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Genetic variations of strawberry cultivars of *Fragaria* x *ananassa* and *Fragaria vesca* based on RAPD

GANIES RIZA ARISTYA[1,♥], RINA SRI KASIAMDARI[2], RACHMI SETYONINGRUM[3], BENING LARASATI[3]

[1]Genetic and Breeding Laboratory, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sleman 55281, Yogyakarta Indonesia. ♥email: ganies_riza@ugm.ac.id.
[2]Laboratory of Plant Systematics, Faculty of Biology. Jl. Teknika Selatan, Sleman 55281, Yogyakarta Indonesia
[3]Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sleman 55281, Yogyakarta Indonesia

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Abstract. *Aristya GR, Kasiamdari RS, Setyoningrum R, Larasati B. 2019. Genetic variations of strawberry cultivars of* Fragaria x ananassa *and* Fragaria vesca *based on RAPD. Biodiversitas 20: 770-775.* In Indonesia, the increasing market demand for strawberries (*Fragaria* spp.) is not comparable to increased strawberry productivity. One of the efforts made to increase strawberry productivity with superior quality is plant breeding. The purpose of this research was to determine the genetic variation, lineage, and similarity index in some strawberry cultivars using molecular markers of Random Amplified Polymorphic DNA (RAPD). Eleven strawberry cultivar samples were taken from Indonesian Citrus and Subtropical Fruits Research Institute (Balitjestro), Batu City, East Java, Indonesia and Strawberry Agritourism in Banyuroto Village, Magelang District, Central Java, Indonesia. DNA isolation using modified CTAB buffer method. DNA amplification using PCR-RAPD method with 5 primers, namely UBC-516, UBC-594, OPA 10, OPA 16, and OPG 11. Strawberry lineage dendrogram construction was analyzed with clustering of Unweight Pair-Group Using Arithmetic Average (UPGMA) software Multi-Variate Statistical Average (MVSP). The research results showed that the 5 RAPD primers used in 11 strawberry cultivars produced 30 polymorphic DNA bands and 20 monomorphic DNA bands so it can be concluded that the genetic variation among 11 strawberry cultivars can be detected using RAPD molecular markers. The lineage of 11 strawberry cultivars that have the highest similarity index is found in Earlibrite and Rosalinda II cultivars of 98.85%.

Keywords: *Fragaria* spp, RAPD, similarity index

INTRODUCTION

Strawberry is one of the plants that can grow and has a high economic value in Indonesia. Indonesian farmers, especially in the highland commercially make strawberry commodities as agribusiness and agroindustry opportunities. Based on Balitjestro data (Anggraeni, 2017) the strawberry cultivation in Indonesia is initially still limited to production center areas such as West Java (Sukabumi, Cianjur, Cipanas, and Lembang), East Java (Batu) and Bali (Bedugul), but along with the increasing market demand, strawberry businesses have been carried out commercially in other areas, such as Banyuwangi, Magelang, and Purbalingga. According to Central Bureau of Statistics (2016), strawberry production volume was 10.8 million flats that had been brought in from fields at this point in 2017. But it was increasing 16.6 million flats was produces as of mid-March 2018. Domestic strawberry production has not been able to cover the high market demand so that in 2018 there is an increase in strawberry import by 29.7%, increasing from 452 tons to 764 tons (Central Bureau of Statistics 2018). Due to the lack of the government's attention to strawberry plant quality, it is necessary to research the genetic diversity, plant breeding, and genetic engineering of strawberry plants to improve its quality in Indonesia. The plant breeding researches in Indonesia produce some strawberry cultivars and now are widely cultivated in several areas.

Polymerase Chain Reaction (PCR) is an enzymatic synthesis technique to amplify DNA in vitro. PCR technique can be used to amplify DNA segments in million times in just a few hours. RAPD is one of the PCR-based molecular markers. This technique detects nucleotide segment polymorphism on DNA using a single primer that has a random nucleotide sequence. Therefore, the purpose of this study was to know the genetic variation and the lineage of strawberry cultivars cultivated in Indonesia.

MATERIALS AND METHODS

Study area

Eleven strawberry cultivar samples were taken from Indonesian Citrus and Subtropical Fruits Research Institute (Balitjestro), Batu City, East Java, Indonesia and Banyuroto Strawberry Agritourism, Magelang District, Central Java, Indonesia. Materials used 8 cultivars taken from Indonesian Citrus and Subtropical Fruits Research Institute, namely Californica, Festival, Rosa Linda, Salsa, Holly bride, Holland, Aeorut, and Shantung; and 3 cultivars from Banyuroto Strawberry Agritourism, namely Earlibrite, Rosa Linda, and Festival. Raspberry (*Rubus idaeus*) serves as outgroup. RAPD primers used here are UBC-516, UBC-594, OPA 16, OPA10, and OPG11 (Martelli et al. 1999; Morales et al. 2011).

Procedures

DNA extraction

DNA extraction was done using the modified protocol of Doyle and Doyle (1990). After that, DNA qualitative test was done using 0.8% agarose gel electrophoresis visualized under transilluminator UV light and quantitative test using an Ultraviolet-Visible spectrophotometer (UV-Vis).

DNA amplification

DNA amplification was done with Polymerase Chain Reaction-Randomly Amplified Polymorphism DNA (PCR-RAPD) method, a composition in PCR buffer, namely KAPA Extra HotStrart 12.5 µL, RAPD primer 3 µL (75µM), DNA template 3 µL (50 and 75 ng), MgCl2 1 µL (2.5 µM), and aquabidest 5.5 µL. Furthermore, they were amplified with PCR machine, and arranged in accordance with the procedure in the protocol of KAPA Taq Extra Hotstart ReadyMix PCR Kit, namely predenaturation 95°C for 5 minutes, denaturation 95°C for 30 seconds, annealing 37-51°C for 30 seconds, elongation 72°C for two minutes with a repetition of 35 cycles, then post elongation 720C for 5 minutes.

Data analysis

Furthermore, DNA band size data were processed into a matrix using Microsoft Excel program. DNA bands were converted into matrix 0-1. DNA bands that appear on the certain molecular weight are written with the number "1" and the bands that do not appear written as number "0", then the strawberry lineage dendrogram construction is analyzed using a clustering of Unweight Pair-Group Using Arithmetic Average (UPGMA) on Multi-Variate Statistical Average (MVSP) software 3.1A.

RESULTS AND DISCUSSION

DNA Isolation from strawberry leaves

DNA Isolation of strawberry leaves (Figure 1) shows that all the genome DNA bands are successfully isolated as indicated by the DNA genome bands under the well and its sizes are above 1 kB. The bar chart results in Figure 2 shows that DNA purity ratio is still below 1.8, only the Festival cultivar (Balitjestro) has a DNA purity ratio of 1.8. The low value of DNA purity ratio can be caused by the existence of the contaminants such as polysaccharides, proteins, and phenols (Tan and Yiap 2009). While the ratio on Rosa Linda cultivar (Balitjestro) is above 1.8, namely 3.5. This might be caused by the existence of the contaminants with light molecular weight, namely RNA in DNA isolates.

DNA concentration value of 699-3.696 ng/µL

Figure 2 shows DNA concentration value of 699-3,696 ng/µL. This concentration value can be used for further analysis, namely DNA amplification as the optimization of DNA concentration for DNA amplification process in this research generated an optimal DNA concentration of 50-75ng/µL.

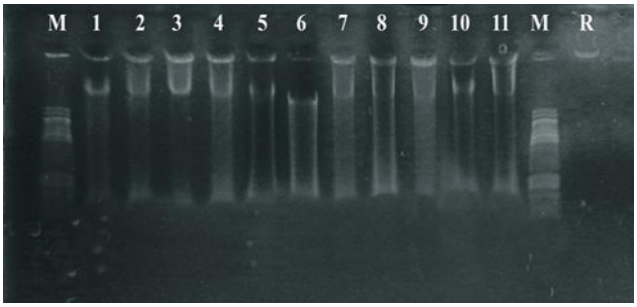

Figure 1. Isolated Genome DNA of 11 cultivars of strawberry leaves and raspberry. Note: M: Marker 1 kb; 1. Festival (Balitjestro); 2. Rosa Linda (Balitjestro); 3. Californica (Balitjestro); 4. Holland (Balitjestro); 5. Salsa (Balitjestro); 6. Holly bride (Balitjestro); 7. Santung (Balitjestro); 8. Aerut (Balitjestro); 9. Festival (Banyuroto); 10. Earlibrite (Banyuroto);11. Rosa Linda (Banyuroto); R. Raspberry

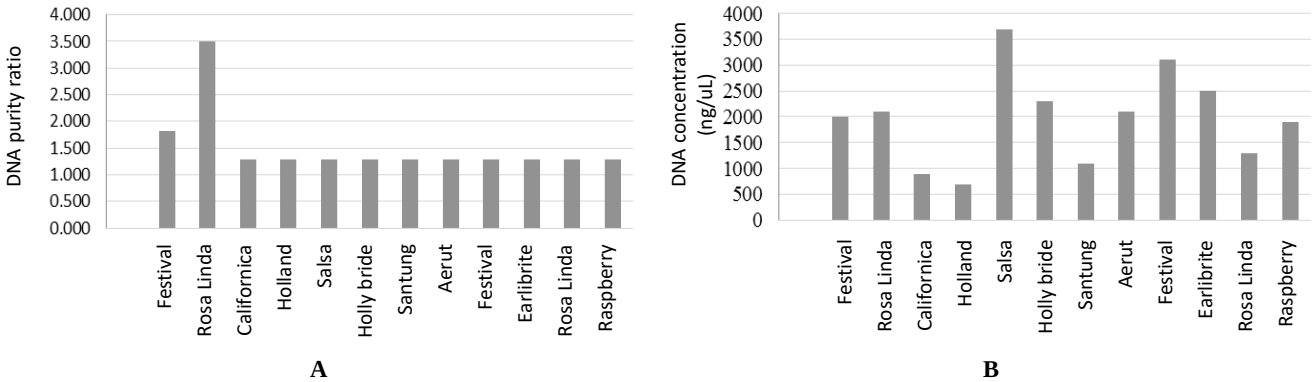

Figure 2. The quantitative test results of isolated DNA of 11 strawberry cultivars and raspberry: A. DNA purity ratio; B. DNA concentration

DNA band formation in 11 strawberry cultivars and 1 raspberry

The UBC-516 primer generated 18 DNA bands of 130-2000 bp. Of the 18 DNA bands, 6 of which are monomorphic bands and 12 polymorphic DNA bands. Figures 3.A and B showed the existence of the specific DNA bands which are solely owned by the raspberry in the sizes of 230 bp, 800 bp, and 2,000 bp. There were conservative DNA bands as family markers in the sizes of 180 bp, 280 bp, 400 bp, 480 bp, 550 bp, and 700 bp. The specific DNA bands as the marker of species *Fragaria* spp, are in the size of 450 bp. In addition, Figures 3.A and B showed that the DNA bands formed in the raspberry have similarities with DNA bands of 11 strawberry cultivars. This was because the strawberry and raspberry are the plants belonging to one family, Rosaceae. Overall results of PCR-RAPD amplification using this primer produced a diverse genetic variation, which is marked by the DNA band formation in 11 strawberry cultivars and 1 raspberry with varying sizes in each cultivar.

The UBC-594 primer (Figures 3.C and D) displayed 13 DNA bands in size of 170-2.000 bp. Of the 13 DNA bands, 4 of which are monomorphic bands and 9 polymorphic bands. Figures 3.C and D show existence of specific DNA bands which are solely owned by the raspberry in the sizes of 900 bp, 1.200 bp, 1.500 bp, and 2000 bp. The DNA bands specific to Holland cultivar is in the size of 700 bp. The conservative DNA bands as family marker are in the sizes of 180 bp, 240 bp, 400 bp, 500 bp, and 650 bp. There are also specific DNA bands as marker of species *Fragaria* spp. in the size of 500 bp. Overall results of PCR-RAPD amplification using this primer produce a diverse genetic variation, marked by the DNA bands formation in 11 strawberry cultivars and 1 raspberry with varying sizes in each cultivar. OPA-10 primer (Figure 3.E and F) displayed 9 DNA bands in the size of 320-1,750 bp. Of the 9 DNA bands, 3 of which are monomorphic bands and 6 polymorphic bands. Figures 3.E and F show the existence of specific DNA bands which are solely owned by the raspberry in the sizes of 320 bp, 650 bp, and 750 bp.

Conservative DNA bands as family marker are in the sizes of 400 bp, 420 bp, and 500 bp. Specific DNA bands as marker of species *Fragaria* spp. are in the size of 700bp. In addition, Figures 3.E and F show that the DNA bands formed in raspberry have similarities with DNA bands of

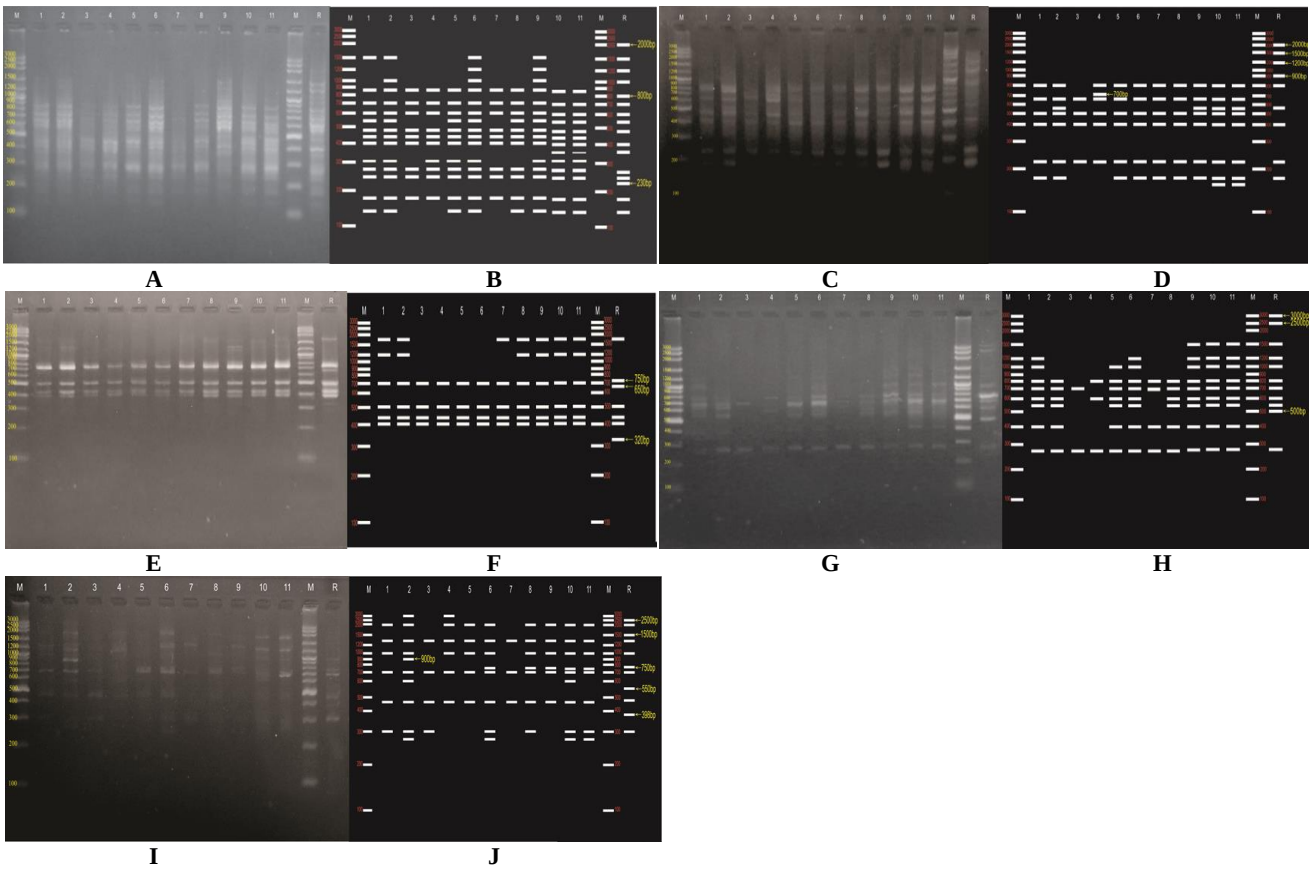

Figure 3. Amplified DNA of 11 strawberry cultivars and 1 *raspberry* based on the 5 RAPD primers, namely (A) UBC-516 (C) UBC-594 (E) OPA-10 (G) OPA-16, and (I) OPG-11; A, C, E, G, I electrophoregram and B, D, F, H, J electropherogram construction. Note: M: Marker 1 kb; 1. Festival (Balitjestro); 2. Rosa Linda (Balitjestro) ; 3. Californica (Balitjestro); 4. Holland (Balitjestro); 5. Salsa (Balitjestro); 6. Holly bride (Balitjestro); 7. Santung (Balitjestro); 8. Aerut (Balitjestro); 9. Festival (Banyuroto); 10. Earlibrite (Banyuroto);11. Rosa Linda (Banyuroto); R. Raspberry

11 strawberry cultivars. This is because the strawberry and raspberry are the plants belonging to one family, Rosaceae. OPA-16 primer (Figure 3.G and H) displayed 12 DNA bands in the size of 280-3,000 bp. Of the 12 DNA bands, only one band is monomorphic. Figures 3.G and H show the existence of specific DNA bands solely owned by the raspberry in the sizes of 500 bp, 2,500 bp, and 3,000 bp. Conservative DNA bands as family marker are in the size of 280 bp.

In addition, it appears that the DNA bands formed in raspberry have similarities with DNA bands of 11 strawberry cultivars. This is because the strawberry and raspberry are belonging to one family, Rosaceae. Overall results of PCR-RAPD amplification using OPA-16 primer produce a high genetic variation level compared to other primers, marked by the DNA bands formation in 11 strawberry cultivars and 1 raspberry with varying sizes in each cultivar. OPG-11 primer (Figure 3.I and J) displayed 16 DNA bands in the size of 280-3,000 bp. Of 16 DNA bands, 3 bands are monomorphic. Figure 3.I and J show the existence of specific DNA bands solely owned by the raspberry in the sizes of 398 bp, 550 bp, 750 bp, 1,500 bp, and 2,500 bp. Conservative DNA bands as family marker are in the sizes of 450 bp, 700 bp, and 1,220 bp. Specific DNA bands in the Rosa Linda cultivar are in the size of 900 bp. In addition, Figures 3.I and J show that the polymorphic DNA bands formed are more than monomorphic DNA bands, so it can be said that the genetic variation level in OPG-11 primer is high.

Percentage of polymorphisms DNA

Overall results of PCR-RAPD amplification using this primer produce a diverse genetic variation, marked by the DNA bands formation in 11 strawberry cultivars and 1 raspberry with varying sizes in each cultivar. The scoring results of each primer generate DNA polymorphism data for 11 strawberry cultivars and raspberry as shown in Table 1.

Regarding the data of Table 1, the 5 primers used in 11 strawberry cultivars and 1 raspberry generated total 68 DNA bands amplified, with 29 polymorphic DNA fragments and 19 monomorphic DNA fragments.

In line with the percentage of polymorphic DNA from the five primers, the average of the polymorphic DNA percentage is greater than 50%, so it can be said that these five primers have a high polymorphism level and can be used in the genetic variation analysis to compare between strawberry and raspberry. However, the percentage of the polymorphic DNA can change, when only total DNA bands of 11 strawberry cultivars are used, as seen in Table 2.

Based upon the data in Table 2, the percentage of polymorphic DNA above 50% is only found in UBC-516 (53.33%), OPA16 (88.89%), and OPG11 (72.73%) primers, while percentage of polymorphic DNA in the OPA10 primer (33. 33%) and UBC-594 primer (44.44%).

Dendrogram construction using MVSP 3.1A

Phenetic lineage of 11 strawberry cultivars in this research is seen from the similarity values resulting from cluster analysis of binary data based on DNA band profile that appears from DNA amplification in the five primers. The similarity level is then used to construct the dendrogram with UPGMA (Unweighted Pair Group Method with arithmetic mean) method. The dendrogram obtained from the result of binary data construction using MVSP 3.1A software can be seen in Figure 4.

Dendrogram of the phenetic lineage of 11 strawberry cultivars and raspberry (Figure 4) shown that there are two main groups formed in the percentage of similarity of 64.94%, consisting of groups A and B. Group A consists of raspberry and group B consists of 11 strawberry cultivars. These two groups are separated genetically on the similarity percentage of 64.94% due to the difference in genus and species in both groups.

According to Weeden et al (1992), the intensity of amplified DNA bands in each primer is strongly influenced by the purity and concentration of DNA template. DNA template contains many contaminant compounds such as polysaccharides and phenolic compounds, and the smaller concentration of DNA template often generates thin or unclear amplified DNA bands. In addition, different environmental conditions can lead to adaptation. The adaptations that occur continuously may cause speciation, which gives rise to new properties (Puspaningtyas 2014). In this research, cultivars which have the highest similarity index are Rosa Linda II and Earlibrite of 98.85%. This is because the Earlibrite cultivar is a result of a crossbreeding between Rosa Linda and FL 90-38 (Table 3). In addition, both cultivars are belonging to the same species, that is *Fragaria* x *ananassa* having octoploid chromosome number of (2n=8x=56), so they have a close lineage.

Table 1. DNA polymorphisms detected on 5 RAPD primers for 11 strawberry cultivars and raspberry

Primers	Number of DNA bands	Polymorphic DNA bands	Monomorphic DNA bands	Percentage of Polymorphic DNA bands (%)
UBC-516	18	12	6	66.67
UBC-594	13	9	4	69.23
OPA10	9	6	3	66.67
OPA16	12	11	1	91.67
OPG11	16	13	3	81.25

Table 2. DNA polymorphisms detected on 5 RAPD primers for 11 strawberry cultivars

Primers	Number of DNA bands	Polymorphic DNA bands	Monomorphic DNA bands	Percentage of Polymorphic DNA bands (%)
UBC-516	15	8	7	53.33
UBC-594	9	4	5	44.44
OPA10	6	2	4	33.33
OPA16	9	8	1	88.89
OPG11	11	8	3	72.73

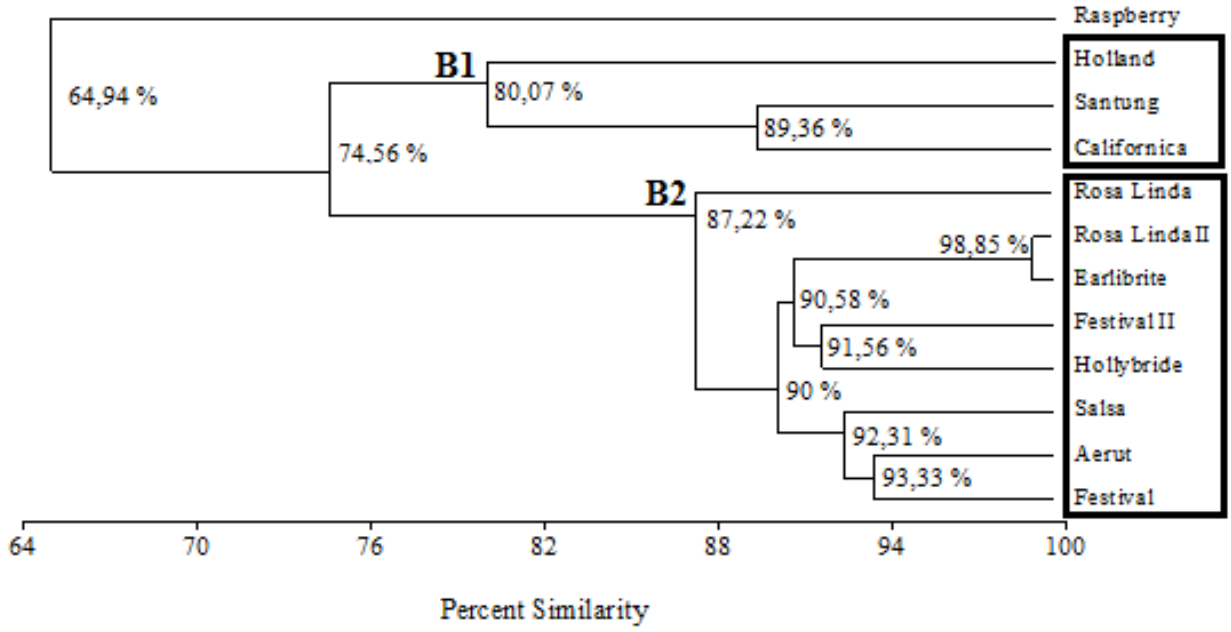

Figure 4. Dendrogram of phenetic lineage of 11 strawberry cultivars and raspberry based on RAPD marker with UPGMA methodPercentage of Polymorphisms DNA

Table 3. Crossbreeding each cultivar

Cultivar	Crossbreeding	Species
Californica	-	*F. vesca* L.
Santung	-	-
Holland	-	-
Rosa Linda	FL 87-418 x FL 8-200	*F.* x *ananassa* Duch.
Earlibrite	Rosa Linda x FL 90-38	*F.* x *ananassa* Duch.
Festival	Rosa Linda x Osso grande	*F.* x *ananassa* Duch.
Hollybride	-	*F.* x *ananassa* Duch.
Salsa	-	-
Aerut	-	*F.* x *ananassa* Duch.

Discussions

The polymorphism in genomic DNA of twelve cultivars were determined by RAPD method. Five random RAPD markers were effectively used to assess the genetic similarities between the strawberry genotypes. All the cultivars differ in size, color and flavor. Raspberry (*Rubus ideus*) is a member of the Rubus genus, while strawberry is a member of the *Fragaria* genus. However, both are belonging to the same family, Rosaceae, so both of them are capable to generate similarity index despite in the low level. This is consistent with Singh's opinion (1999) that a taxon is said to be one genus if it has a similarity index of ≥65% and ≥85% for the species. The similarity percentage of 74.56% in group B is divided into two small groups, B1 and B2. Group B1 consists of Holland, Santung, and Californica cultivars; and group B2 consists of Festival, Rosa Linda, Salsa, Hollybride, Aerut, Festival II, Earlibrite, and Rosa Linda II cultivars. Festival II, Rosa Linda II, and Earlibrite cultivars of strawberry leave samples are taken from Banyuroto Agrotourism, while the other strawberry cultivars are taken from Balitjestro. Both groups are separated from the similarity percentage of 74.56%. In the hierarchical classification, if the degree of similarity is higher than the lineage is closer. A taxon is said to be one genus when it has a similarity index of ≥65% and ≥85% for the species. When the degree of similarity of ≥85% describing the variety or cultivar to be compared has a close similarity, so that the genetic variation is getting lower or it can be said that if the character similarity between species is higher, then the diversity level and its variation are lower (Singh 2004). One of the factors that lead to these two groups are separated at similarity index of 74.56%, is that when seen from the number of its chromosome in Californica cultivar (*Fragaria* vesca), it is a diploid strawberry plant that has a chromosome number of 2n=14. Festival cultivar (*Fragaria* x ananassa) is an octoploid strawberry plant that has a chromosome number of 8n=56 (Nathewet et al. 2009). But both are belonging to the same genus, *Fragaria*, so both of them are capable to generate similarity index. Group B1 consists of Holland, Santung, and Californica cultivars that are clustered in similarity index of 80.07%. Based on the morphological characters according to Inayati (2015), Holland and Santung cultivars have common similarity that is the Ovoid-Conic fruit shape, the fruit's outer color is bright and free flower attachment, thereby both show the lineage. Santung and Californica cultivars are clustered in the similarity index of 89.36%, this may be due to the high similarity of morphological characters between Santung and Californica when compared with Santung and Holland. Similarity in morphological characters between Santung and Californica lies on the flat calyx attachment type, compound flower attachment type, and the petal size is larger than the crown. However, these three cultivars have similarities in white core and hermaphrodite flower type. In

addition, it can be assumed that Holland and Santung belong to the species of *Fragaria vesca* L. Group B2, Rosa Linda cultivar has a close lineage with Earlibrite, Rosa Linda II, Festival II, Hollybride, Salsa, Aerut, and Festival cultivars in group B2 with similarity index of 87.22%. This might occur as these cultivars are the members of the species *Fragaria* x *ananassa* (Chandler et al. 2000). In this research, Rosa Linda cultivar taken from Balitjestro is separated with Rosa Linda II cultivar that was taken from Banyuroto on the similarity percentage of 87.22%.

Table 3. Strawberry cultivar crossbreeding data (Chandler et al. 2000). This might occur as the plant age at the time of sampling between Balitjestro and Banyuroto was different. The strawberry leave age of Rosa Linda in Balitjestro was already old, it is seen from the morphology of leaves that were not fresh and dark green. While strawberry leaves of Rosa Linda in Banyuroto were still young and fresh. This leave age affects the DNA purity, because when the leaves are older, the contaminants such as polysaccharides, phenol, and protein are will be numerous. This is seen in the spectrophotometry results in Figure 2, showing that the DNA purity ratio of Rosa Linda cultivar (3,500) is greater than Rosa Linda II cultivar (1,444).

Genetic variation among the 11 strawberry cultivars can be detected using RAPD marker with 5 primers, which are UBC-516, UBC-594, OPA-10, OPA-16, and OPG-11 that generated 30 polymorphic DNA bands and 20 monomorphic DNA bands. On UBC-594 primer, there is specific DNA band with a size of 700 bp on Holland cultivar. OPG11 primer, there is a specific DNA band with a size of 900bp on Rosa Linda cultivar. Group B1 consists of Holland, Santung, and Californica cultivars that are clustered in similarity index of 80.07%. Santung and Californica cultivar are clustered on similarity index of 89.36%. Group B2, Rosa Linda cultivar has a close lineage with Earlibrite, Rosa Linda II, Festival II, Hollybride, Salsa, Aerut, and Festival cultivars in group B2 with similarity index of 87.22%. Earlibrite and Rosalinda II cultivars have the highest similarity level, with similarity index of 98.85%.

For valuable cultivar identification in the results indicated that the RAPD technique is effective to develop genotype-specific banding patterns. The results verified the effectiveness and suitability of RAPD markers for strawberry cultivars identification. Cultivar identification and genetic diversity analysis studies using RAPD techniques are considered as the one used techniques because it does not require previous DNA sequence information and uses a very small quantity of DNA (Bir et al. 2018). Therefore, for the further research program, especially for breeding, a genotype selected from different clusters will provide maximum heterosis regarding yield (Congiu et al. 2000). Gaafar and Saker (2006) also found three clusters in seven varieties of the genetic stability of the tissue culture-derived strawberry plants. Random molecular markers were better suited for discriminating between genotypes (individuals) rather than for revealing relationships among wild populations (Harrison et al. 1997; 1997. The RAPD markers were coherent in grouping the cultivars, and up to a certain point, with the genealogy and country of origin, and can be used to study genetic similarity (Conti et al. 2002). The similarity values obtained in the analyses reinforced that there was genetic divergence among the cultivars, especially due to the use of genotypes derived from different breeding programs.

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