

Extract of Dragon Fruit Pulp (*Hylocereus polyrhizus*) Potentially Stain Chromosomes of Red Onion (*Allium ascalonicum*)

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ABSTRACT

Side effect of aceto-orcein as a dye of chromosome structures remains some risks. This dye may remain tissue damage on eyes mucous membrane, burnt by skin contact, severe irritation of respiratory by spray mist. Exploration of natural dye is an alternative solution. Betacyanine of red dragon fruits *Hylocereus polyrhizus* pulp might have a potential role to stain chromosome. Aim of this study is to extract dragon fruit pulp to stain chromosomes of mitotic cells of red onion *Allium ascalonicum*. The method used in this research is qualitative description of application squash method with aceto-orcein as synthetic dye and two ratio of extract ratio of red dragon fruits pulps as natural dye to mitotic cells of red onion roots. The result revealed that 2:1 ratio of red dragon fruits and distilled water could stain chromosomes of red onion somatic cells. Further specific solvent is required to further investigation to get best betacyanine extract of red dragon fruits pulps.

Keywords: betacyanine, chromosome, dragon fruit pulp, natural dye, staining

INTRODUCTION

In plant breeding, study of chromosomes morphology is an important key to identify plant species. Karyotype is a tool to determine plant species by discovery of the specific numbers of chromosomes. Karyotype data will be analyzed quantitatively using multivariate analysis techniques, which provide systematic position of species. Distinguish quantitative traits of chromosomes morphology of subgenus *Vicia* species (Gianfranco *et al.*, 2008; Lysak *et al.*, 2001).

A stain-fixative method for that study of chromosomes structures was found called aceto-orcein. Standard solution of this stain contains 45% of acetic and 1% of orcein. Staining root tip could be used successfully by using aceto-orcein with addition of 1 to 10 mL of HCl solution, and heating of the tissues (Cour, 2009; Maluszynska, 2003).

For plants, a preparation called squash method combined with dye of aceto-orcein could observe chromosome structures (Anjali *et al.*, 2012; Pan *et al.*, 2008). Meanwhile, side effect of aceto-orcein as a dye remains some risks. This dye may remain tissue damage on eyes mucous membrane, burnt by skin contact, severe irritation of respiratory by spray mist

(Elgubbi and Asayh, 2007; MCCSD, 2009). Therefore, safety of researchers who works in the squashing method with aceto-orcein is required an attention.

Exploration of natural dye is an alternative solution. Extraction of plant organ could be the material of natural dye (Elgubbi and Asayh, 2007; Gilbert and Cooke, 2001; Kasiri and Safapour, 2015). Some example of major categories of plant pigments are betalain, anthocyanin, carotenoids, and chlorophylls (Tanaka *et al.*, 2008). *Hylocereus polyrhizus* commonly called dragon fruit or pitaya originated from Latin America is a member of Cactaceae family. Special characteristics of this deep color pulp fruit could be discovered in European, United States market and Asia such as Vietnam, Malaysia, Taiwan, and China. The color pulp is nitrogen-containing pigments (Rebecca *et al.*, 2010). Red dragon pulp contains of betacyanine fractions, which highly reduce radical scavenging capacities (Tenore *et al.*, 2012). This pulp extract exhibits the maximum peak at 230 and 537 nm which indicates presence of betalain compound. The result reveals the compound which responsible for purple appearance is betacyanine (Woo *et al.*, 2011). This betacyanine might have a

potential role to substitute aceto-orcein as synthetic dye. According to study of karyotype, Ramesh (2015) stated that Genus *Allium* is generally interesting karyotypic object for microscopy because of large genomes in medium size of chromosome and polyploidy. Therefore, aim of this study is to extract of dragon fruit pulp (*Hylocereus polyrhizus*) and used it to stain chromosomes of mitotic cells of red onion *Allium ascalonicum*.

METHOD

This research was conducted at Laboratory of Biochemistry, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta. The method used in this research is qualitative description by using squash method with aceto-orcein as synthetic dye and two ratio of natural dye from red dragon fruits pulps.

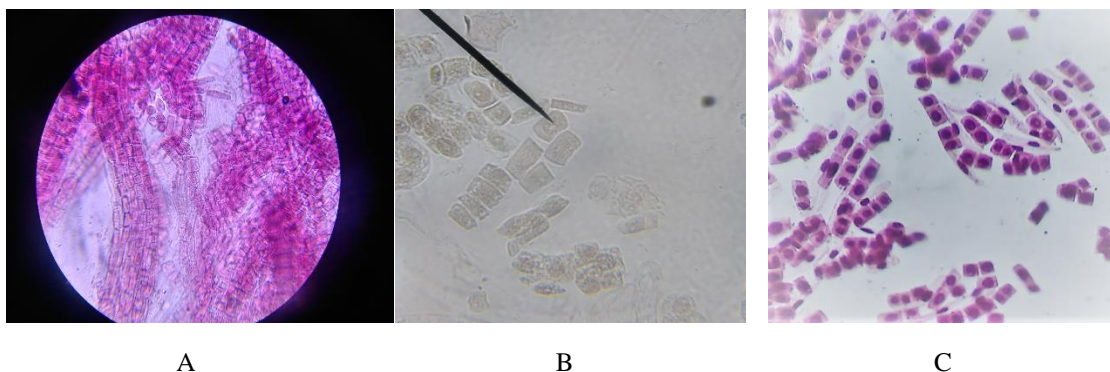
Extraction of dragon fruits pulps.

Dragon fruits (*Hylocereus polyrhizus*) were used for material of extraction when reaching full ripening stage, i.e. 30-35 days after pollination. The pulp was separated from the skin using a stainless, then cut off the pulp into smaller. Direct extraction of pulp was by homogenization using a blender with different volume of distilled water as a solvent. There were 2 ratio of extraction process based on ratio of pulp weight (gr) and volume of ddH₂O (mL). Ratio A was 2:1 (w/v) which ratio of pulp weight (200 gr) and volume of distilled water (100 mL). Ratio B was 1:1 (w/v) which ratio of pulp weight (100 gr) and volume of distilled water (100 mL). Then, extraction result of dragon fruit pulps was filtered using 150 mm qualitative filter paper.

Chromosomes visualization of red onion (*Allium ascalonicum*). Preparation of chromosomes visualization of red onion used Squash method. First, somatic cells of the root were collected by cutting 0.5 cm of root tip to upper roots. This collection was conducted at 08.00 to 12.00 A.M to reach best mitotic time. The roots were fixated in 45% of glacial acetic acid (45 ml glacial acetic acid in 55 ml of distilled water) at 4°C for 15 minutes and washed gently with distilled water in three times. Then, the roots were processed to maceration process in 1 N of Hydrochloride acid (5 ml HCl in 55 ml distilled water) at 55°C for 15 minutes and washed until three times using distilled water. Staining process conducted by using ratio A and B which mentioned previous paragraphs, and positive control of 1% aceto-orcein conducted for 24 hours. Squash method with 1% of aceto-orcein dye was used in this research as a positive control. One percent of aceto-orcein was mixture of 1 gram of orcein in 100 ml of 45% acetic glacial acid. Stained roots were put into slide glass and treated by glycerin before glass covering. Squashing by wooden sticks was conducted on the glass into form of single layer cells. Then, fixed specimens were observed using digital microscopy for reach best visual of chromosomes.

RESULT

In this research, application of natural dye from simple extraction of red dragon fruits peels to somatic cells of red onions roots was done. Two ratios of extracted pulps and control as dyes revealed image of the chromosome staining which presented in Figure 1.



A

B

C

Figure 1. Image of stained chromosome of red onions *Allium ascalonicum* with different ratio of natural dye using 40x magnification: A. Stained chromosome of mitotic cells by squashing method with Ratio A contains 2:1 (w/v); B. Stained chromosome of mitotic cells by squashing method with Ratio B contains 1: 1(w/v); C. Red stained chromosome of the mitotic cells by squashing method with 1 % of aceto-orcein as positive control.

DISCUSSION

Staining chromosomes usually is utilized to make contrast between different cellular components then allow appropriate visualization with different capturing technique. In order to study of banding to chromosome structure, it requires understanding of how stains or dyes bind to the chromosomes. A stain could bind to DNA by intercalation, minor groove or major groove binding or external binding which can either be covalent or noncovalent (Armitage, 2005; Karlsson *et al.*, 2003; Muhlisyah dkk., 2014; Nafisi *et al.*, 2007).

Mechanism of using aceto-orcein dye is interaction process of an acid pH and negative charged groups or possible interact hydrophobically with chromatin. It binds to histon proteins at chromatin and stains its structure. Nucleoli could not be stained with acetic orcein, in meanwhile the chromatins which could be stained at clear area (K *et al.*, 2014).

According to Figure 1, three views reveal three different quality of stained chromosomes in red onions which used 40x of magnification of digital microscopes. Ratio A contains of 2:1 ratio (w/v) of red dragon fruit pulps and distilled water could produce reddish specimen of mitotic cells of *Allium ascalonicum*, although the stained chromosomes is not quite clear to determine the structures and numbers of chromosomes. In meanwhile, ratio B contains of 1:1 ratio (w/v) of red dragon fruit pulps and distilled water could only create different contrast between cells to cells. In meanwhile, the chromosomes clearly can be stained by using 1% aceto-orcein dye as positive control.

Ratio A contains of 2:1 ratio (w/v) of the pulps and distilled water could help creating some dots in the middle cells that nucleus containing chromosomes and some stage of mitotic phase could be found in that microscopic images. This phenomenon might

be happened as binding modes of major groove binding. However, staining of those chromosomes remains blurring images of mitotic cells. Binding modes which depend interaction between stain and Deoxyribonucleic acid (DNA). Major groove binding happens with special characteristics. This binding is formed because of binding modes with the corresponding of hydrogen bonding interaction, the functional group of electronegative atoms such oxygen and nitrogen of the stain responsible for the interaction, and the DNA site where H donor and H acceptor of the base pairs (Armitage, 2005; Maheswari and Palaniandavar, 2004; Yan *et al.*, 2000; Zipper *et al.*, 2004).

In this research, image of Figure 1A might give explanation that functional group of electronegative atoms such nitrogen in the betacyanine of red dragon fruits could interact with the DNA site of chromosomes of *Allium ascalonicum* somatic cells. Based on (Rebecca *et al.*, 2010) explained that betacyanine is nitrogen-containing pigments. This interaction called major groove binding which hydrogen interaction classified as noncovalent binding with DNA site where H donor and H acceptor of the base pairs. The H-donor sites of the base pairs are positions 6 of adenine, 4 of cytosine, and 2 of guanine while the H-acceptor sites are positions 3 and 7 of adenine and guanine, and 2 of thymine (Asensio *et al.*, 2003; Gorb *et al.*, 2004).

In meanwhile, specimen in Figure 1A and 1B could be stained in the surrounding of the nucleus and cell wall. It might because of acid part of red dragon fruits could stain base parts of nucleus and cell wall. Based on Nurwanti dkk. (2013), anthocyanin in the red dragon fruits which has low pH (acid condition) could stain cell walls where in the base condition. Therefore, further extraction by specific solvent for red dragon pulps need to investigate.

CONCLUSION

Extraction of dragon fruit pulp (*Hylocereus polyrhizus*) potentially could stain chromosomes of mitotic cells of red onion *Allium ascalonicum* with ratio A contains 2:1 (w/v) of red dragon fruit pulps (gr) and distilled water (v). Specific solvent of red dragon fruit pulp is required to further investigation to get best betacyanine extract of red dragon fruits pulps.

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