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Genetic diversity of strawberry cultivars in Banyuroto, Magelang, Indonesia based on Cleaved Amplified Polymorphic Sequence

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Abstract. *Arif MF, Aristya GR, Kasiamdari RS. 2019. Genetic diversity of strawberry cultivars in Banyuroto, Magelang, Indonesia based on Cleaved Amplified Polymorphic Sequence. Biodiversitas 20: 1721-1728.* Banyuroto Village, Magelang, Central Java is the center of strawberry (*Fragaria* spp.) development and cultivation program. The mild climate makes the location very suitable for developing sub-tropic horticulture such as strawberry. Various kinds of cultivars have been developed as part of the agrotourism program. However, genetic variation research of those cultivars is still rarely done. Therefore, the molecular study of strawberry was conducted to determine the genetic variation using CAPS markers. DNA of Five strawberry cultivars from Banyuroto and seven strawberry cultivars from Indonesian Citrus and Subtropical Fruits Research Institute, Malang were isolated using the CTAB method. DNA amplification was performed by PCR using four pairs of primers named APx, OLP, F3H2, and CTI2. The amplification results were cut with three kinds of restriction endonuclease enzymes named *Mbo*I, *Mlu*I, and *Taq*I. Restriction product was used to analyze the genetic variation of twelve cultivars and to construct the dendrogram using MVSP software with the UPGMA algorithm. The result showed that the percentage of polymorphic was 45% from 9 polymorphic bands. Dendrogram result showed that there were four clusters. Cluster A consists of Stroberi Hitam, Cluster B consists of Californica Cultivars, cluster C consists of Osso Grande and Osso Purbalingga cultivars, and cluster D consists of Rosalinda, Deeprose, Dorit, Earlibrite, Tristar, Festival, Brastagi, and Aerut. This research concluded that CAPS markers can be used as a method for study genetic diversity of strawberries.

Keywords: CAPS, *Fragaria*, genetic variation, molecular markers, strawberry, restriction enzyme

Abbreviations: CAPS: Cleaved Amplified Polymorphic Sequence, PCR: Polymerase chain reaction, MVSP: MultiVariate Statistical Package, SNP: Single Nucleotide Polymorphisms

INTRODUCTION

Banyuroto Village, Magelang is the center of strawberry development in Central Java, Indonesia (Aristya et al. 2017). It is located on the valley of Merabu, with an altitude of around 1300 m above sea level (Falah et al. 2018). The mild climate makes the location very suitable for developing sub-tropic horticulture such as strawberry. There are several cultivars developed for agrotourism programs. The development is carried out in order to improve the quality of strawberry production which continues to decrease. It is also helpful for improving the living standards of natives there.

According to Suhariyanto (2015) data, Indonesia still needs to import strawberries from other countries because it has not been able to fulfil the market demands that have increased yearly. Strawberries consumed by the public today are cross-products of various kinds of progenitors (Faedi et al. 2002). Each of them has different phenotype characters. Phenotypic variations indicate genetic variation up to the molecular level. Analysis of variations up to the molecular level will help strawberry farmers deal with their agricultural problems. Molecular variation is characterized by the presence of polymorphisms (Qi et al. 2014). The numbers of developed cultivars make the genetic variation of strawberry is an interesting topic to research and to explore. Aristya et al. (2019) has analyzed the genetic diversity of strawberries cultivated in Banyuroto Village, Magelang using RAPD markers. The result showed that there are several polymorphisms which can be detected specifically. However, to improve the result, we still need other research using other molecular markers.

Molecular markers are a technique that can be used to detect genetic variation, genetic inheritance, allele combinations, and genetic polymorphism in a group of plants (Hussain et al. 2015). It also can be used as a tool for phenetic and phylogenetic analysis. There are several kinds of molecular markers that can be applied. Each of the markers has its own advantages and disadvantages (Weising et al. 2005). CAPS has a simple principle (Shavkurov 2015). CAPS is developed from conventional RFLP technique. PCR product from gen of interest is cut using restriction enzyme, then separated using gel agarose (Lu et al. 2010). CAPS is co-dominant, which makes homozygous and heterozygous individuals are easily recognized. This technique provides advantages in genetic studies and a perfect fit as an additional tool for more detailed and accurate analyses (Akbari et al. 2006). Kunihisa et al. (2006) stated that this method provides stable results and highly reproducible especially for

detecting polymorphisms in strawberries.

The primers used in this study were compiled from research conducted by Kunihisa et al. (2005). The primers amplify genes that play an important role for the strawberries. In this study four pairs of primers named APx, OLP, CTI2, and F3H2 were used. Each primer consists of a forward and reverse primer. Therefore, amplified DNA was produced in a specific fragment that encoded a particular gene. The APx primer amplified the gene encoding the ascorbate peroxidase enzyme. The OLP primer amplified the gene encoding osmotin like protein. The CTI2 primer amplified the chitinase enzyme-coding gene. The F3H2 primer amplified the flavanone 3-hydroxylase coding gene. According to Caverzan et al. (2012), ascorbate peroxidase enzyme is an enzyme that catalyzes the conversion of H_2O_2 into H_2O because of its ability to provide electron donors. Plants that experience environmental stress will produce Reactive Oxygen Species (ROS), which can damage their own cells. The presence of ascorbate peroxidase enzyme makes plants able to detoxify H_2O_2 through the cycle of ascorbate-glutathione. Osmotin like protein is produced when plants experience injury, response to ethylene, environmental stress, or defense against pathogens (Zu et al. 1995). Increased of Osmotin Like Protein has been shown to increase resistance against pathogens such as bacteria, fungi, and viruses. The chitinase enzyme is produced by plants as a self-defense response from attacks of fungal and insects. The chitinase enzyme is able to degrade chitin which is the main structure of fungi's cells and insect's cells (Sharma et al. 2011). Flavanone 3-hydroxylase is one of the nuclear enzymes that has the role to catalyze the formation of flavonoid reaction pathways such as naringenin to dehydroflavonol (Britsch and Grisebach 1986). Flavonoids play an important role in plant self-defense systems in the environment.

The purpose of this study is to study the genetic diversity of strawberry cultivars in Banyuroto Village using CAPS molecular markers.

MATERIALS AND METHODS

Study area

This study used twelve strawberry cultivars. Five strawberry cultivars were collected from Banyuroto Village, Magelang, Central Java and seven strawberry cultivars were collected from Indonesian Citrus and Subtropical Fruits Research Institute, Malang, East Java. (Table 1). The fifth young leaf from each cultivar was used to be extracted. There were four pairs of primers used for this study (Table 2). Three kinds of restriction enzyme with different cut-off points were used to cut the amplification product (Table 3).

Procedures

DNA extraction

Samples were stored at -20°C. DNA was extracted from leaves of each cultivar using the modified CTAB method (Doyle and Doyle 1987). 0.1 gram samples were ground using mortar and pestle. Liquid nitrogen was used to make it easier. Ground samples were placed into 1.5 microtube. 0.02 gram of PVP and 700 µL of 2% CTAB (1M Tris-HCl; 0.5M EDTA; 5M HCl; 0,2% β-mercaptoethanol) were added into microtube. Samples were incubated at 65°C for 15 min. 700 µL of chloroform-isoamyl alcohol (24: 1) was added into the mixture, and then all of the samples were homogenized for 30 min. Samples were centrifuged at 13.000 rpm for 10 min. The formed supernatant was moved into the new microtube. Cold isopropanol was added into the supernatant with ratio 1: 1. Samples were precipitated for 60 min. After that, the samples were centrifuged at 13,000 rpm for 10 min. Formed pellets were washed by using ethanol absolute for two times. The washing process was done by centrifuging samples at 13,000 rpm for 10 min. The pellets were dissolved in 50 µL TE buffer solution and stored at -20°C. The modified methods were adding PVP, incubation time and temperature, and centrifugation speed and time.

Table 1. Strawberry cultivars used in the study

Cultivars	Species	Origin of sample
'Californica'	*F. vesca* L.	Banyuroto, Magelang
'Earlybrite'	*F.* × *ananassa* Duch.	Banyuroto, Magelang
'Rosalinda'	*F.* × *ananassa* Duch.	Banyuroto, Magelang
'Tristar'	*F.* × *ananassa* Duch.	Banyuroto, Magelang
Stroberi Hitam	*F. moschata* Duch.	Banyuroto, Magelang
'Aerut'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Berastagi'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Dorit'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Deeprose'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Festival'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Oso Grande'	*F.* × *ananassa* Duch.	ICSFRI, Malang
'Oso Purbalingga'	*F.* × *ananassa* Duch.	ICSFRI, Malang

Table 2. Primers and sequence of primers used in this study (Kunihisa et al. 2005)

Primers		Primer Sequence 5'-3'
CT12	Forward	GTCAAACCTCTCACGAAACCACT
	Reverse	GTTRCTAAGAAAATGAAAGAGCTGATG
OLP	Forward	TGTGTCCAAAACCGATCAGTATTGC
	Reverse	TCTTTCAGAGTGGTACGTACCCC
F3H	Forward	TAATAGGGTCTAGGTGCGTGG
	Reverse	GAGTTCACTACKGCCTGGTGATC
APX	Forward	GTGGTCACACCTTGGTGC
	Reverse	AGTATAATATTTAAGCAGAATGCAGACTTC

Table 3. Restriction Enzymes used and its cut-off point (Kunihisa et al. 2005)

Restriction enzymes	Cut-off point	Incubation temp. (°C)
MboI	5' ↓GATC 3'; 3' CTAG↑5'	37
MluI	5' A↓CGCG T 3'; 3' T GCGC↑A 5'	37
TaqI	5' T↓CG A 3'; 3' A GC↑T 5'	60

Quantitative analysis using spectrophotometry

Each of isolated DNA was analyzed using nanodrop spectrophotometry UV Vis. A total of 2 µL DNA was taken from the microtube. The isolate was dripped into the spectrophotometer (NanoVue Plus). The process was done 3 times for each cultivar for the repetition. Each repetition was taken from a different individual. The average DNA concentration and purity were calculated.

DNA amplification

DNA amplification was performed with four pairs of the primer by PCR (Table 2). Each reaction contained 12,5 µL KAPA hot start ready-mix, 1 µL forward primer, 1 µL reverse primer, 8,5 µL nuclease-free water, and 2 µL DNA template. The template concentration was 10 ng/µL and the primers concentration was 25 µM. PCR was carried out by several processes using thermal cycler (Veriti Thermal Cycler, Applied Biosystems). Pre-denaturation temperature was 95°C for 3 min, denaturation temperature was 95°C for 30 secs, annealing temperature was 54°C-58°C for 30 secs, elongation temperature was 72°C for 1 min, post-elongation temperature was 72°C for 1 min, and hold temperature was 4°C. Denaturation-elongation processes were repeated up to 40 cycles. Amplicons were visualized using electrophoresis with 2% agarose gel.

Digestion of amplicons

Each of amplicons was given restriction enzyme treatment. Each reaction contains 3.5 µL of nuclease-free water, 1 µL of reaction buffer, 5 µL of amplicon, and 0.5 µL of restriction enzyme (4U). Samples were incubated using thermocycler for 120 min with specific temperature (Table 3). Restriction products were separated and visualized using electrophoresis with 2% agarose gel.

Data analysis

The polymorphic percentage was calculated using the band's pattern. Restriction products were converted into a matrix using Microsoft Excel. DNA bands were converted into the binary matrix. Present bands were converted into "1" and absent bands were converted into "0". The dendrogram was constructed with Jaccard's coefficient using the Unweighted Pair-Group Using Arithmetic Average (UPGMA) algorithm on Multi-Variate Statistical Average (MVSP) 3.22 software.

RESULTS AND DISCUSSION

DNA Isolation of Strawberry Cultivars

In this study, the modified CTAB method successfully isolated all 12 cultivars. The isolated DNA was generally large in size. Isolated DNA was larger than 10 kb. This can be seen on the results of agarose gel electrophoresis with 0.8% concentration (Figure 1). Generally, the successfully isolated genomic DNA has not moved far because of its large size. The larger the molecular size, the slower the transfer rate at the agarose. The average concentration and purity of isolated DNA were shown in Table 4. Various level of concentration was detected. The lowest concentration was on 'Tristar' with an average of 174.83 ± 30.15 ng/µL. The highest concentration was on 'Earlybrite' with an average of 1174.33 ± 46.47 ng/µL. The purity result (Table 4) showed that all of the isolated were pure and free from contaminants. All of the DNA purity was near to 1.8.

DNA Amplification of CAPS Markers

We have successfully amplified all of the samples using 4 pairs of primer. The size of amplified fragments was different for each primer (Figure 2; Table 6). The APx primer amplified the target with 567/488 bp length. The OLP primer amplified the target by 520 bp length. The CTI2 primer amplified the target by 312 bp length. The F3H2 primer amplified the target by 390 bp length.

Digestion of amplicons using restriction enzyme

From this study, 4 pairs of primer and 3 restriction enzymes produced in 12 cutting combinations. Not all of the enzymes can cut the amplicons. From the 12 presented cutting combinations, five of them did not produce a cut pattern (Table 6). Each strawberry cultivar also provided a different and unique cutting pattern (Figure 2). Amplicon which was successfully cut will be reduced in size according to its initial size.

Table 4. The quantitative analysis result of DNA from 12 cultivars with 3 repetition

Cultivars	Concentration (ng/µL)	Purity (Å260/Å280)
'Californica'	656.50 ± 85.62	1.98 ± 0.05
'Earlybrite'	1174.33 ± 46.47	1.99 ± 0.02
'Rosalinda'	269.67 ± 51.69	1.83 ± 0.04
'Tristar'	174.83 ± 30.15	1.80 ± 0.05
Stroberi Hitam	526.50 ± 47.56	1.64 ± 0.06
'Aerut'	1157.83 ± 210.16	1.81 ± 0.06
'Berastagi'	589.33 ± 108.44	2.02 ± 0.03
'Dorit'	852.33 ± 8.08	1.75 ± 0.12
'Deeprose'	528.83 ± 199.95	1.83 ± 0.10
'Festival'	838.83 ± 118.74	1.93 ± 0.13
'Oso Grande'	823.50 ± 172.66	1.94 ± 0.04
'Oso Purbalingga'	530.00 ± 130.05	1.88 ± 0.02

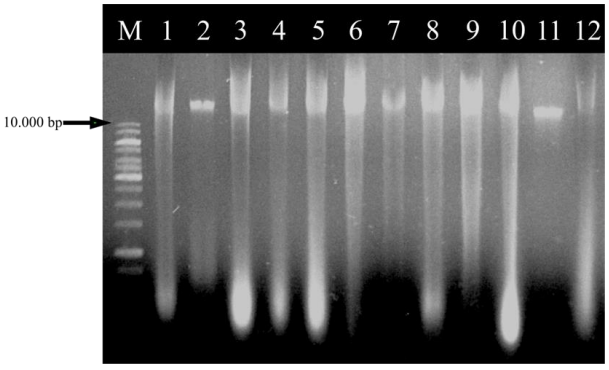

Figure 1. Electrophoresis of DNA Genome of 12 strawberry cultivars. Note: M1: 1 kb marker, 1: Aerut, 2: Brastagi, 3: Californica, 4: Earlibrite, 5: Dorit, 6: Deeprose, 7: Festival, 8: Stroberi Hitam, 9: Rosalinda, 10: Tristar, 11: Osso Purbalingga, 12: Osso Grande

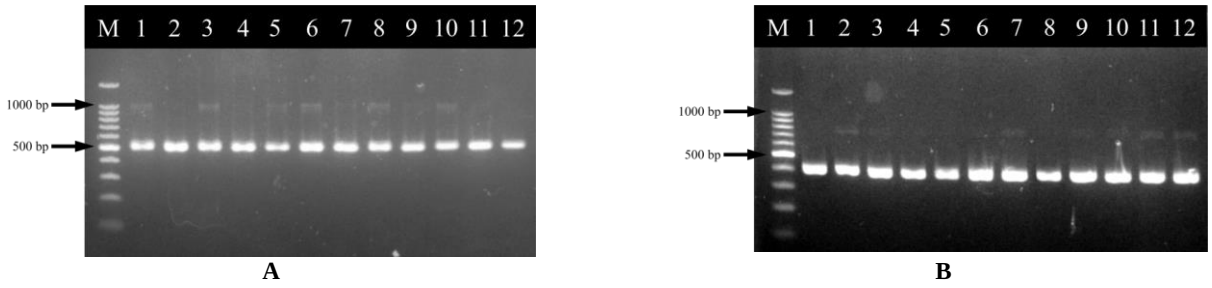

Figure 2. Amplicons of (A) OLP primer and (C) F3H2 primer. M1: 100 bp marker, 1: Aerut, 2: Brastagi, 3: Californica, 4: Earlibrite, 5: Dorit, 6: Deeprose, 7: Festival, 8: Stroberi Hitam, 9: Rosalinda, 10: Tristar, 11: Osso Purbalingga, 12: Osso Grande. Note: Not all of the data are shown

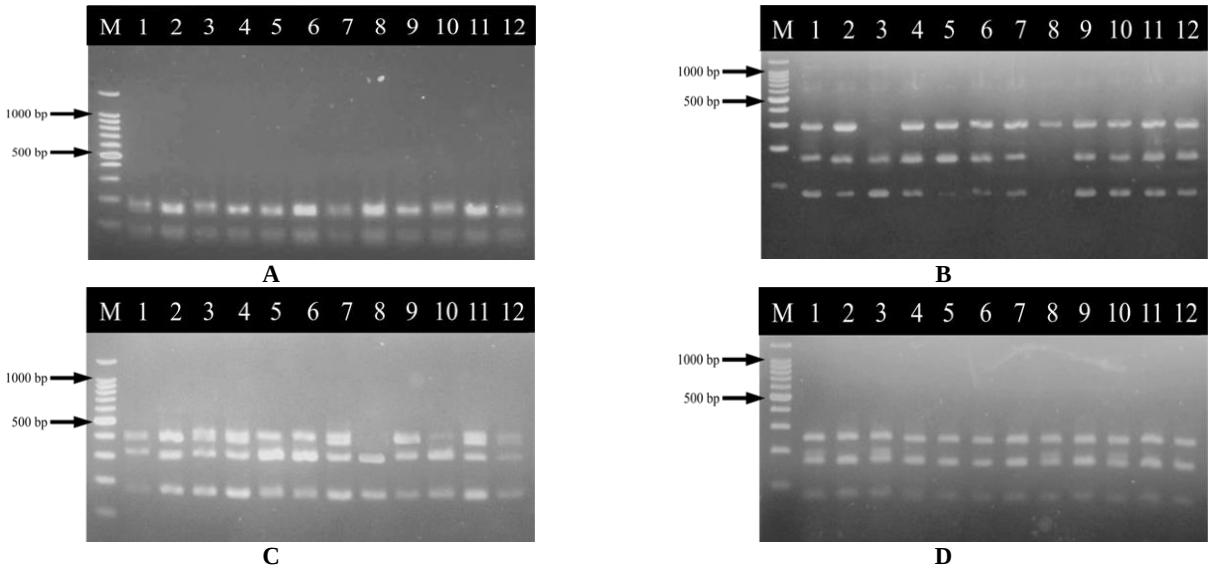

Figure 3. Restriction products of (A) OLP-*Taq*I, (B) CTI2-*Mlu*I, (C) APx-*Taq*I, and (D) OLP-*Mbo*I. M1: 100 bp marker, 1: Aerut, 2: Brastagi, 3: Californica, 4: Earlibrite, 5: Dorit, 6: Deeprose, 7: Festival, 8: Stroberi Hitam, 9: Rosalinda, 10: Tristar, 11: Osso Purbalingga, 12: Osso Grande. Note: Not all of the data are shown

Table 5. Percentage of polymorphism of CAPS markers

Primers-restriction enzyme	Total numbers of DNA bands	Mono-morphic bands	Poly-morphic bands	Percentage of polymorphism
CTI2-*Mlu*l	3	2	1	33 %
F3H2-*Mlu*I	3	0	3	100 %
OLP-*Mbo*I	3	3	0	0 %
APx-*Taq*I	3	2	1	33 %
APx-*Mlu*I	3	2	1	33 %
CTI2-*Mbo*I	3	0	3	100 %
OLP-*Taq*I	2	2	0	0%
Total	20	11	9	45%

Digested amplicons gave a total of 20 DNA bands with different sizes. The total number of the monomorphic band was 11. The total number of the polymorphic band was 9. Based on that result, it can be seen that the CAPS molecular marker gave the polymorphic percentage of 45% (Table 5).

After digested using three kinds of enzyme, each of amplicon were visualized on the agarose gel (Figure 3). Digested amplicons gave different length result from the original amplicon bands (Table 6). The original size of APx amplicon was 567/488 bp. Different cultivar resulting a different amplicon size. After digested using *Taq*I and *Mlu*I enzyme, the band's size was 387/305/152 bp and 567/489/395 bp. The APx amplicons cannot be digested by *Mbo*I enzyme. The original OLP amplicon size was 520 bp. After digested using *Taq*I and *Mbo*I enzyme, it digested into about 156/99 bp and 250/155/82 bp. The OLP amplicons cannot be digested by *Mlu*I enzyme. The CTI2 amplicons original size were 312 bp. After digested using *Mlu*I and *Mbo*I, its size became 312/178/92 bp and 312/170/89 bp respectively. The CTI2 amplicons cannot be digested by *Taq*I enzyme. The original size of F3H2 amplicons were 390. After digested using *Mlu*I enzyme, its size became 384/242/119 bp. The F3H2 amplicons cannot be digested by *Mbo*I and *Mlu*I enzyme.

Table 7. Estimate genotype of each cultivar from the digested pattern

Primer-enzyme	Cultivars											
	Aerut	**Brastagi**	**Californica**	**Earlibrite**	**Dorit**	**Deeprose**	**Festival**	**S. Hitam**	**Rosalinda**	**Tristar**	**Oso Purbalingga**	**Oso Grande**
CTI2-*Mlul*	HR	HR	HM	HR	HR	HR	HR	HM	HR	HR	HR	HR
F3H2-*Mlu*I	HM	HM	HR	HM	HM	HM	HM	HM	HM	HM	HR	HR
OLP-*Mbo*I	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
APx-*Taq*I	HR	HR	HR	HR	HR	HR	HR	HM	HR	HR	HR	HR
APx-*Mlu*I	HR	HR	HM	HM	HM	HM	HR	HM	HM	HR	HM	HM
CTI2-*Mbo*I	HR	HR	HM	HR	HR	HR	HR	HM	HR	HR	HR	HR
OLP-*Taq*I	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM

Note: HR: Heterozygote, HM: Homozygote

Table 6. Restriction products length of CAPS markers

Primer	Restriction enzyme	Amplicon length	Restriction length
	Taq*I*	567/488	387/305/152
APx	*MluI*	567/488	567/489/395
	MboI	567/488	-
	TaqI	520	156/99
OLP	*MluI*	520	-
	MboI	520	250/155/82
	TaqI	312	-
CTI2	*MluI*	312	312/178/92
	MboI	312	312/170/89
	TaqI	390	-
F3H2	*MluI*	390	384/242/119
	MboI	390	-

Note: "-" indicated that the amplicons cannot be cut by the restriction enzymes

Digested patterns can be used as a tool to estimate the type of genotype from each cultivar. If there are two different types of digested patterns resulted from one digestion enzyme, it can be assumed that the cultivar is heterozygous. If there is just one kind of digested pattern resulted from a digestion enzyme, it can be assumed that the cultivar is homozygous (Konieczny and Ausubel 1993). The assumed genotype can be seen in Table 7.

Dendrogram result between 12 strawberry cultivars

Based on the dendrogram result (Figure 4), twelve cultivars of strawberry were grouped into 3 clusters. Cluster I consists of Stroberi Hitam which has 63 % similarity with the other cluster. Cluster II consists of Californica cultivar which has 84 % similarity with cluster III. Cluster III consist of 10 cultivars which are Osso Grande, Osso Purbalingga, Rosalinda, Deeprose, Dorit, Earlibrite, Tristar, Festival, Brastagi, and Aerut. The dendrogram was constructed using Jaccard's coefficient with the UPGMA algorithm.

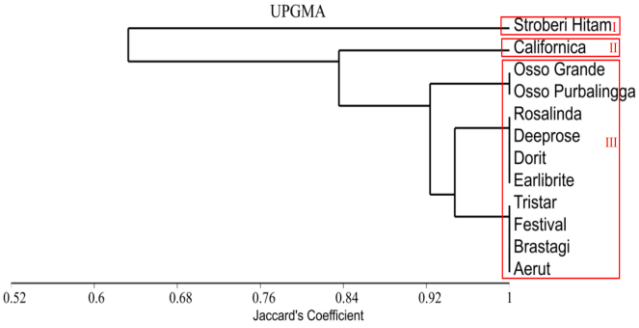

Figure 4. Dendrogram constructed between 12 strawberry cultivars

Discussion

DNA Isolation of 12 Strawberry Cultivars

Leaf was used as the DNA source for extraction in this study. We used young leaves. According to Achakzai et al. (2009) older leaf generally produces higher amounts of polyphenols, tannins, and polysaccharides than the young ones. These compounds reduce the quality of isolated DNA. Polysaccharides tend to bind nucleic acids in low ionic buffers (Wang and Stegemann 2010). This made the isolated pellets thick and textured like glue. If those happened, the pellets would be difficult to pipette and also interfered the activity of *Taq* polymerase enzyme in the PCR reaction (Spangler et al. 2009). Our isolation method used CTAB as the main material for extracting DNA from the cell nucleus. PVP was added which acted as a strong antioxidant that prevented the binding of DNA and polyphenols (Puchooa 2004). β-mercaptoethanol also used as the mixture component of CTAB to prevent the occurrence of polyphenol oxidation and disulfide bonds (Chang et al. 1993). Isopropanol precipitated DNA by the mechanism of dehydration. The water content in DNA can be deposited because the solubility is lowered than isopropanol by pulling out water from the DNA. Therefore, isopropanol made the solubility of DNA was decreased. The formed pellet was stored into Tris-EDTA buffer until ready for amplification.

The quantitative analysis was done using spectrophotometry UV Vis to measure DNA concentration and purity. The DNA and the contaminant molecules absorb different wavelength specifically (Nunes et al. 2011). The wavelength data were converted to measure concentration and purity. Our modification method success isolated DNA with a high amount of concentration and purity (Table 4). This made we must perform DNA dilution using TE buffer for PCR reaction. The average concentration yield was various for each cultivar. The Å260/Å280 ratios were nearly 1.8. That indicated that the isolated DNA was not free from contaminants but still can be used for PCR reaction. The pH of the solution will lead the Å260/Å280 to be different (Wilfinger et al. 1997). The presence of EDTA in the solution of DNA also might interfere with the absorbance in wavelengths of 280nm (Nunes et al. 2011).

DNA Amplification of CAPS Markers

DNA amplification was done by PCR technique. The PCR technique can multiply the specific fragment of nucleic acid by regulating the reaction temperature (Joshi and Deshpande 2010). The double-stranded DNA was denatured into a single-stranded DNA when it was given high-temperature treatment (around 94^0C or more). Right after denatured, the DNA was given a low-temperature treatment (around 37^0C) then the target primer attachment would begin. The *Taq* polymerase enzyme extended the primer sequence attached to the DNA template through 5'-3` direction (Campbell et al. 2008). All of the components for amplification such as *Taq* polymerase enzyme, dNTP, $MgCl_2$, and loading dye were gathered in the PCR kit. This research used 10 ng/µL concentration of DNA template. The concentration level of DNA templates and primers influence the success of the DNA amplification process (Lorenz 2012). Kunihisa et al. (2005) stated that the CAPS marker did not require a high concentration of DNA.

Digestion of amplicons using restriction enzyme

Restriction enzymes have a different cut off point which able to recognize certain sequences to cut. Each restriction enzyme generally has 4 to 6 identification sequences (Roberts et al. 2003). The recognition site of restriction enzymes makes the enzyme only able to cut DNA fragments in certain parts specifically. Restriction enzymes were incubated at 37°C for *Mlu*I and *Mbo*I, while the *Taq*I enzyme was incubated at 60°C. Incubation process was done for 6 hours to complete the restriction process. The difference in incubation temperature is caused due to differences in the origin of the enzyme obtained. The *Taq*I enzyme required a higher incubation temperature because it was obtained from the thermophilic bacteria *Thermus aquaticus* YT I (Sato 1978). The *Mbo*I enzyme was obtained from the *Moraxella bovis* bacteria and the *Mlu*I enzyme was obtained from the *Micrococcus luteus* (Gelinas 1977).

Based on the results obtained in this study (Table 6), not all of the enzymes were able to cut amplified fragments. This can happen because the amplified fragments do not have sequences capable of being recognized by the restriction enzymes. Restriction enzyme works by inspecting the DNA sequence to find its specific recognition sequence. If it cannot find the specific recognition sequence, it will not bind to the DNA and the two strands of the double helix cannot be cut (Buckhout-White et al. 2018). The *Taq*I enzyme was only able to cut APx and OLP amplification fragments. The *Mlu*I enzyme was able to cut APx, CTI2 and F3H2 amplification fragments. The *Mbo*I enzyme only cut OLP and CTI2 amplification fragments. These things ensure that the CAPS marker method is very specific. Therefore, CAPS markers can be used as an alternative for SNP analysis with large sample quantities. The polymorphism can be analyzed without the sequencing process (Shavrukov 2016). SNP has a very important function as a molecular marker that describes the level of genetic variation and genetic changes made by breeders (Edwards and Batley 2010).

Dendrogram and similarity index between 12 strawberry cultivars

Based on the dendrogram result (Figure 4), three cluster groups were obtained. The grouping and clustering pattern illustrated how the sequence differences from the four primers using for amplification. Research on genetic variation in strawberry cultivars has been done previously by Aristya et al. (2019) using the RAPD marker. In that study, strawberries are grouped into two large clusters separated according to the morphological character types of each individual. In addition, the results of cluster grouping in the study showed a close similarity relationship between cultivars with the same number of chromosomes. In this study, clusters formed also follows the pattern of the origin of each cultivar. This is related to the inheritance of genetic material from the parent to the offspring. Strawberries 'Osso Grande', 'Osso Purbalingga', 'Aerut', 'Brastagi', 'Californica', 'Earlibrite', 'Dorit', 'Deeprose', 'Rosalinda', and 'Tristar' are grouped in a cluster because the digested fragments have a similarity value of 95 %. All of these cultivars are individuals classified into *F.* × *ananassa* Duch. This hybrid results from a cross between species from North America, namely *F. virgiana* Duch. and species from Chile, *F. chiloensis* (L.) Mill (Chandler 2012). The possibility that causes a small difference in these cultivars is the presence of environmental factors. All primers used in this study amplified genes that play a role in defence mechanisms from pathogens and environmental stresses. Excessive environmental stress can cause genetic mutations in the form of insertions or deletions (Elena and Viser 2013). The strawberry 'Osso Purbalingga' is cultivated of the 'Osso Grande' strawberry in the Purbalingga area. This makes the two cultivars have more similar value compared to the eight others cultivars. Stroberi Hitam and 'Californica' strawberry were separated from the ten other cultivars. Unlike the other cultivars, both of them are not hybrids.

The clustering results in the twelve strawberry cultivars also grouped following the number of ploidy of each cultivar. Hancock et al. (2008) stated that 'Aerut', 'Brastagi', 'Festival', 'Tristar', 'Earlibrite', 'Dorit',

'Deeprose', 'Rosalinda', Osso Grande, and Osso Purbalingga are octaploid individuals (2n=8x=56). The 'Californica' is diploid individuals (2n= 2x=16). It was separated from the previous ten cultivars. Stroberi Hitam which are the furthest separated cultivars with the eleven other cultivars is hexaploid individuals (2n=6x=48). It came from allopolyploidization or autopolyploidization is still unclear. Strawberries are still actively cultivated and hybridized by humans. This makes strawberries have many variations in the number of chromosome sets. Kunihisa et al. (2005) stated that CAPS markers can detect the polymorphic bands from the octaploid cultivars. Cultivars with less number of alleles have low dose of alleles that can generate polymorphic bands among them. Some of cultivars cannot be distinguished from the dendrogram because we only use 4 pairs of primers with 3 kinds of restriction enzymes. However our research has successfully distinguished diploid and hexaploid cultivars from other cultivars.

Based on this research, we concluded that the CAPS marker gave 45% of polymorphism (Table 5). That means primers and restriction enzymes used in this research still give low of polymorphic bands. The percentage is smaller than the study before using RAPD marker that has been done by Aristya et al. (2019). However, the CAPS molecular marker can provide complementary data for analysis of the relationship and genetic variation of strawberry cultivars in Banyuroto, Magelang. Although the method is different, the generated data can correlate well with the other data such as cytogenetic and morphological analysis. Each method has its own advantages and disadvantages; the application can be adjusted with the analysis needed.

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