetic Laboratory, Faculty of Biology, Universitas Gadjah Mada.

Chromosome preparation of Festival strawberry control and result of induction. The tip put in flacon bottle and fixation with glacial acetic acid (AAG) 45% at temperate 4^{o}C for 24 hours. Then rinsed with aquades as much as three times and maceration with hydrochloric acid solution (HCl) 1N at temperate 60^{o}C for 11 minutes. Further rinsed with aquades as much as three times and soaked with AcetoOrcein 1% for 3 hours. This method refers Nathewet *et al.*, (2009) have been modified. Chromosomes are observed with a light microscope with 100x10 magnification on Olympus BX-41 microscope.

Karyotyping. Select the best images of prometaphase then measured and cut by using Image Raster 3. Subsequently size of the data being processed by using the application of Microsoft excel 2007 until produced an ideogram. Karyotyping character i.e short arm of chromosome (p), long arm of chromosome (q), centromere index (IS=p/(p+q)), ratio of long arm of chromosome against short arm of chromosome (RLK=q/p), and chromosome form corresponds to the description of Levan *et al.* (1964).

Flow Cytometry. The method of flow cytometry used refers from Ochatt (2008) which combined with Otto (1990). The festival strawberry young leaf prepared and then cut into small slabs by using a razor blade and placed into petridish contains 500 µl otto buffer 1 (400 µl 0.1 M citric acid monohydrate and 0.5% Tween-20). Then sample was filtered using a nylon mesh sieve 100 µm and centrifuge with speed of 5000 rpm for 10 minutes. The discarded supernatant, and pellet added otto buffer II and 500 µl PI (Propidium Iodide) and then incubated for 20 minutes in room temperature and the dark room. Then analyzed sample using flow cytometry machine Apple which has been connected with computer. The results are presented of histogram with reading of result refer to Schepper *et al.* (2001).

RESULT AND DISCUSSION

Based on research, the chromosome number of Festival strawberry control (Figure

1a) is 2n=4x=28. This result isn't in accordance with Owen & Miller (1996) research that *Fragaria* x *ananassa* var. Chandler has chromosome number is 2n=8x=56. *Fragaria moschata* (2n=8x=56) doesn't produce a hybridized when growing up were in natural condition or same region (Nosrati *et al.*, 2011; Scott, 1951). Based on the description of some strawberries species in Banyuroto farm, it didn't affect chromosomal change. The first possibility crossed between two species from different chromosome number is not produce fertile strawberry plant. The second possibility of chromosome number difference between *Fragaria* x *ananassa* var Chandler in America with *Fragaria* x *ananassa var. Festival* in Indonesia is a mutation. Mutation can occur natural and artificial. A natural mutation due to the presence of natural radiation comes from comic rays and radioactive mineral. Mutation is rare in natural. But if it occurs, it can produce a change that will be passed to the next generation. While the artificial mutation is a mutation that was done intentionally by humans to create the diversity of plants that can produce superior crop varieties (Wang *et al*., 2009). But until recently, there hasn't been information or publication in utilization of artificial mutation of Festival strawberry plant in Indonesia.

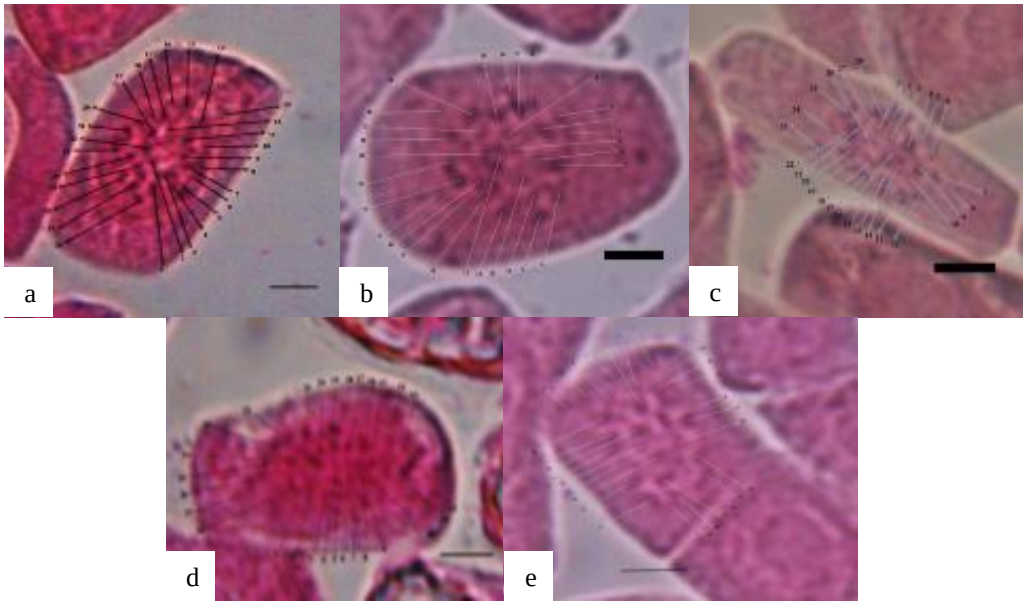

Figure 1. Chromosome number of Festival strawberry: a. control and result of induction with colchicine treatment; b. 0.05% 36 hours root and leaf induction; c. 0.05% 24 hours root and leaf induction; d. 0.05% 36 hours leaf induction; e. 0.01% 36 hours root and leaf induction

Based on chromosome number of all Festival strawberry induction with colchicine is 2n=4x=28 (figure 1b, 1c, 1d, and 1e). These result showed that chromosome number does not occur chromosomal duplication. This possibility caused colchicine has been exhausted and colchicine induction of plants did not work on active mitotic division. Therefore probability of cells are tetraploid or have not chromosomal duplication. Colchicine induction on plants that are undergoing active mitotic division will cause most cells with certain phase are not affected with colchicine (Caperta *et al*., 2006; Dhooghe *et al*., 2011). While induction colchicine on plants that are undergoing interphase will more effectively produce most cells that polyploidy cell after effect colchicine has been exhausted and mitotic division runs normally.

Based on karyotype (Figure 2), karyotype formula of Festival strawberry control is 2n=4x=28m with the range of absolute length chromosome is 1.20-3.31 µm. Karyotype formula of Festival strawberry induction with colchicine Treatment 0.05% 36 hours root and leaf induction is 2n=4x=28m with the range of absolute length chromosome is 1.49-4.64 µm. Karyotype formula of Festival strawberry

induction with colchicine treatment 0.05% 24 hours root and leaf induction is 2n=4x=28m with the range of absolute length chromosome is 1.83-2.97 µm. Karyotype formula of Festival strawberry induction with colchicine treatment 0.05% 36 hours leaf induction is 2n=4x=28m with the range of absolute length chromosome is 1.73-3.78 µm. Karyotype formula of Festival strawberry induction with colchicine treatment 0.01% 36 hours root and leaf induction is2n=4x=28m with the range of absolute length chromosome is 1.56-4.51µm.

Karyotype different become two types i.e. symmetrical karyotype and asymmetrical karyotype. Asymmetrical karyotype considered more advanced than symmetric karyotype (Eroğlu, 2015)(Lavia *et al.*, 2009). Asymmetric karyotype consists of chromosome form which diverse and considered the result of new cultivation. If matched with this result show that karyotype of festival strawberry control or induction has kind of symmetrical karyotype with metacentric chromosome form.

In previous studies reported that there may be difference effect phenotype character of Festival strawberry with treatment colchicine 0.01% 36 hours of root and leaf induction and 0.05% 36 hours of root and leaf induction (Aristya & Daryono, 2014). If the comparison result is associated with long and short sleeve chromosome of Festival strawberry between control and treatment, maybe phenotype character affected by the content chromosome difference.

Flow cytometry is applied method for approximate quantity DNA in nucleus cell and determines ploidy level in the organism (Cires *et al*., 2011). Basic principles of flow cytometry are the analysis of fluorescent microscopy and optical properties from the movement of particles in suspension.Then the suspension will pass flow chamber and radiated light source and produce fluorescent signal that is detected by series of chronic mirrors then photomultiplier will convert electrical signals into digital value and displayed in histogram form (Ochatt, 2008)(Doležel & Bartoš, 2005). Figure 3a show emergence two peak formed as reported by Schepper *et al.* (2001) that can be taken to mean that the first peak is cell in the G1 phase, when analyzed cell is diploid plant (2n=2x) so the first peak show 2C DNA while this sample research is tetraploid plant (2n=4x) which means the first peak show 4C DNA.The reading of the second peak represents cell in G2/M phase of cell cycle. When analyzed cell is diploid plant (2n=2x) thus second peak show 4C DNA while this sample research used is tetraploid plant (2n=8x) which means the second peak show 8C DNA.According to Ochatt (2008), the ploidy position of x along histogram can be used for determination ploidy process of plant cell. Object method for determining range of fluorescent intensity is Festival strawberry control, while for determination phase of Festival strawberry induction based on Festival strawberry control.

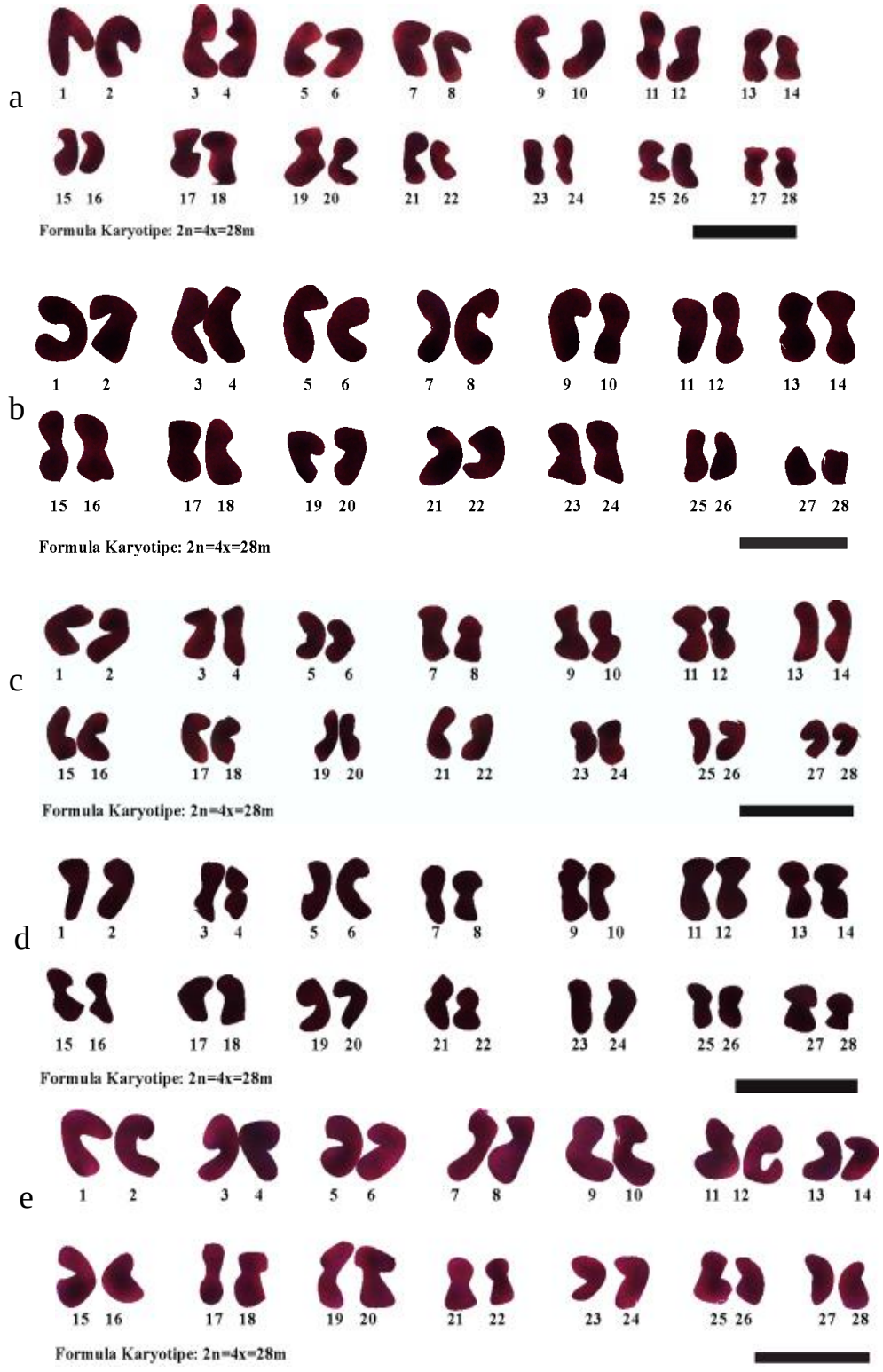

Figure 2. Karyotype of Festival strawberry: a. control and result of induction with colchicine treatment; b. 0.05% 36 hours root and leaf induction; c. 0.05% 24 hours root and leaf induction; d. 0.05% 36 hours leaf induction; e. 0.01% 36 hours root and leaf induction. Bar line = 5 µm

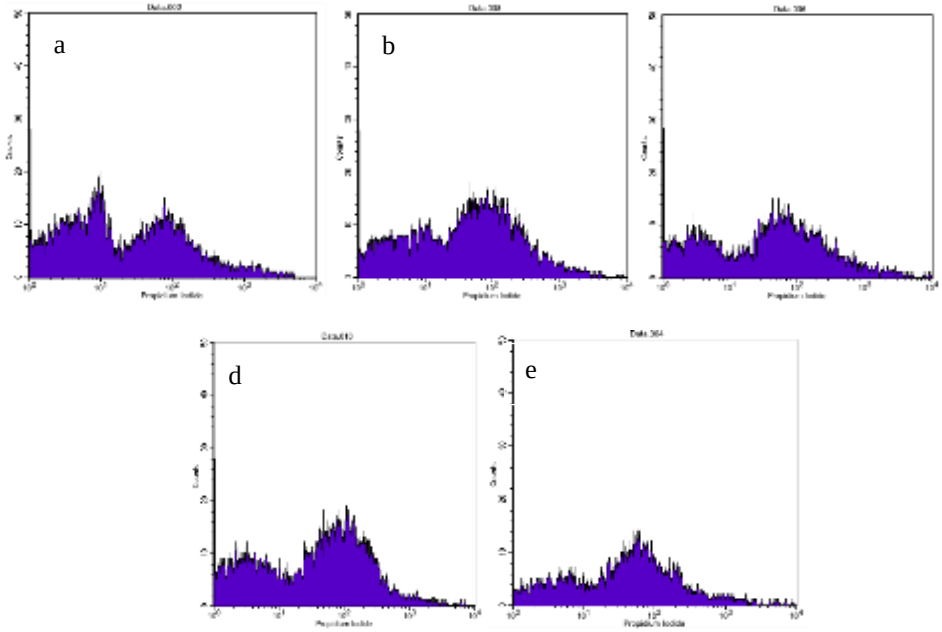

Figure 3. Flow cytometry histogram typical of Festival strawberry: a. control and result of induction with colchicine treatment; b. 0.05% 36 hours root and leaf induction; c. 0.05% 24 hours root and leaf induction; d. 0.05% 36 hours leaf induction; e. 0.01% 36 hours root and leaf induction

In figure 3b-e is flow cytometry histogram typical of Festival strawberry with colchicine treatment, appear two peak with the x-axis position equal the x-axis position of Festival strawberry control that means show fluorescent intensity form of Festival strawberry induction equal Festival strawberry control, so that it can be interpreted that the ploidy number of Festival strawberry induction is tetraploid. According to Ochatt (2008), if leaf isolation comes from the treatment plant with substance mutagen will produce endoreduplication process or mixoploid. If endoreduplication process then the number peak form has three peaks declined and drastic descent. However, if that happens is mixoploid, the number peak form is three peak with description of first peak show 2C DNA of G1 phase. The second peak represents cell in G2/M phase of cell cycle or 4C DNA of G1 phase. The three peaks represent 4C DNA of G2/M of cell cycle.

CONCLUSION

Festival strawberry control and induction have chromosome number 2n=4x=28 while the analysis flow cytometry of Festival strawberry control and induction showed that ploidy degree is tetraploid. Colchicine treatment needs to be done continuously at Festival strawberry for improving quality and quantity of strawberry plant.

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