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Genetic Variation in Local Cultivar of Singgalang Cabbage (*Brassica oleracea* L. var. *capitata*) Using RAPD Marker

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Abstract: The local cultivar of Singgalang cabbage is an endemic vegetable in West Sumatra Province, Indonesia, it has three variants, namely *Biaso*, *Batang Hitam* and *Senggan* which is favored by consumers. This local cultivator is threatened with extinction due to lack of interest by farmers regarding the low productivity and susceptibility to pest and disease attacks compared to modern cultivars. To conserve the local germplasm and its use as a breeding material have been analyzed genetic variation and genetic differentiation by RAPD technique using 17 primers. The result showed that three primers (OPAC-11, OPA-02, and OPA-9) were able to amplify DNA and produce polymorphic bands. The percentage of polymorphic bands using OPAC-11, OPA-02 and OPA-09 was 100%, 90%, and 100%, respectively. The *Batang Hitam* variant has highest heterozygosity value (H) is 0.2725 with 0.3932 of the Shanon diversity index value (I), while the *Biaso* variant shows the lowest value of H (0.1400) and I (0.2070). Genetic variation between variants (Gst) is categorized as low (0.3917), and the gene flow (Nm) is high (0.7763). The cluster analysis showed that between *Biaso* and *Senggan* variants have the most distant genetic distance of 0.4057, and followed by *Biaso* and *Batang Hitam* variants with the genetic distance of 0.3273.
Keywords*:* cabbage, local cultivar, genetic variation, genetic differentiation, RAPD.

INTRODUCTION

The local cultivar of *Singgalang* cabbage (*Brassica oleracea* L. var. *capitata*) is an endemic cabbage in Mount Singgalang, Agam Regency, and West Sumatra Province, Indonesia. The local cultivar is estimated to have been cultivated since the Dutch colonial era about the 1800s, but it is unknown to its origin.

There are three variants of this local cultivar in Singgalang mountain, namely variants *Biaso*, *Batang Hitam* and *Senggan*, but currently, these variants are rarely found today [1]. The local cultivar of Singgalang cabbage is a favorite vegetable among the people of West Sumatra Province and surrounding provinces because it has a coarse fiber texture, a distinctive taste and tasty when compared with modern cultivars. Although the local cultivars of Singgalang cabbage have a high selling value because consumers favor it, its production is much reduced. This condition is caused by the lack of interest of farmers to grow local cultivars of Singgalang cabbage are less productive and susceptible to disease and pest attacks. Consequently, the popular vegetable germplasm will be endangered in the future.

The decrease in productivity of local varieties can be caused by the loss of genes or alleles in a population due to genetic erosion [2]. The factors causing genetic erosion include modernization of agriculture, the presence of modern cultivars, limited landholdings, population decline, and social engineering changes [3]. The extinction of cultivation crops due to genetic erosion has been reported in other local cultivar vegetables. Some traditional vegetables in Tanzania were found to be threatened with extinction due to genetic erosion due to a decline in crop populations on converted agricultural land for settlements [4]. The genetic erosion in tomato local cultivars has occurred in Valencia Spain due to the decline of the crop population associated with the presence of modern cultivars and plant disease attacks [5]. Changes in soil pH may also cause genetic erosion in *B. oleracea* [6]. In recent years, the fact of genetic erosion has occurred globally as the implications of the decline or loss of genetic diversity [7].

The rate of genetic erosion and extinction of germplasm should be anticipated; it is necessary to carry out monitoring and evaluation of genetic variation as a basis for conserving and breeding of crops [7]. One method to detect genetic variation is The Random Amplified Polymorphic DNA (RAPD) technique. RAPD markers have been used to analyze genetic diversity in plants from the Brassicaceae family,

including homogeneity and genetic diversity analysis both within and between populations [8], genetic diversity assessment [9, 10], evaluation of pure cultivar of F1 cabbage cultivars [11, 12], and identification of crosses [13]. Primers have been known for amplifying DNA and exhibiting polymorphism in the *B. oleracea* are OPA-03, OPA-04, OPA-09, OPA-11, OPA-15, and OPA-18 [14]. OPA-08 and OPA-16 primers also showed polymorphism in cabbage (*B. oleracea* var. *capitata*) DNA [15], OPB-04 and OPB-12 for evaluation of hybrid F1 broccoli (*B. oleracea* var. *italica*) [11], OPB-18, OPB-20, OPC-14 and OPE-14 on flower cabbage DNA [13]. OPA-02, OPC-09, OPC-14, OPC-18, OPO-02, OPD-07, OPO-20, OPN-10 and OPY-15 primer shows unique bands in some broccoli varieties [16].

MATERIALS AND METHODS

Collection of samples

Crop leaf samples were collected in Padang Kudo and Padang Laweh Villages, Sungai Puar Subdistrict, Agam Regency, West Sumatra Province, Indonesia. Young leaves were taken ten individuals at random for each variant on farmer's cropland. Pieces of leaves are stored in a plastic bag containing silica gel to maintain damage to the sample during the trip to the laboratory.

Isolation and amplification of DNA

DNA was extracted from the leaves using a modified method of Doyle and Doyle [17] using liquid nitrogen, CTAB buffer, chloroform, isoamyl alcohol (24: 1), isopropanol, 70% ethanol, and TE buffer. DNA was analyzed using SENSOQUEST PCR machine, agarose gel, PCR Bioline kit, and DNA ladder 100 bp. DNA was analyzed using Random Amplified Polymorphism DNA (RAPD) markers with modified methods from Williams *et al*. [18]. DNA amplification uses six primers that are known to show polymorphism in cabbage DNA (OPA-03, OPA-04, OPA-09, OPA-11, OPB-15, OPB-18), and 15 other primers available in the laboratory (OPA-01, OPA-02, OPA-05, OPA-10, OPA-12, OPA-13, OPA-16, OPAC-11, OPAC-12, OPAC-15, OPB-08, and OPB-10).

DNA was amplified by a PCR machine through an initial denaturation cycle at 94 °C for two minutes, followed by 40 cycles at 94 °C for 30 seconds, annealing at 36 °C for one minute, and extended at 72 °C for 80 minutes seconds. Each 4 µL DNA sample was mixed with 12.5 µL Go Taq Green, added 6.5 µL nuclease-free water, and 2 µL primer. To see the quantity and quality of DNA fragments are separated in a 1.6% agarose gel (dilute in 10 X TBE) for two hours (60 volts, 150 mA, and 20 Watt). The gel is stained with ethidium bromide and photographed using a Polaroid camera under UV light-illuminators.

DATA ANALYSIS

RAPD marker was scored visually of their present (1) or absent (0), separately for eachindividual of three variants and each primer. The score obtains using all primers in RAPD analysis were pooled for constructing a single data matrix. This matrix used for estimating loci polymorphic, Nei's [19] genetic variation, gene flow (Nm), genetic differentiation (Gst), genetic distance (D), and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Mean) dendrogram between variants using POPGENE (version 1.32) computer program.

RESULTS AND DISCUSSION

Polymorphism

The results of the primer selection showed that three of them (OPAC-11, OPA-02, and OPA-9) of 17 primers used were able to amplify DNA and produce polymorphic bands (Fig. 1, 2 and 3). The OPAC-11 produces the most bands (13 bands), followed by OPA-02 (10 bands) and OPA-09 (8 bands). The three primers produced a total of 31 bands with an average of 10, 33 bands per primer (Table 1). The percentage of polymorphic bands using OPAC-11, OPA-02 and OPA-09 was 100%, 90% and 100%, respectively (Table 2). Primers of OPA-09 have produced polymorphic bands in *B. oleracea* [14], OPA-02 in *B. juncea, B. rapa* [20] and *B. oleracea* var. *italica* [16]. A higher number of bands for each primer indicated the existence of more substantial genetic diversity among the genotypes [21]. Primers with higher polymorphic bands are essential in studying genetic diversity and discrimination of the genotypes [22].

In the *Biaso* variant were found three specific bands (196 bp, 44 bp, and 567 bp) using OPAC-11, and one band of 172 bp using OPA-02 and one 1060 bp band using OPA-09. In the *Batang Hitam* variant, only one specific band (1600 bp) was found using OPA-02 (Table 1). Specific bands have been identified using RAPD primers in canola (*Brassica napus*) so that the primers can be used more effectively for genotype identification and assessment of genetic variation [23].

Genetic variation

The *Batang Hitam* variant has highest heterozygosity value (H) is 0.2725 with 0.3932 of the Shanon diversity index value (I), while the *Biaso* variant shows the lowest value of H (0.1400) and I (0.2070) (Table 3). Genetic variation between variants (Gst) is categorized as low (0.3917), and the gene flow (Nm) is high (0.7763) (Table 4). The low GST and the Nm height are estimated that the three variants are introduced from the same center of origin or centers of genetic diversity. Despite the propagation of these cabbages by local farmers through buds so as not to allow gene flow to occur, but the fact that genetic differentiation among variants is still low. The low genetic differentiation of these three variants may be due to growth in the same habitat although it is estimated to have been cultivated in Singgalang Mountain since the 1880s. Cultivated goldenseal (*Hydrartis canadensis* L.) population with propagation

through vegetative had lower genetic variation compared to the wild population which reproduced generatively [24]. Local cabbages that have been cultivated by farmers have improved through mass selection for centuries causing low variations in the population [25]. In addition, factors as population isolation, mating system, genetic drift, selection and shift in distribution are responsible for alteration in complex genetic constituting and further cause variations hereditary multiplicity within a population [26].

The cluster analysis showed that between *Biaso* and *Senggan* variants have the most distant genetic distance of 0.4057, and followed by *Biaso* and *Batang Hitam* variants with the genetic distance of 0.3273, while between the *Batang Hitam* and *Senggan* variants showed the closest genetic distance of 0.1242 (Table 5). endrogram (Fig. 4) shows that the Biaso variant is a separate group of *Batang Hitam* and *Senggan* variants. Groupings based on UPGMA reflected the geographic similarity of specific accession germplasm of locally *Brassica* species [27].

The three variants of Singgalang cabbage are a valuable genetic resource and should be conserved for use in future breeding programs. Based on the genetic variation and the genetic distance could be useful to select a parent to be cross between the *Biaso* and *Senggan* variants or the *Biaso* and the *Batang Hitam* variants. To be useful for plant breeder, the genetic resources must be characterized by morphological and agronomic traits [28].

Table-1: RAPD primers that successfully amplify the DNA fragment at all three variants of a local cultivar of Singgalang cabbage

No.	Primer	Band size (bp) in variants:			Number of bands
		Biaso	*Batang Hitam*	*Senggan*	
1	OPAC-11	196, 390, 440, 500, 567, 800, 1000, 1120, 1500	216, 335, 390, 500, 650, 800, 1000, 1120, 1400, 1500	216, 335, 390, 500, 650, 800, 1120, 1400, 1500	13
2	OPA 02	172, 260, 326, 500, 600, 971, 1100	260, 326, 400, 500, 600, 737, 971, 1100, 1600	260, 326, 400, 500, 600, 737, 971, 1100	10
3	OPA 09	300, 467, 600, 700, 1030, 1060, 1200	300, 467, 600, 700, 1030, 1200, 1600	300, 467, 600, 700,1030, 1600	8
Total number					31

Notes: bp (base pairs); bold printed band sizes show specific bands on each variant and primer

Table-2: Number of polymorphic bands by using three primers in the three variants of a local cultivar of Singgalang cabbage

No.	Primer	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphic bands
1	OPAC-11	13	13	0	100%
2	OPA-02	10	9	1	90.00%
3	OPA-09	8	8	0	100%
Total number		31	30	1	96.67%
Average		22.67	22.67	0	96.67%

Table-3: Number of alleles, heterozygosity and Shannon's diversity index value in three variants of a local cultivar of Singgalang cabbage

No	Variant	Na	Ne	H	I
1	Biaso	1.3871	1,2458	0.1400	0.2070
2	Batang Hitam	1.6452	1,4939	0.2725	0.3932
3	Senggan	1.4839	1.3619	0.1991	0.2877

Notes: Na = average number of observed allels; Ne = Number average of effective allels; H = heterozygosity/Nei's genetic diversity; I = Shanon's diversity index value;

Table-4: Gene flow and genetic differentiation between variants of a local cultivar of Singgalang cabbage

No.	Jumlah Individu	Hs	Dst	Ht	Nm	Gst
1	30	0.2039	0.1313	0.3352	0.7763	0.3917

Notes: Hs = value of intra-variant heterozygosity; Dst = value of inter-variant heterozygosity; Ht = total value of heterozygosity; Nm = gene flow; Gst = genetic differentiation inter-vatiant

Table-5: Matrix of genetic distance between three variants of Singgalang cabbage local cultivar

Variant	Biaso	Batang Hitam	Senggan
Biaso	-		
Batang Hitam	0.3273	-	
Senggan	0.4057	0.1242	-

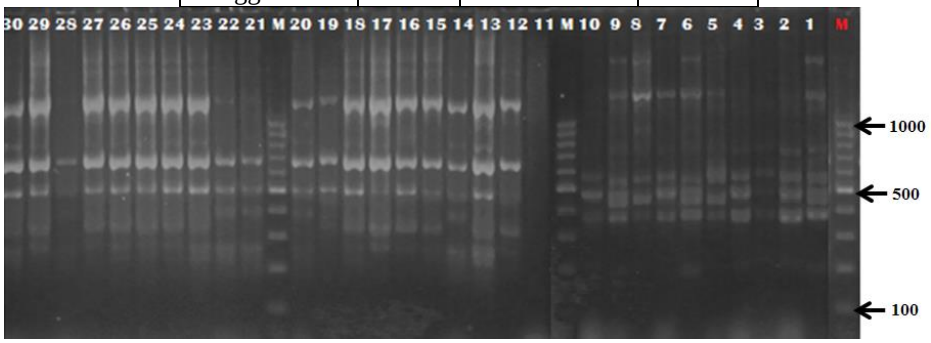

Fig-1: RAPD profile of local cultivar of Singgalang cabbage was amplified using OPAC-11: Accessions 1-10 (*Biaso* variant), 11-20 (*Batang Hitam* variant), and 21-30 (*Senggan* variant)

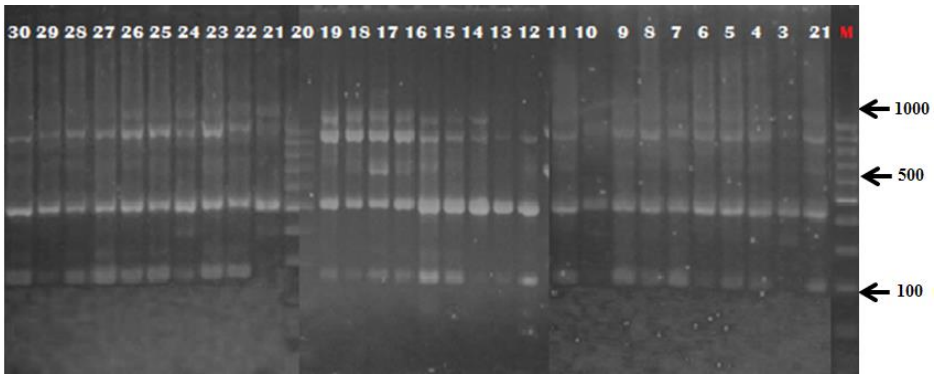

Fig-2: RAPD profile of local cultivar of Singgalang cabbage was amplified using OPA-02: accessions 1-10 (*Biaso* variant), 11-20 (*Batang Hitam* variant), and 21-30 (*Senggan* variant)

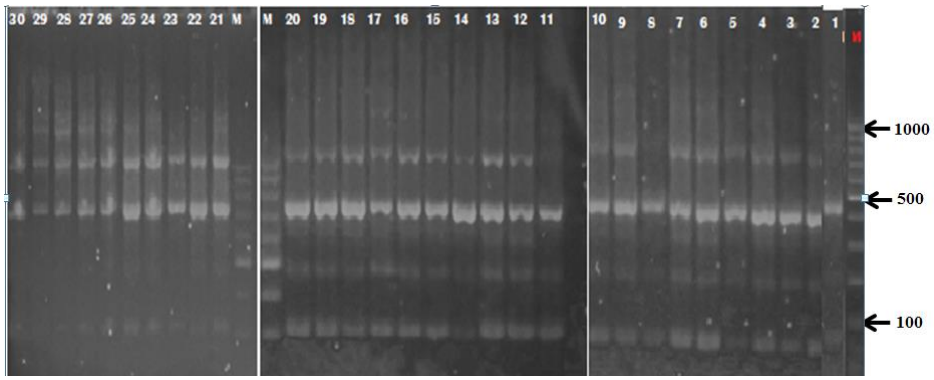

Fig-3: RAPD profile of local cultivar of Singgalang cabbage was amplified using OPA-9: accessions 1-10 (*Biaso* variant), 11-20 (*Batang Hitam* variant), and 21-30 (*Senggan* variant)

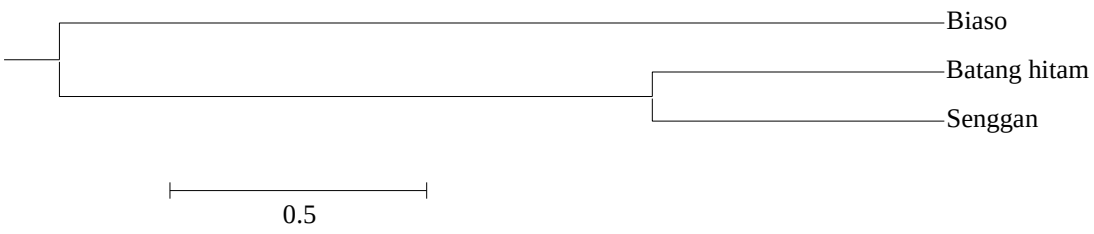

Fig-4: UPGMA dendrogram based on Nei's genetic distance showing differentiation between three variants of a local cultivar of Singgalang cabbage

CONCLUSION

The *Batang Hitam* variant has highest heterozygosity value (0.2725) with 0.3932 of Shanon diversity index value dibandingkan dua variants lainnya compared to the other two variants. Genetic variation among variants are categorized as low (0.3917) but the gene flow showed highest (0.7763). Genetic distance between *Biaso* and *Senggan* variants have highest value (0.4057), and followed by *Biaso* and *Batang Hitam* variants with the genetic distance of 0.3273.

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