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Genetic Characterization of Maize Kisar Var. Kuning Genjah and Maize Var. Bisi-II-Hibrida Based On Molekular RAPD Marker

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Abstract

The variety of maize in Southwest Maluku (MBD) has an ability to grow in the extremely dry land, especially at Kisar island, because those areas were dominated by dry land. Dry resistance very useful to be used for genetical characterization in order to provide accurate data about the character of local maize Kisar var. Kuning Genjah. To identify the characteristics at the genome level of this local maize variety, one of the molecular methods that can be applied was RAPD molecular method. The advantage of the RAPD marker is it's technically simpler and fast in testing, does not require DNA sequence information, hence this marker becomes widely used, it only requires a small sample of DNA, the primary is commercially available and does not use radioactive compounds. The aim of this study was to find out the bands profile that has the potential to be used as a marker of dry resistance. The method applied was RAPD (Random Amplified Polymorphic DNA) using OPA-02, OPA-07, and OPC-12 primers and produces 22 DNA bands. The measurement of bands by estimating the molecular weight based on marker exponential regression. The polymorphic percentage was 90.9% between var. Kuning genjah and var. BIZI-II-Hybrid. The percentage of polymorphism showed the potential of bands that can be used as molecular markers for markers of dry resistance that can be utilized in plant breeding

Keywords**:** genetic, maize kisar and BISI-II-Hibrida, RAPD

A. Introduction

Maize (*Zea mays* L.) is a staple food in the world besides rice and wheat.In Indonesia, some region such as Madura, Gorontalo, West Nusa Tenggara (NTB), East Nusa Tenggara (NTT), Minahasa, Maluku and Papua consumemaize as a staple food. In Maluku, maizehas been cultivated as a local commodity which is not less competitive compare to other local commodities. The cultivation area of maize in Maluku are divided into three, namely cultivation area I, covering West Southeast Maluku Regency (maize as staple food); cultivation area II, covering Southeast Maluku Regency, Aru Islands and Central Maluku (maize as a side business); and cultivation area III, including West Seram Regency, Buru Regency and North Seram District (maize as a commercial business) (Susanto & Sirappa, 2005). The maizeoccupies the second largest commodity cultivated in Maluku, which is 6.463 ha, mainly in West Southeast Maluku, West Seram, Buru and Central Maluku (Pesireron and Senewe, 2011).

The maize varieties in Southwest Maluku (MBD)growing at the range of tolerance to the dry land, especially at Kisar Island, because the area is dominated by dry land. Most of the land is dry land overgrown with forest plants, shrubs, grasslands, and shifting cultivation (Susanto and Sirappa, 2005). The maize in this area is really diverse in variety and morphology than maize in general. Specific local maize varieties found on Kisar Island, Southwest Maluku Regency are yellow, kuning genjah, red-pomegranate brown-cob, red-pomegranate white-cob, blood-red, white, and red (Susanto & Sirappa, 2005). The variety ofmaize used in this study wasvar.kuning genjah.

The characteristic genome level of local maize Maluku can be determine with the RAPD method (Random Amplified Polymorphic DNA). The RAPD marker is technically simple and fast to testing, does not require DNA sequence information, used widely, the requirement for only a small of DNA, the primary is commercially available and does not use radioactive compounds (Zulfahmi, 2013).The primers selected are often used in molecular research on maize or those that are closely related, the selection was done by using the RAPD method with a high level of polymorphism, high rates, and producing a clear band pattern. Several studies on the genetic characterization of maize had been repoted, i.e. the characteristics and kinship of local Bebo maize from Sangalla Tana Toraja, South Sulawesi with carotenoid syn 3 origin from simmyt based on molecular markers simple sequence repeat (SSR) (Siga, Juhriah, Masniawati, & Asnadi, 2015). Genetic variation and relationship between Turkish Flint Maize landraces by unclear RAPD markers (Okumus, 2007) and morphological and molecular characterization of strawberry and yellow popmaize (*Zea mays* L. Everta group) (Indhirawati, Purwantoro, & Basunanda, 2015). Considering the investigation, the author are interested to identify the morphological character of maize Kisar var Kuning Genjah and band profile (DNA band pattern) of the maize Kisar var Kuning Genjah with maize var. BISI-II-Hybrid using RAPD marker.

B. Methodology

The study was conducted from Mei 2016 tothe end of January 2017 in Kisar Island (Ambon) and Biotechnology center research and Bioindustry Indonesia (PPBBI), Bogor. The sample used was maize Kisar (*Zea mays* L) var. Kuning Genjah from Kisar Island which was cultivated in Ambon and a control var. BISI-II-Hibrida The tools used in this study were, millimeter block, stationery, camera, scissor, mortar and pastel, eppendorf tube, vortex, incubator, fume hood, centrifuge, refrigerator, freezer, laminar air flow cabinet or blower, analytical scale, tweezers, autoclave, oven, 0.5-10µl micropipette, 10-100µl micropipette, 100-1000µl micropipette, 2-20µl micropipette, 500-5000µl micropipette, NanoDrop Spectrophotometer, PT-100 MODEL PCR machine (MJ Research) thermocycler, 1.5% agarose gel, uv transluminator. The materialsused in this study were label paper, aluminium foil, wrapping, medicinal plastic, cotton, gauze, tissue, ice crystal, dish soap, dishwashing sponge, brown envelope, glove, mask, liquid nitrogen, poly (1-ethenylpyrrolidin-2-one) (PVPP), extraction buffer, β-mercaptoethanol 1%, chloroform: isoamil alcohol (24: 1), cold

isopropanol, TE buffer, Na-acetate, absolute ethanol, 70% ethanol, RNase, 70% alcohol, TAE buffer, KAPA2G Fast ReadyMix PCR reagent, Nuclease-free water.

The sample preparation began with washing the leaves of maize and then being dried using tissue paper. The dried sample was cut and the leaf bone was removed to facilitate the smoothing process. The equipments used were previously wet sterilized using an autoclave and followed by dry sterilized with an oven to avoid contamination. The leaf samples of maize were mashed until smooth by using a mortar while being added to liquid nitrogen. Then 0.1 gram (poly1-ethenylpyrrolidin-2-one) (PVPP) was added to the mortar. Samples and PVPP were added liquid nitrogen slowly then the sample was groundedfurther. Adding liquid nitrogen continuously during the smoothing process. Approximately 0.1 gram sample was then transfered into a cold eppendorf tube and put it into liquid nitrogen. DNA isolation started with 5 mL of the extracted buffer and 500 µL β-mercaptoethanol 1% which both had been heated were mixed, then shaken with vortex and incubated for 30 minutes at 65ºC. Every 5 minutes the tube was shaken tofasten the reaction. The sample was left to cool in a fume hood, then 5 mL of chloroform solution: isoamilalcohol (24: 1) was added. Samples were centrifuged at a speed of 11.000 rpm for 10 minutes at 25ºC. The supernatant was removed, then added 5 mL of chloroform solution: isoamilalcohol (24: 1), shaken with vortex and centrifuged again at a speed of 11.000 rpm for 10 minutes at 25ºC. The supernatant was removed and then 1x volume of cold isopropanol was added. The sample was homogenized by flipping the tube and then stored in a refrigerator (4ºC) for 30 minutes then centrifuged again at a speed of 11.000 rpm for 10 minutes at 25ºC. The obtained supernatant was removed while the obtained pellets were dried. After drying, the pellet was dissolved with 1 mL TE buffer and then shaken. One-tenth of 3M's volume of Na-Acetate pH 5.2 and 2.5 mL absolute ethanol were added and shaken until a collection of white DNA fibers was seen. Samples were stored in a freezer -20ºC for 30 minutes or overnight. Samples were centrifuged at a speed of 12.000 rpm for 10 minutes at 4ºC. The supernatant obtained was removed and the pellets were dried. Pellets were washed with 70% ethanol as much as 100 µL. The mixture is centrifuged again at a speed of 8,000 rpm for 5 minutes at 25ºC. The supernatant was removed and the pellets were dried in a laminar airflow cabinet or the blower was activated. The dried pellet was added a TE buffer solution of 30 µL and homogenized to a pellet and a homogeneous solution.

The RNase around 1/10 of the volume of DNA was added to the DNA. The 3 µL of RNase was added to DNA and incubated at 37°C for 30 minutes. Test the quantity and quality of DNA using Absorbance (A) measured at wavelengths of 230 nm, 260 nm, and 280 nm. Ratios of A260: A280 and A260: A230 were used for DNA purity measurements.

The amplification carried out on the PT-100 MODEL (MJ Research) thermocycler. In the tube, 0.2 ml added 12.5 µl of Fast ReadyMix KAPA2G PCR reagent, 1 µl of 10 µM primer, 2 µl of DNA template, and 9.5 µl of Nuclease-free water (total the reaction of 25 µl).

PCR was programmed as follows: pre-denaturation at95^{o}C for 3 minutes, denaturation at 95^{o}C for 15 seconds, annealing at 35^{o}C for 15 seconds, extension at 72^{o}C for 15 seconds, post-extension at 72^{o}C for 7 minutes. The PCR reaction was carried out in 45 cycles. The 18 primers was used to amplification, namely OPA-02, OPA-04, OPA-07, OPA-11, OPA-14, OPA-17, OPB-06, OPB-08, OPB-13, OPC- 04, OPC-05, OPC-08, OPC-12, OPC-15, OPC-19, OPD-04, OPJ-02, OPN-09. The PCR results were electrophoresed on 1.5% agarose gel for 90 minutes then visualized under UV transluminator and photographed with black and white polaroid. Data analysis used the descriptive by giving a score as the presence (1) or absence (0) of a DNA band.

C. Results and Discussion

The morphological character can be identified physically. The character will provide data information that is intended as one of the distinguishing characteristics of other plants. Based on the morphology observation both of maize kisar var. Kuning Genjah and maize var. Hibrida such as vegetative and generative characters can be identified. Observation on these two characters was

conducted on maize plants at the age of fruiting. Vegetative characters (Table 1) observed were leaf color, leaf apex, leaf lamina direction on the stem, stem color, total leaf number, leaf length, leaf width, and plant height. For generative characters (Table 2) observed were cob length, cob shape, cob width, seed row arrangement, number of seed rows, seed color, grain length, grain width, and grain thickness.

Table 1. Vegetative characteristics of maize Kisar var. Kuning Genjah and maize var. BISI- II Hibrida.

Characteristics	Kisar Kuning Genjah	Bisi II-Hibrida
Leave colour	Green	Green
Leave apex	Acute	Acute
Leave Lamina	Melengkung	Melengkung
Stem colour	Green to reddish	Green to yellowish
Total number of leaves	11. 13	11. 15
Leaf length (cm)	43,2-85,5	38-96
Leaf width (cm)	4,2-7,25	4,5-7,4
Plant hight (cm)	189,3-220	215- 245

Table 2. Generative characteristics of maize Kisar var. Kuning Genjah and maize var. BISI- II- Hibrida

Characteristics	Kisar Kuning Genjah	BISI-II-Hibrida
Cob shape	Conical cylindrical	Conical cylindrical
Cob length(cm)	6,9. 13,5	21.21,9
Cob width (cm)	3. 4	5,7. 5,9
seed row arrengement	curved	straight
Number of seed row	13.14	16.18
Seed colour	Kuning genjah	Yellow
Grain lenght(cm)	0,6 - 0,8	0,8 - 1,2
Grain width(cm)	0,3-0,6	0,5-0,9
Grain thickness (cm)	‘0,5 - 0,7	0,9 - 1,2
Cob width (cm)	3 - 4	5,7 - 5,9

The morphology character comparison is recessive, there was no difference between the same type of plants. stated that morphological character more effectively to measure diversity on plant based on the phenotype in the vegetative and generative phases (Kuswandi, Sobir, & Suwarno, 2014). The morphological character both of the maize Kisar var Kuning genjah and the maize var BISI-II-Hybrid showed similarity in vegetative (Table 1) and generative (Table 2). The identical characterson vegetative phasewere leaf color, leaf apex, the direction of leaf strands on the stem, the total number of leaves and leaf width. The different characterswere stem color, leaf length, and plant height. The identical character on generative phase was cob shape. The different characters include cob length, cob width, seed row arrangement, number of seed rows, seed color, grain length, grain width, and grain thickness.

The observation of maize Kisar var. Kuning Genjah and maize var. BISI-II-Hibrida were carried out in separate place, thus the environment has given side-effect in morphological characters (Singh, Srivastava, Srivastava, & Srivastava, 2011). Therefore, morphology identification only shows the character as a natural character or a character that has been affected by the environment, hence it was difficult to be distinguished morphologically. Based on morphological character observed, there was a difference between the maize Kisar var. Kuning Genjah and maize var. BISI-II-Hybrid. It can be due to maize var. BISI-II-Hybrid is a result of plant breeding from crossing maize superior elders and produce a variety with superior character, whereas the difference in morphology can be

seen clearly, however, both of maize have some similar character. Genetic assay has been done to identify diversity and distinguish character through morphological observations.

The process isolation of DNA was purification of DNA sample by separating DNA compounds from impurities such as RNA, phenol compound and other polysaccharides. Furthermore, calculated the quantity and quality of DNA in RAPD-PCR process using nanodrop spectrophotometer. For quantitative test, the DNA concentration used for amplification was the Ambonesia sample (KG) with a concentration of 62,8 ng/µl and BISI II Hybrid with a concentration of 86 ng/µl. In addition, the DNA sample used had purity level of A260/ 280 1,83 for the Ambonesia sample (KG) and 1,81 for the BISI II Hybrid sample, A260/230 2,55 ratio for the Ambonesia sample (KG) and 2,41 for the BISI II Hybrid sample. Through this test, the results of total DNA genomic electrophoresis from both types of maize can be seen (Figure 2).

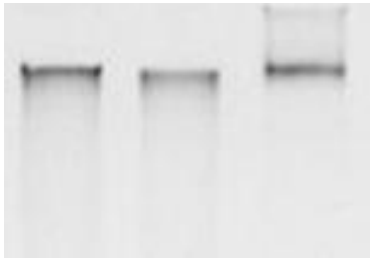

Figure 1. Genome profile of DNA Ambonesia KG & Bisi II Hibrida.(M) Marker Lambda DNA, (1) Ambonesia KG sample, (2) BISI II-Hibrida sample.

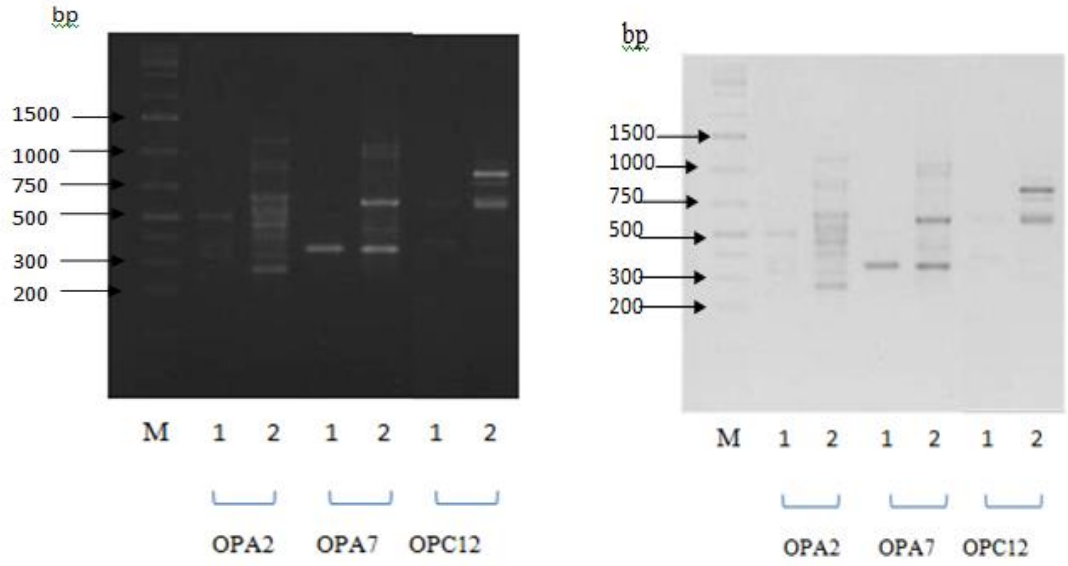

Figure 2. Maize DNA banding pattern using OPA-02, OPA-07, and OPC-12. primers -2-hybrid. Description: (M) marker 1 kb pluss ladder thermo, (1) maizeKisar var. Kuning Genjah sample, (2) maize var BISI-2-Hibrida sample.

In the RAPD-PCR reaction of 18 primers tested, only 3 primers amplified the DNA of both plants properly. The amplification result revealed the profile of DNA bands from maize Kisar var. Kuning Genjah and maize var. BISI-II-Hybrid using 3 primers namely OPA-02, OPA-07 and OPC-12 (Figure 2). From those results, another step to determine the DNA band size in each primer had to be calculated manually using a ruler on a computer screen and using Microsoft Excel in processing data to estimate the DNA band size produced in each primer with a regression approach. The

regression approach is used to estimate the DNA band size from the amplification results based on marker ladder. The estimation of using this approach must be close to precision as if the size of the DNA band is the same as the marker ladder. After getting the estimated size of the bands then validated to determine whether the size is close to precision or not. The non-linear (exponential) graph is chosen as a model in estimating DNA band size because it has an R-value that is close to precision (0.991) and higher than other graphs, hencefrom the graphic showed the regression equation of $y = 4472.e^{-0.96x}$, then it can be used to estimate the size of each ribbon by determining the distance of each band first as a variable x. From the results of the regression analysis, polymorphic bands and monomorphic bands can be determined.

The results of total DNA genome amplification (Figure 1) show thick and clear bands, hence the use of the research method based on the Orozco Castillo method is able to isolate genomic DNA properly. The qualitative testing of DNA also shows that isolated DNA is pure. The results of DNA band amplification can be clearly seen to facilitate the scoring of DNA bands (Figure 2). The resolution of each DNA band from electrophoresis is not always clearly visible, depending on the number of fragments amplified in the plant genome. The difference in DNA band intensity was influenced by the distribution of the primary attachment site in the genome, the purity and concentration of the genome in the reaction. (Sembiring, Putri, & Setiado, 2015). The purity of DNA was determined by the level of contamination of proteins in solution larutan (Sinaga, Putri, & Bangun, 2017). DNA molecules are pure if the ratio A260 to A280 ranges from 1,8 – 2,0. If the ratio smaller than 1,8, there is still contaminant of protein or phenol in the solution. If the ratio value greater than 2,0, there is still RNA contaminant in the solution.

The result of the qualitative test showed that the A260/280 DNA sample used was pure DNA with 1,83 for the maize kisar var. Kuning Genjah and 1,81 for maize var. BISI-II-Hybrid. Meanwhile, for A260/230 DNA sample contaminated with polysaccharide compounds, namely 2,55 for Kisar maize var. Kuning Genjah and 2,41 for maize var. BISI-II-Hybrid. In DNA isolation stage to separate DNA from other compounds such as polysaccharide can be done by adding reagents such as NaCl. The contaminant can interfere with enzyme activity and DNA amplification process. The purity DNA is closely related to concentration DNA sample to be tested in the RAPD-PCR. If the DNA concentration is high, the purity DNA will below, it means the impurities in the DNA are in large quantities. Conversely, if the DNA concentration is low, the DNA purity will be high due to the little amount of impurity. The number of bands produced is dependent on primer to recognizing the homolog in the DNA mold (Rahayu & Handayani, 2010). The size of DNA product bands amplification ranges from 263-1200 bp for the three different primers .

Table 3 Score of DNA bands, size (bp), and polymorphic percentage

No.	Primer	Amount of band		Size (bp)
		Monomorphic	Polimorphic	
1.	OPA-02	-	10	498; 1200; 944; 891; 618; 525; 433; 386; 307; 263
2.	OPA-07	-	7	496; 331; 1144; 1029; 578; 425; 328
3.	OPC-12	2	3	567; 365; 809; 273; 567
Polimorphyc (%)		2	20	

Polymorphism is amplification description that obtained from difference in DNA fragment studied and scoredits different sequence to show any variation(Oktavia, Munir, Suryatingtyas, & Kuswanhadi, 2011). The number of polymorphic DNA bands in genetic diversity analysis influences the diversity ona population, because the number of polymorphic DNA bands will be more capable to clearly describe the situation of plant genomes and reduce the bias due to the non-representation of the genome parts of (Kawengian, Lengkong, & Mandang, 2016).

The percentage of polymorphism bands analysis was calculated to gain a percentage of the polymorphism bands formed in each primer was used (Carsono, Lukman, Damayanti, Susanto & Sari, 2014) In this study, the percentage of polymorphism was 90.9%. Based on DNA amplification that the maize var. BISI-II-Hibrida was contributed more polymorphic bands compared to maize Kisar var.Kuning Genjah. Itcan be due to the maize var. BISI-II.Hibrida is a plant breeding product with significant character such as superior elders controlled crosses and controlled environment conditions, meanwhile maize Kisar var Kuning Genjah is local maize which still retained its own natural character and is not plant breeding product. High polymorphic percentages indicate a high genetic variation of maize BISI-II-Hybrid. Maize Kisar var. Kuning Genjahis very different from BISI-II-Hybrid maize both morphologically and genetically. Genetic variations are very influential in the selection process in plant breeding strategies. Thus, the higher genetic variation the chance to obtain gene sources that characterize the desired plant is higher(Hijria, Boer, & Wijayanto, 2012)

D. Conclusions

The results showed that maize Kisar var. Kuning Genjah is very different to maize var. BISI-II-Hibrida, and DNA band profile from the RAPD analysis using the three primers showed 22 DNA bands with a high genetic variation of 90,9% in size of 263-1200 bp.

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