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Chromosome analysis of cayenne pepper (*Capsicum frustescens* L.) in colchicine induced mutation

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Abstract

The demand for cayenne pepper is constantly increasing with the growth of the food industry, pharmaceuticals, and cosmetics use, which needs to be accompanied with efforts to increase the production. One effort that can be done is to improve the quality of fruit by mutation breeding using colchicine mutagen induction. Colchicines are an alkaloid that affects microtubule synthesis, and cause doubling of the chromosomes number (polyploidy). Therefore this research aimed to detect polyploidy in cayenne pepper plants (*Capsicum frustescens* L.) as a results of mutation induction with colchicine. The seed of three genotypes of local cultivars of cayenne pepper were soaked in colchicine with the concentration of 0.00% (K0), 0.015% (K1) and 0.025% (K2) for 6 hours. Chromosomes were observed in root tip of eight days old seedings using squash technique and stained using acetoorcein. Ploidy analysis showed that genotype III concentration of 0.015% showed 2n lowest percentage at 13%. The concentration of 0.015% on the chili was able to induce triploids at 60%. Besides inducing triploid plants, colchicine was also able to induce polyploidy such as, tetraploid and mixoploid. Colchicine treatment did not significantly affected tetraploid whereaas chromosome mixopolid was significantly affected at the concentration of 0.015%. The results of this study indicated that the treatment of colchicine was able to induce polyploid in cayenne pepper plants. Polyploids found in this study were triploid (2n = 3x = 36), tetraploid (2n = 4x = 48), and mixoploid (2n = 2x = 24, 2n = 3x = 36 and 2n = 4x = 48).

Key words: *Capsicum frutescens* L., colchicine, chromosome, aceto-orcein.

Introduction

Cayenne pepper (*Capsicum frutescens* L.) is a vegetable commodity and its existence can not be abandoned by the people of Indonesia in daily life. Cayenne pepper is used as cooking ingredients, the main ingredient of the sauce industry, chili powder, and also in pharmaceutical industry. Production of cayenne pepper plant is highly important agribusiness around the world, because by planting chilli, farmers could boost employment and incomes (Dias *et al*., 2013). Thus chili is one of vegetable crops with high economic value and has a good prospect for improving the living standards of farmers.

Cayenne pepper belongs to the family of Solanaceae (Supalkova *et al*., 2007). It is the most common species cultivated in Indonesia with production in 2014 reached to 0.800 million tonnes (BPS, 2015). The social demand for cayenne pepper is constantly increasing with the growth of the food industry, pharmaceuticals, and cosmetics use, which needs to be accompanied with efforts to increase the production. One effort that can be done is to improve the quality of fruit by mutation breeding using colchicine mutagen induction.

Colchicine ($C_{22}H_{25}O_6N$) is an alkaloid that is most widely used as an agent to induce polyploidy. Colchicine affects and inhibits the activity of spindle fibers that cause cellular effects such as inhibition of cell division because of lack of segregation of chromosomes and chromosome doubling lead the production of polyploidy plants (Rao *et al*., 1987; Saisingtong *et al*., 1996).

Polyploidy plant is a plant that has three or more sets of chromosomes. Polyploid chromosomes showed more than two paired sets (homologus) of chromosomes (Burns, 1972). Each plant has different range of concentration and treatment time which effectively cause polyploidy. Appropriate concentration of colchicine will increase the number of chromosomes, and produce polyploidy (Suminah *et al*., 2002). Induction of polyploidy also have been done on green beans (Haryanti *et al*., 2009), melon (Anggraito, 2004), tomato (Santosa & Anggorowati, 1993), peanuts (Harahap, 1996), and red chilli (Murni, 2010).

The purpose of this study was to detect polyploidy resulted from colchicine-induced mutation at the concentration of 0.015 % and 0.025 % in three genotypes of cayenne pepper (*Capsicum frutescens* L.) with different character and fruit shapes.

Materials and methods

Treatment with colchicine: Seeds of three genotypes of local cultivar of cayenne pepper (*C. frutescens* L.) namely genotype I, II, and III were collected from the area of Dau District, Malang, East Java, Indonesia. The seeds were soaked in water to select good seeds. The good quality seeds will sink. Seeds then soaked in cochicine solution with a concentration of 0.0% (K0), 0.015% (K1), and 0.025% (K2) for 6 hours. Seeds were germinated on tissue paper, which had been moistened with water.

Chromosome preparation and analysis: Chromosome preparation was performed using the squash method (Evans and Reed, 1981) which included the following steps; root tip tissue of eight days old seedling was excised and fixed at 11:30

a.m. in solution of ethanol and glacial acetic acid at a ratio of 3: 1 for 24 hours. After that, samples were soaked in a solution of 0.002 M hydroxyquinoline for 4 hours. Maceration of the sample was done with a solution of HCl 1 N for 15 minutes at 55 °C. Subsequently samples were soaked in Carnoy solution (absolute ethanol, glacial acetic acid and chloroform) for 30 minutes, then in aceto-orcein staining solution for 16 hours (Harijati *et al*., 2013). The root tip of 3 mm was cut and placed on an object glass and covered with a cover glass, then it was squashed by pressing and flatten the material in object glass. The samples then were observed on light microscope and documented.

Research design and data analysis: This study used completely randomized factorial design of two factors and three replications. The first factor is genotypes of cayenne pepper consisting of genotype I, II, and III. The second factor is colchicine concentration 0.0% (K0), 0.015% (K1), and 0.025% (K2). The data obtained were statistically analyzed using Statistical Product and Service Solutions (SPSS).

Results

Polyploidy on cayenne pepper (*C. frutescens*) plants: Based on microscopic observation of the root tips of cayenne pepper, the controls (K0) and the treatment of colchicine concentrations 0.015% (K1), and 0.025% (K2) showed the existence of diploid, there were chromosome triploid (2n = 3x = 36), tetraploid (2n = 4x = 48) and mixoploid chromosomes (2n = 2x = 24) (2n = 3x = 36); (2n = 2x = 24) (2n = 4x = 48); or (2n = 2x = 24) (2n = 3x = 36) (2n = 4x = 48) (Fig. 1A-D).

The percentage of diploid chromosome of genotypes III was significantly affected by the treatment of colchicine 0.015% wich showed the lowest percentage (13 %) (Fig. 2).

Application of 0.015% colchicine on cayenne pepper genotype III was able to induce maximum percentage of triploids cells (60 %) which was significantly higher than control plant of each genotype, plants treated with 0.015 % in genotype I, and 0.025 % in genotype II. Application of colchicine with a concentration of 0.025 % in genotype II and 0.015 % on the genotype I showed lower percentage of triploid cells (20 %) compared to other treatment (Fig. 3).

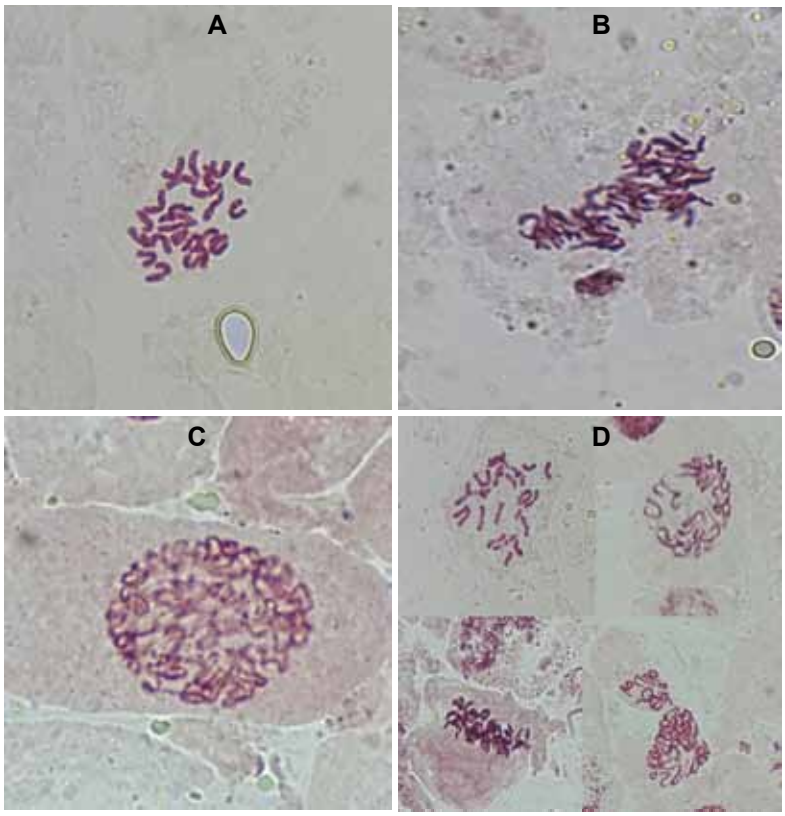

Fig. 1. Cayenne pepper chromosome resulted from mutation induction of colchicine. (A) diploid (2n = 2x = 24), (B) Triploid (2n = 3x = 36), (C) Tetraploid (2n = 4x = 48), and (D) Mixoploid (2n = 2x = 24, 2n = 3x = 36, 2n = 4x = 48).

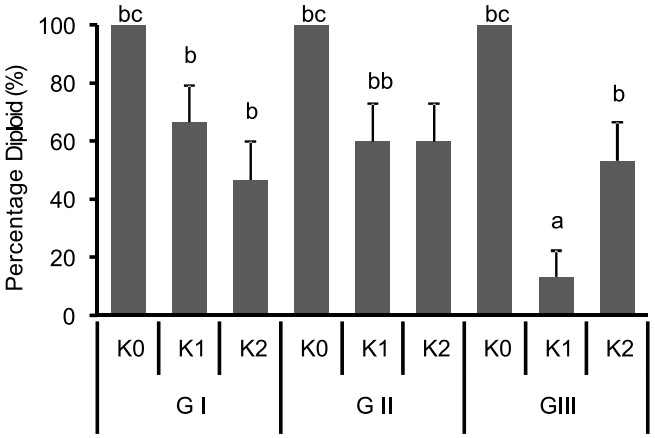

Fig. 2. The percentage of diploid chromosome of cayenne pepper resulted from mutation induction with colchicine. Notes: The same letter for each bar shows no significant difference in the Duncan test (α = 0.05). Control (K0), colchicine concentration 0.015% (K1), colchicine concentration 0.025% (K2), genotype I (GI), genotype II (GII), and genotype III (GIII).

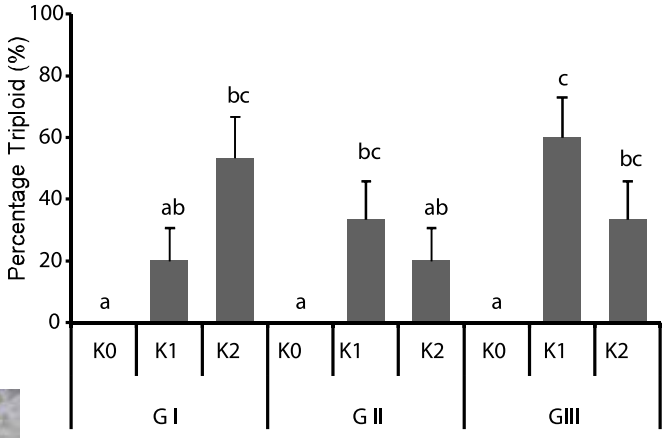

Fig. 3. The percentage of triploid cayenne pepper resulted from mutation induction with colchicine. Notes: The same letter for each bar shows no significant difference in the Duncan test (α = 0.05). Control (K0), colchicine concentration 0.015% (K1), colchicine concentration 0.025% (K2), genotype I (GI), genotype II (GII), and genotype III (GIII).

In addition to the ability to induce triploid plants, colchicine is also able to induce polyploidy such as, tetraploid and mixoploid. However the analysis showed that the percentage of tetraploid chromosomes was not significantly affected by either interaction of two factors or each single factor (Fig. 4).

The application of 0.015% colchicine significantly produced higher percentage of mixoploid by 13% than the control plants, but not significantly different with the plants treated with 0.025% colchicine. The percentage of mixoploid chromosomes was not significantly different in all genotypes used (Fig. 5).

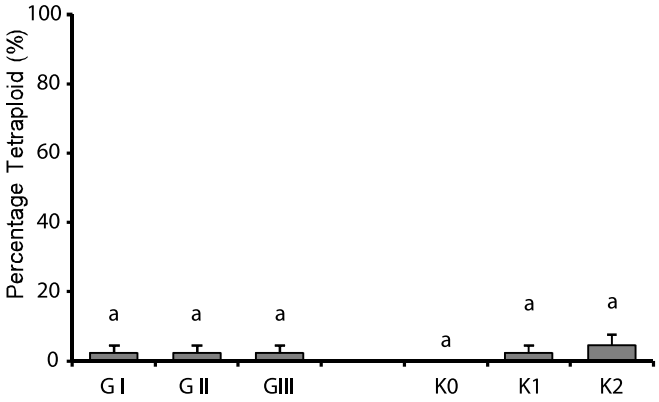

Fig. 4. The percentage of tetraploid cayenne pepper resulted from mutation induction with colchicine. Notes: The same letter for each bar shows no significant difference in the Duncan test (α = 0.05). Control (K0), colchicine concentration 0.015% (K1), colchicine concentration 0.025% (K2), genotype I (GI), genotype II (GII), and genotype III (GIII).

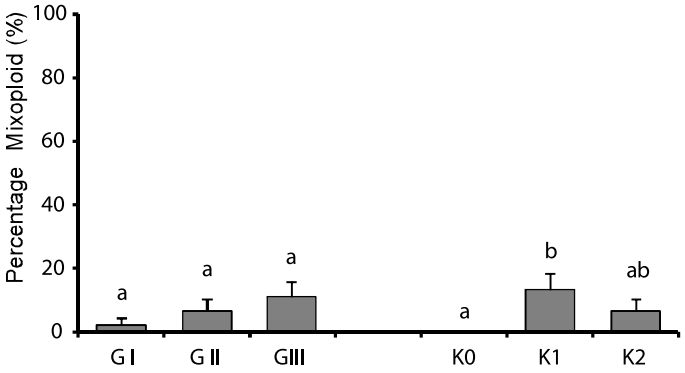

Fig. 5. The percentage of mixoploid cayenne pepper resulted from mutation induction with colchicine. Notes: The same letter for each bar shows no significant difference in the Duncan test (α = 0.05). Control (K0), colchicine concentration 0.015% (K1), colchicine concentration 0.025% (K2), genotype I (GI), genotype II (GII), and genotype III (GIII).

Discussion

The basic chromosome number of cayenne pepper observed in this study was 2n = 2x = 24, as found by Sousa *et al*. (2015) that cayenne pepper has 24 as diploid number of chromosomes (2n = 2x). Based on microscopic observation, application of colchicine with the concentation of 0.015% and 0.025% resulted in the increase of chromosomes number of cayenne pepper to become triploid (2n = 3x = 36). In addition to triploid, colchicine also induce tetraploid (2n = 4x = 48) and mixoploid (2n = 2x = 24) (2n = 3x = 36); (2n = 2x = 24) (2n = 4x = 48); or (2n = 2x = 24) (2n = 3x = 36) (2n = 4x = 48). Polyploid is a process of doubling number of chromosomes to produce a new organism that has doubled sets of chromosomes (2x). In general, normal plant has two pairs of chromosomes called diploid (2n = 2x) in somatic cells, but some plants have more than two pairs of chromosomes as the potatoes which has four pairs chromosome or tetraploid (2n = 4x) and wheat bread (2n = 6x) (Syukur *et al*., 2015).

In this study, cells that did not produce polyploid after the treatment with 0.015 and 0.025% colchicine were also observed. It is caused by the nature of the method of colchicine treatment that induces random mutations, thus providing a different effect on each cell. Some cells were not affected by treatment of colchicine and remained diploid (2n). It was evident that not all the initial chromosome cells induced by mutagens colchicine would have to double, but only partially induced then some cells remain in a state of diploid (Barnabas *et al*., 1999).

Colchicine can work effectively at a concentration range from 0.001 to 1% with 6-72 hours of soaking time. On onion plants (*Allium ascolinum* L.) chromosome number increased to tetraploid, pentaploid, hexaploid, octaploid, and nanoploid after treatment of colchicine for 6 hours (Suminah *et al*., 2002). Similar result was reported by Ariyanto *et al*. (2011) on ginger plant (*Zingiber officinale* Rosc.) that colchicine treatment with the concentration from 0.025 to 0.50% for 3-6 hours lead to doubling the number of chromosomes to form tetraploid, pentaploid, hexaploid, and octaploid.

Colchicine inhibits cell division (Rao *et al*., 1987), by inhibiting the development of microtubule and causes microtubule depolymerization. Colchicine causes discontinuation of the addition of tubulin subunits which merge at the end of the assembly of microtubule polymers. Microtubule depolymerization causes a distrubance in the spindle thread formation which resulted in the separation of chromosomes but the cells fail to produce new cells and cause chromosome doubling (Wiendra *et al*., 2011). Chromosomes can be separated from the centromere through c-anaphase stage with the formation of the core wall and cause chromosome doubling. Induction of polyploidy using mutagen colchicine was also conducted in *Triticum aesativum* (Soriano *et al*., 2007), *Zea mays* (Saisingtong *et al*., 1996) and *Sorghum bicolor* (Ghaffari, 2006).

In this research it was concluded that colchicine was able to induce polyploid in cayenne pepper plants (*C. frutescens*). Polyploid found in this study is a triploid (2n = 3x = 36), tetraploid (2n = 4x = 48), and mixoploid (2n = 2x = 24, 2n = 3x = 36 and 2n = 4x = 48). Colchicine induced mutation with the concentrations of 0.015% tend to increase triploid and mixoploid chromosome cells, but did not significantly increased the percentage of tetraploid cells.

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