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Original Research Article

Genetic Variation in Purple Passion Fruit (*Passiflora edulis* f. *edulis* Sims.) in West Sumatra, Indonesia by RAPD Markers

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Abstract: Three purple passion fruit populations from West Sumatera, Indonesia were compared some fruit characters and determined the genetic variation. The fruit weight, sugar content and vitamin C were calculated by a quantitative method. The genetic variation was analyzed by the RAPD markers to determine Shannon's index, heterozygosity, genetic differentiation and gene flow. An Alahan Panjang population has the best fruit characteristics based on fruit weight, sugar content and vitamin c content respectively 100.22 gram, 14.72 % Brix and 0.47 mg. The test result of 17 primers showed that three primers (OPA01, OPA03, and OPB10) can amplify DNA of purple passion fruit, with 22, 24 and 16 polymorphic bands respectively. The highest average value of heterozygosity and Shannon diversity index value found in Alahan Panjang population respectively 0.1784 and 0.2802. Value of intra population heterozygosity (Hs = 0.1349) is higher than value of inter population (Dst = 0.0191). Cluster analysis with dendrogram in purple passion fruit denoted low genetic distance. According to the result, Alahan Panjang population has best fruit characters and higher genetic variation compare to other population in West Sumatra.
Keywords: *Passiflora edulis* f. *edulis* Sims., RAPD, genetic variation, heterozygosity.

INTRODUCTION

Purple passion fruit (*Passiflora edulis* f. *edulis* Sims.) has two excellences compare to other passion fruit because its vitamin C content and pleasant smell [1]. Because of its advantages, this plant is been a long time cultivated in South Sulawesi and North Sumatra, Indonesia as the raw material to make the syrup or juice. In West Sumatera, purple passion fruit is growing displaced due to not growing the home industry of syrup [2].

Small populations of purple passion fruit still found in Alahan Panjang, Batusangkar and Sijunjung districts, West Sumatra. Lately, the home industry of syrup began to grow, so it is need lots of quality fruits. Therefore, The Department of Agriculture has been intensified purple passion fruit thought quality seeds. The genetic resources of three purple passion fruit populations are a germplasm to study their genetic variation to be utilized the selection of superior plant.

Some morphological important characters which are used a quality fruit that is fruit size, content of sugar and vitamin C [1]. Besides that, heterozygosity is an important genetic resource in early breeding program because it is usually associated with the present of genetic variability [3]. In Alahan Panjang population was found 30 phenotype accessions of purple passion fruit [2] but their genetic variation are not reported. Genetic variation in passion fruit (*Passiflora* spp.) by RAPD technique has analyzed in Brazil for genetic resources [4]. Based on the technique was analyzed the genetic variation in Sumatra Barat population to get an information of heterozigosity as basis on the conservation and breeding programs.

MATERIALS AND METHODS

Sample Collection

The population of purple passion fruits were observed in three districts that are Alahan Panjang, Batusangkar and Sijunjung, West Sumatra, Indonesia (Fig. 1). Young leaf of 10 individual plants was collected from each population. The temporary preservation of leaf is used to the gel silica in plastic bag during travelling from field to laboratory.

Fruit Characterization

Fruits from each population were assessed some characters that are fruit weight, total content of sugar and vitamin C of juicy. The sugar content of was

measured by Luff Schoorl's method and vitamin C by iodine titration method [5].

DNA extraction and PCR amplification

The DNA was extracted from leaf by a modified method from Doyle and Doyle [6] with materials that are liquid nitrogen, CTAB buffer, chloroform, isoamilalcohol (24:1), isopropanol, 70% ethanol, and buufer TE. The DNA was analysed by *Random Amplified Polymorphic DNA* (RAPD) technique, use to PCR SENSOQUEST machine, gel agarose, PCR Bioline kit, DNA ladders 100 bp, and 17 primers (Table 1). DNA was amplified by PCR machine through initial denaturation cycle at 94ºC for two minutes followed by 40 cycles at 94ºC for 30 seconds, annealing at 36ºC for one minute, and extension at 72º C for 80 seconds. Each of 4 µL DNA samples were mixed with 12.5 µL Go Taq Green, added 6.5 µL Nuclease Free Water, and 2 µL primer. RAPD fragments were separated in 1.6% agarose gel (dilute in 10 X TBE) for two hours (60 volt, 150 mA and 20 Watt). The gel stained with ethidium bromide and photographed by Polaroid camera under UV trans-illuminator.

Data analysis

The amplified DNA was calculated with data provided by the observation of the present (1) or absence (0) of bands between pairs of individuals using the Popgene 32 program. Genetic diversity was analysed through Nei's formula [7], Shannon's index, heterozygosity, genetic differentiation and gene flow [8].

RESULTS

Fruit characteristics

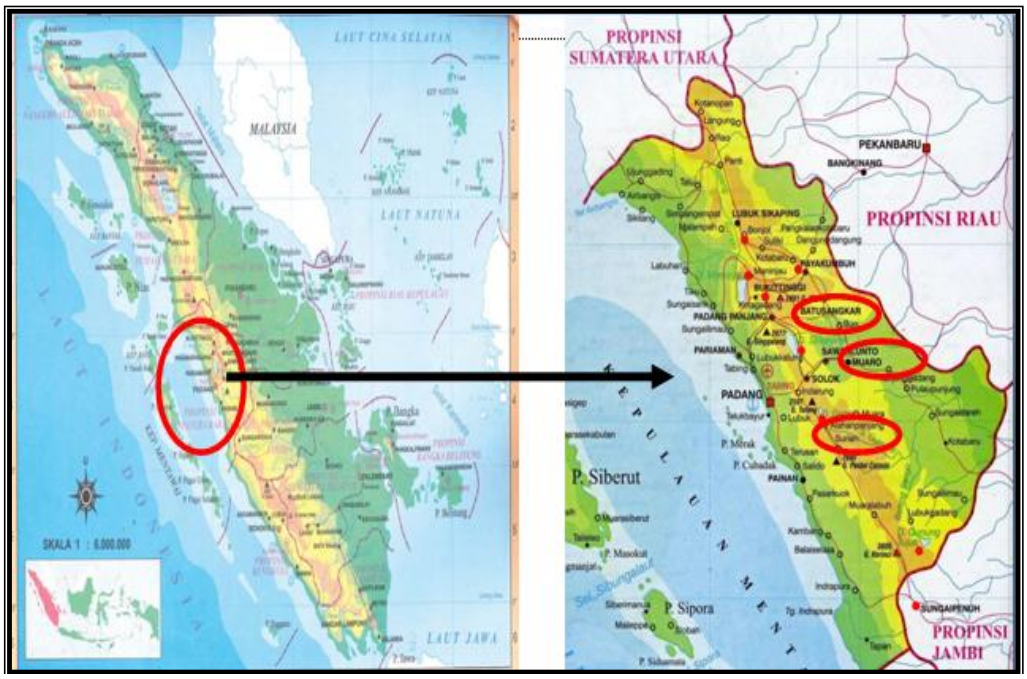
The average weight of the fruit, sugar and vitamin C contents of the juicy are different in three populations (Table 2, Figure 2, 3 and 4). The highest average weight of the fruit obtained from Alahan Panjang population with 100.22 g, and the lowest is Sijunjung population with average weight 60.34 g. The vitamin C content of the juicy, Alahan Panjang population have the highest average value 0.47 mg, and Sijunjung population 0.26 mg became the lowest. The sugar contents of Alahan Panjang and Batusangkar population almost similar respectively are 14.72% Brix and 14.50% Brix, but in Sijunjung population only 11.45% Brix.

Genetic variation

The test result of 17 primers showed that three primers (OPA01, OPA03, dan OPB10) can amplified DNA of purple passion fruit (Fig. 5, 6 and 7), whereas other 14 primers cannot display DNA band. Total bands were amplified by OPA01, OPA03, and OPB10 are 22, 24 and 16 polymorphic bands (Table 3).

The highest average value of heterozygosity (H) and Shannon diversity index value (I) found in Alahan Panjang population respectively 0.1784 and 0.2802 (Table 4). While the lowest heterozygosity found in Sijunjung population 0.0997 meanwhile Shannon diversity index value 0.1651. High heterozygosity value of this population is parallel to high proportion of polymorphic loci of Alahan Panjang population, 64.52%.

Table 5 showed that value of intra population heterozygosity (Hs=0.1349) is higher than value of inter population (Dst=0.0191). Gst value or genetic differentiation 0.1243 with gene flow value (Nm) 3.5216. UPGMA cluster analysis resulted the grouping of purple passion fruit in West Sumatra divided into two big group. The one group consists of Alahan Panjang population and the second one composed by Batusangkar and Sijunjung populations. Cluster analysis with dendrogram in purple passion fruit (Fig. 8) denoted low genetic distance. The nearest genetic distance found between Batusangkar and Sijunjung population with 0.0168, meanwhile the furthest genetic distance found between Alahan Panjang population and Batusangkar populations are 0.0350.



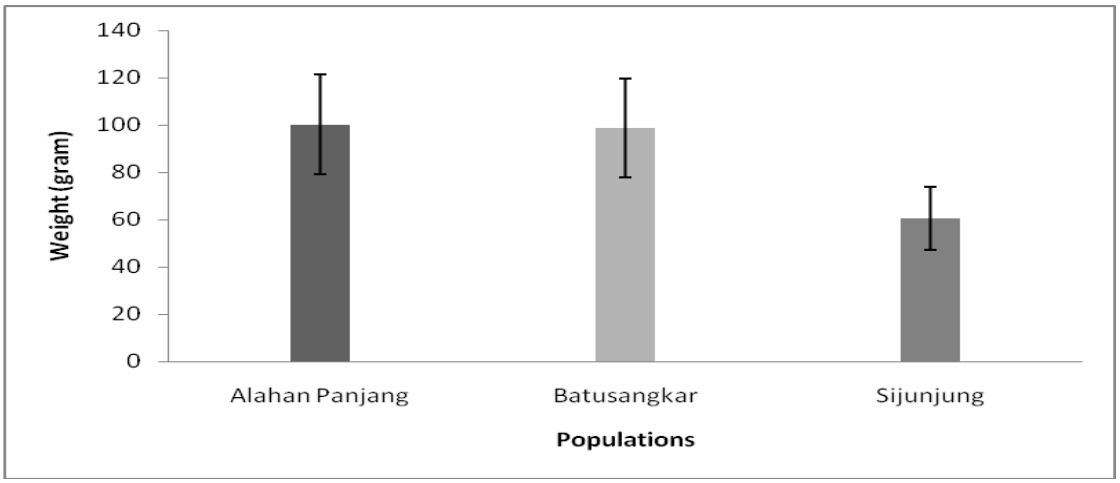
Fig−1. Location map of Alahan Panjang, Batusangkar and Sijunjung in West Sumatra, Indonesia

Table−1. The primers using to amplify the DNA

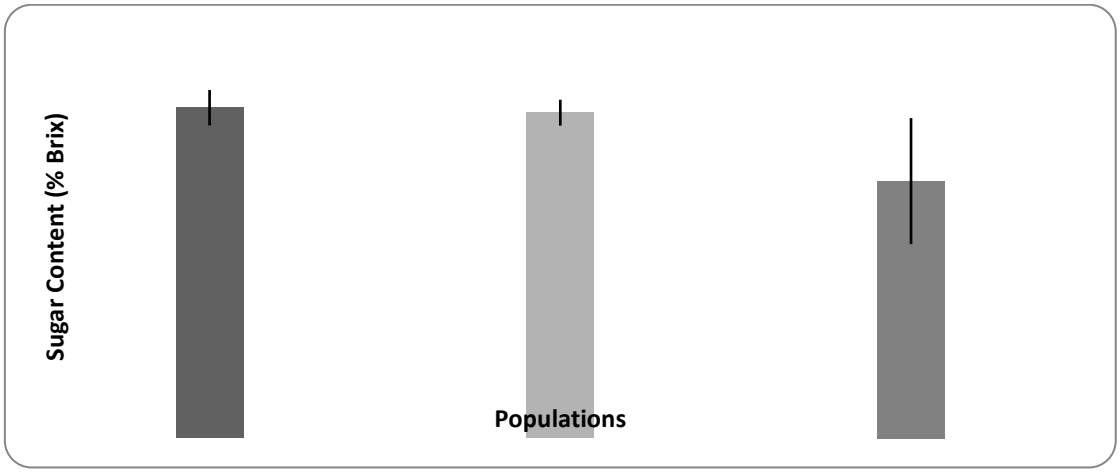
Primer	Sekuen Primer 5'----------3'	Referensi
OPA01	CAGGCCCTTC	(Almeida *et al*, 2010)
OPA02	TGCCGAGCTG	(Almeida *et al*, 2010)
OPA03	AGTCAGCCAC	(Almeida *et al*, 2009)
OPA04	AATCGGGCTG	(Simatupang, 2015)
OPA05	AGGGGTCTTG	(Almeida *et al*, 2009)
OPA06	GGTCCCTGAC	(Almeida *et al*, 2009)
OPA07	GAAACGGGTG	(Almeida *et al*, 2010)
OPA08	GTGACGTAGG	(Almeida *et al*, 2010)
OPA09	GGGTAACGCC	(Almeida *et al*, 2010)
OPA10	GTGATGGCAG	(Almeida *et al*, 2010)
OPA11	CAATCGCCGT	(Almeida *et al*, 2009)
OPA12	TCGGCGATAG	(Almeida *et al*, 2009)
OPA13	CAGCACCCAC	(Almeida *et al*, 2009)
OPA16	AGCCAGCGAA	(Almeida *et al*, 2009)
OPAC12	GGCGAGTGTG	-
OPB08	GTCCACACGG	(Crochemore *et al*, 2003)
OPB10	CTGCTGGGAC	(Almeida *et al*, 2009)

Table−2. The average of fruit weight, content of sugar and vitamin C of fruit juicy of three purple passion fruit populations in West Sumatra

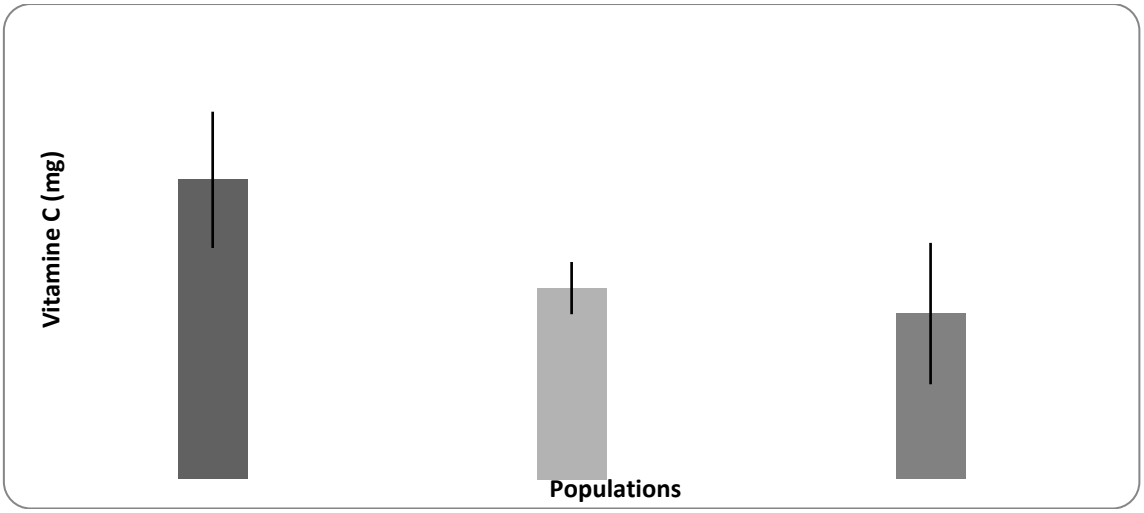
Population	Fruit Weigh (g)	Content of vitamin C (mg)	Content of sugar (% Brix)
Alahan Panjang	100.22± 21.063	0.47± 0.789	14.72± 0.107
Batusangkar	98.77± 21.062	0.30± 0.577	14.50± 0.041
Sijunjung	60.34± 13.352	0.26± 2.807	11.45± 0.111



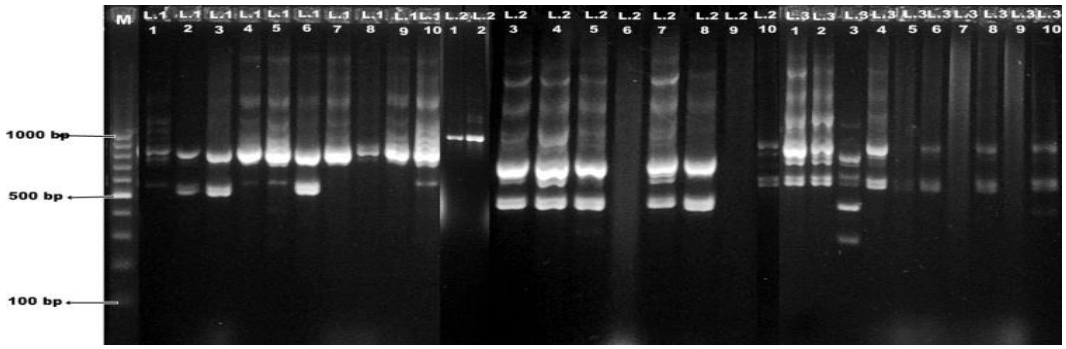
Fig–2. Weigh fruit of purple passion fruit in three populations in West Sumatra



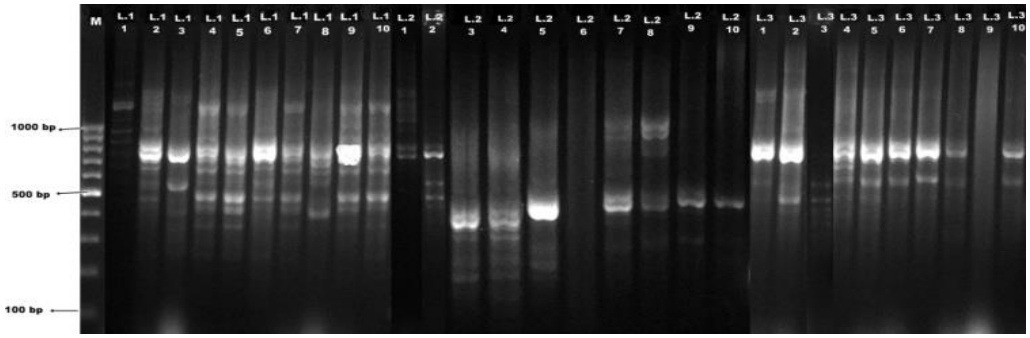
Fig–3. Sugar content (% Brix) of purple passion fruit in three populations in West Sumatra



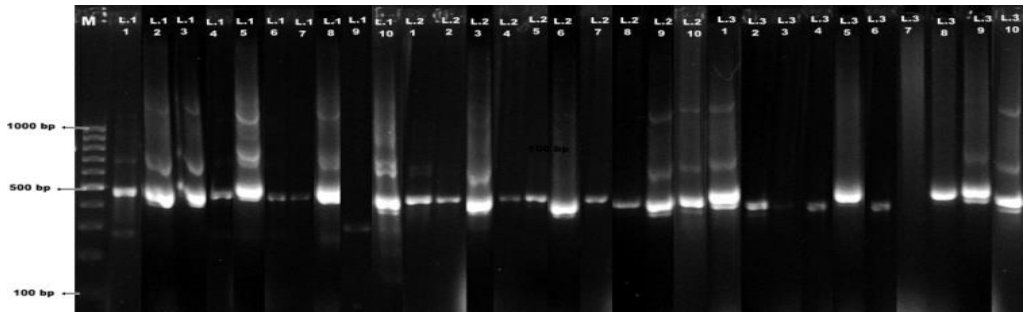
Fig–4. Content of vitamin C (mg) of purple passion fruit in three populations in West Sumatra



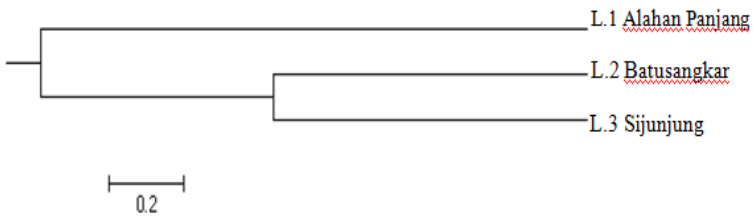
Fig−5. RAPD profile of purple passion fruit DNA were amplified with OPA01



Fig−6. RAPD profile of purple passion fruit DNA were amplified with OPA03



Fig−7. RAPD profile of purple passion fruit DNA were amplified with OPB10



Fig−8. Dendogram of three purple passion fruit populations in West Sumatra

Table −3. The result of amplified DNA of purple passion fruit with three primers

Primers	Number of amplified bands	Number of polymorphic bands	Percentage of polymorphic bands
OPA-01	22	22	100%
OPA-03	24	24	100%
OPB-10	16	16	100%
Total	62	62	
Average	20.67	20.67	

Tabel−4. The value of intra population genetic variation in purple passion fruit in West Sumatra

Populations	Na	Ne	H	I	PLP (%)
Alahan Panjang	1.6452± 0.4824	1.2856±0.3268	0.1784±0.1773	0.2802± 0.2540	64.52
Batusangkar	1.5645±0.4999	1.1774±0.2145	0.1264±0.1370	0.2112±0.2127	56.45
Sijunjung	1.4516±0.5017	1.1471±0.2398	0.0997±0.1411	0.1651±0.2140	45.16

Notes: Na = average number of observed alleles; Ne = number average of effective alleles; H = average heterozygosity/Nei's genetic diversity; I = Shannon's index; PLP = percentage of polymorphic bands.

DISCUSSION

Among three populations of purple passion fruit in West Sumatra, Alahan Panjang population has the best fruit characteristics based on fruit weight, sugar content and vitamin C content respectively 100.22 g, 14.72 % Brix and 0.47 mg. Meanwhile Sijunjung population's fruit weight, sugar content and vitamin c content are the lowest with 60.34 gram, 11.45 % Brix dan 0.26 mg (Table 2, Figure 2, 3 and 4). It showed that the population in Alahan Panjang highland (±1500 m; altitude) have higher fruit weight than two other populations, Batusangkar (±1200 m; altitude) and Sijunjung (±800m m; altitude) which included to moderate land. Based on habitat [9] reported that passion fruit better adapted in tropical highland.

Three primers (OPA01, OPA03, dan OPB10) able to amplified DNA of purple passion fruit (Fig. 5, 6 and 7). The primers were reported [10] can amplify the DNA of several species *Passiflora* in Venezuela (*P. edulis* f. *flavicarpa*, *P. cincinnata*, *P. gibelrti*, *P. subpeltata*, *P. maliformis*, and *P. foetida*) revealed 10, 15 and 16 bands respectively. The proportion of polymorphic loci in three populations are higher compared to reporting by [11] that the proportion of polymorphic loci in same species with 16 primers is 38% polymorphic band.

The highest average value of heterozygosity (H) and Shannon diversity index value (I) found in Alahan Panjang population respectively 0.1784 and 0.2802 (Table 4). While the lowest heterozygosity found in Sijunjung population 0.0997, meanwhile Shannon diversity index value 0.1651. High heterozygosity value of this population parallel with high proportion of polymorphic loci of Alahan Panjang population, 64.52%. The heterozygosity of of Alahan Panjang population (0.0997) lower than the heterozygosity value of wild *P. edulis* population in Brazil were 0.34 [12].

High heterozygosity value and Shannon diversity index value in Alahan Panjang population have similarity with [2], stated in this location found 30 phenotype accessions of purple passion fruit. Thus, high heterozygosity value in Alahan Panjang population might have a relation with high sub population number than the other two populations. According [13] that high genetic variability was found in wild passion fruit (*Passiflora edulis* Sims.) accessions. Therefore, high heterozygosity value in Alahan Panjang population can be used for conservation and plant breeding program. Population with high genetic diversity became target for genetic conservation and as germplasm sources for *Passiflora* spp. breeding [14, 11, 15]. Wild *Passiflora* with high genetic diversity can be utilized to increase genetic diversity of *Passiflora* commercial through breeding programs [14].

The value of intra population heterozygosity (Hs=0.1349) is higher than value of inter population (Dst=0.0191) (Table 5). Genetic variation among intra population is high because purple passion fruit didn't cultivated intensively and there are no selection given by the farmer. Besides that, the pollination system of this plant helped by insects so that the cross-pollination more than self-pollination. Outcrossing species have high genetic diversity intra population than inter population [16]. Gst value or genetic differentiation 0.1243 with gene flow value (Nm) 3.5216. Conceptually, low Gst value or genetic differentiation indicated that rate of gene flow inter population of purple passion fruit. Nevertheless, geographical distance among three populations is not possible to allow cross-pollination assisted by insect, except by wind. Therefore, the high gene flow values concluded Batusangkar and Sijunjung populations probably originated from the Alahan Panjang population.

Cluster analysis with dendrogram in purple passion fruit (Fig. 8) denoted low genetic distance between populations. The nearest genetic distance found between Batusangkar and Sijunjung population with 0.0168, meanwhile the furthest genetic distance found between Alahan Panjang population and Batusangkar population, 0.0350. According to [17] that higher geographical isolation to cause the lower gene flow occurs, and the accessions of same geographic have low genetic distance.

CONCLUSION

Among three populations of purple passion fruit in West Sumatra, Alahan Panjang population has the best fruit characteristics based on fruit weight, sugar content and vitamin C content respectively 100.22 g, 14.72 %

Brix and 0.47 mg. Meanwhile Sijunjung population's fruit weight, sugar content and vitamin c content are the lowest with 60.34 gram, 11.45 % Brix dan 0.26 mg. The highest genetic variation intra population also found in Alahan Panjang population with values (H = 0.1784, I = 0.2802, PLP = 64.52%). Genetic variation inter population is lower (Dst = 0.0191) compared to genetic variation intra population (Hs = 0.1349). Gene flow value inter population (Nm = 3.5216), Gst value = 0.1243. Low Gst value or genetic differentiation indicated high rate of inter population gene flow of purple passion fruit.

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