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RESEARCH ARTICLE

GENETIC VARIATION AMONG THE SCABIES-INFESTED SWEET POTATO CULTIVARS

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ABSTRACT

Sweet potato tuber posses various nutritious content makes them fit for food source, whether consumed as the whole, or their derivatives. These plants are susceptible to diseases including scabies. Current study was aimed to show polimorphism patterns of 10 different scabies-infested sweet potatto cultivars using 10 RAPD-PCR primers. For this purpose, 10healthy individual sweet potato plants were initiallyinfested with the *Sphaceloma batatas* spores then isolated for their DNAs using a modified CTAB.Current data showed the DNAs ranged from 61.5-1011 ng/ml, but contaminated witheither protein or polyphenol. Six primersresulted 100% polymorphism on the 10 cultivars with different total numbers of loci. The highest polymorphismsappeared on OPA-11 which showed 12 loci with 35 bands, while the highest number of bands were 48 bands was produced by OPA-3 with 8 loci. Other primers, however, showed 1-3 loci monomorphic. The cultivars might be grouped into four major groups and two sub groups within group I and III. The Borobudur and Sari cultivars were closely related with genetic distanceof 0.181.

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INTRODUCTION

Sweet potatoes contains nutritious content such vitamins, minerals and carbohydrate but traced in lipid thus fit to be consumed by people with high cholesterol. Because of that, the American farmers cultivate it massively up to 135,000 acres (Naeve, 2015) and was predicted to be increased due to promotion of the tuber's health benefits. In Indonesia, however, sweet potatoes are mainly cultivated in Java and Irian Jaya. As a tropical country, the Indonesian agricultural activities face various plant-disease organisms (Juanda and Cahyono, 2000) lead to a requirement of well management practices from planting to harvesting. Alternatively by cultivating disease-free planting material which so far, about 172.5 million Hectares area have been being used mainly for maize and cotton; a double digit increasement (James, 2013) due to development of new cultivar by changing the plant's genetic characters. The plants' profiles, therefore, change its characters including nutrient contents, defence and survival characters against biotic and abiotic stresses (Bennet, 1993). The genomic DNAs (deoxyribo nucleic acids) might be analysed using the PCR-RAPD (Polymerase chain reaction-rapid amplification of polymorphic DNA) which is able to detect polymorphism among the individuals or cultivars randomly according to their genetic characters in an easy, fast, cheap and simple steps (Williams *et al.,* 1990). stated the PCR-RAPD might be run without any initial information of the template's nucleotide, small amount of DNA and short primers.

The PCR-RAPD has been being reported to be applied for identifying genetic variations in various types of plants and animals (Poerba *et al.,* 2007; Langga *et al.,* 2012; Purnomo, *et al.,* 2018). Current study was aimed to explore the polymorphisms among those 10 scabies-infested sweet potato cultivars using 10 RAPD-PCR primers.

MATERIAL AND METHODS

Source of genomic DNAs: Current study selected 10 different sweet potato cultivars namely: Cangkuang=Ca, Cilembu=Ci, Ungu tua=Utuh, Solosa kuning=So, Kidal=Ki, Borobudur=Bo, Sari=Sa, Sutuh=Si,Antin=An, and Beta=Be and tested for polymorphisms against *Sphaceloma batatas*. The healthy plants were initially infested by *Sphaceloma batatas* spores to cause scabies. Polymorphisms were tested using 10 different 10-mer RAPD primers: OPA-1, OPA-2. OPA-3, OPA-9, OPA-11, OPA-13, OPA-13, OPA-15, OPA-16 and OPA-18. Genomic DNAs were isolated according to a modified-CTAB (cetyltrimethylamonium bromide) as followed: 2 grams scabies-potatoes leaf of each cultivar was taken and cleansed from dirt then dried. The dried leaf was grinded using mortar and pestle and added by 1.500 µl CTAB buffer (Table 1) which was pre-heated for 30 minutes at 65^{o}C in a waterbath then with β mercaptoethanol. Mixture was then transferred into a microcentrifuge tube and warmed in awaterbathfor 80 minutes at 65^{o}C, and centrifuged for 10 minutes at 11,000 rpm.

Supernatant was transferred to new tube and added by 800 µl cold CIAA (chloroform Isoamyl alcohol 24:1) then vortexed for 5 minutes and re-centrifuged for 20 minutes at 11,000 rpm. The supernatant was taken to new tube and added with ammonium acetic as much as 1/10 of the supernatant volume, and cold absolute ethanol 2/3 of the supernatant volume, then incubated over night at -20^{o}C. The next day, re-centrifuged for 20 minutes at 11,000 rpm at 4^{o}C; supernatant was thrown out and kept the pellet and added with 750 µl of 70% ethanol, and centrifuged for 5 minutes at 11,000 rpm. Supernatant was thrown and the pellet was dried up by putting the micro centrifuge tube upside down to dry and added by 100 µl TE (tris-EDTA) 1x buffer and kept at -20^{o}C for further uses. The obtained genomic DNAs were measured for their concentration by spectrophotometer.

Table 1. Components of buffer modified CTAB

Number	Comppounds	Volume 1 x Reaction (µl)
1	CTAB 10%	300
2	Tris-HCl 1M pH 8	150
3	EDTA(ethylenediaminetetraacetic acid) 0,5 M pH 8	60
4	NaCl 5 M	420
5	SDS (Sodium dodecyl sulfate)	270
6	β-mercaptoethanol	15
7	Sterile Aquadestillate	285

PCR-RAPD Amplification: PCR-RAPD was performed in 13 µl total volume containing 1 µL 50 ng/ml genomic DNA, 1 µl primer, 6 µl PCR super mix, and 5 µl ddH2O. Amplification was set as 5 minute at 95^{o}C pre-denaturation, run for 45 cycles consisting of 30secondsdenaturation at 94^{o}C, annealing for 30seconds at 40^{o}C, extension for 1 minute at 72^{o}Cand post extension for 7 minute at 72^{o}C and soaking at 4^{o}C. The obtained genomic DNAs were liquidified to theend concentration of 50ng/ml each prior to. Amplification of the DNA template strongly depends on the primer (s) since each of them has its own position to stick, leading to different polymorphisms (numbers and size of the base pair/bp)as wells the DNA fragment (s). Selected primers were stated on the following table(Table 2) as stated by Maftuchah, (2009) where OPA primers showed better polymorphisms than OPF ones.

Table 2. List of primers used along the study

Primer	Sequence (5'-3')	Product size (bp)
OPA-1	CAGGCCCTTC	250-1,300
OPA-2	TGCCGAGCTG	250-2,000
OPA-3	AGTCAGCCAC	250-1,300
OPA-4	AATCGGGCTG	300-1,450
OPA-9	GGGTAACGCC	250-1,500
OPA-11	CAATCGCCGT	250-1,500
OPA-13	CAGCACCCAC	250-2,300
OPA-15	TTCCGAACCC	250-1,200
OPA-16	AGCCAGCGAA	350-1,400
OPA-18	AGGTGACCGT	250-1,500

RAPD-PCR finger printings: Visualization of PCR-RAPD products were performed in 1% agarose gel and interpreted for their polymorphisms. The fragments were then analysed using a statistical program Mega 7.02 for their genetic variability and relationship.

RESULTS AND DISCUSSION

Total genomic DNA yield and quality: The total genomic DNAs were measured for their quantity and checked for quality using a spectrophotometer applying two absorbance ratios of λ260/λ230and λ260/λ280, for detecting any contaminants (polysaccharides or protein) (Gupta and Preet, 2012). These contaminations might lead to no amplification, because they blockage activity of Taq polymerase. Table 3. Showed the total genomic DNAs of samples ranged from 61.5 to 1,011 ng/µl which were enough for RAPD-PCR (Anggereini, 2008) with absorbances of 0.33-1.17 (λ260/λ230) and 1.06 to 2.01 (λ260/λ280). These absorbances indicating the current genomic DNAs were contaminted by either protein or polyphenol (Sambrook *et. al.,* 1989) which were not surprisingly happened, as these two compounds might interfere in plant's cells walls, thus make them difficult to be split from the genomic DNA without specific precaution on the extraction steps.

Table 3. DNA concentration of the scabies infested-sweet potato cultivars using spectrophotometer

Varieties	Concentration (ng/ml)	λ260/λ230	λ260/λ280
Cangkuang	61.5	0.330	1.139
Cilembu	287	1.174	2.014
Ungutua	1,011	1.000	1.062
Solosa kuning	208	0.935	1.921
Kidal	172	0.520	1.814
Borobudur	330	0.801	1.791
Sari	121	0.339	2.252
Sutuh	104	1.000	1.000
Antin	96.5	0.680	1.874
Beta	78.2	0.476	1.896

Varadarajan and Prakash (1991) stated if the plant's secondary metabolites as latex, polysaccharides, and protein interfere with the extraction of high-quality DNA from sweet potatoes. Polysaccharides are more difficult to be split from DNA since it produces matrix. Isolating genomic DNA, therefore, need a proper step both physically and/or chemically. Some research reports for examples, reported involvement of liquid nitrogen to freeze the cells, adding some homogenised beads, keeping the material in a dark room for up to 2 days, pre heating mortar and pestle or using beads prior to and during grinding the leaf to a powder (Doyle and Doyle, 1987; Goldberg, 2008; Sharma, 2008). Current modified CTAB method did not produced pre genomic DNAs. Others, suggested chemical compounds such as the used of β mercaptoethanol, PVPP, natrium bisulphite, sucrose and ascorbic acid (Surzycki, 2000; Varma *et al.,* 2007; Syafaruddin *et al.,* 2011; and Nugroho *et al.,* 2017). β mercaptoethanol was used to reduce the amount of disulfide bonds during denaturation of protein stick on the cell's wall. Current data involved 5M NaCl, but not fully able to separate the contaminants , indicating the protein content in the scabies infested sweet potato has already fully oxidized and irreversible bind to the nucleic acid (Varadarajan and Prakash, 1991). Furthermore, Fitriya *et al.,* (2015) reported the genomic DNAs of three bacterial cells were contaminated by protein (λ260/λ230= 2.01-2.28). Syafaruddin *et al.* (2011), added 1.5% PVPP resulted in 1.80-1.91 purity λ260/λ280 of their cashew nut DNAs,. Even though β mercaptoethanol applies to overcoming protein binding, but. However, it did not perform well here which might was due to low concentration was added, since the Bio-Rad Laboratories suggested 50 µl to make 1.000 µl solution buffer.

Amplificatin of PCR-RAPD markers: The better quality of DNA templatethe better PCR products would be. Current study used the genomic DNAs and amplified using ten different 10-mer primers which agreed to Poerba *et al.* (2007), supposed to have 10 bp length but without any palindrom sequence. Current study used 13 µl solution consisting of 1 µL DNA, 1

µl primer, 6 µl super mix, and 5 µl ddH2O. Amplification was run for 45 cycles in a Thermo cycler machine (PeqLab, Primus 25 type) and set as follows: 5'95^{o}C pre-denaturation; 30'94^{o}C denaturation, 30'40^{o}C annealing, 1'72^{o}C extension and 7'72^{o}C post extension prior to soaking at 4^{o}C. Table 4. Showed polymorphisms among those genomic DNAs of 10 scabies infested sweet potato using 10-mer RAPD primers with total bands reduced were 359 bands. This table indicating complementary sequences between DNA templates and primers except for OPA-2 and OPA-4 which did not show any band in any locus of the Antin and Cilembu cultivars. Current study was focused on polymorphisms pattern among those cultivars by involving ten different 10-mer RAPD markers. Tingey *et al.* (1994) stated the numbers and intensity of the DNA fragments will strongly depends on how complement the DNA sequence of the primer to the sequence of DNA template. The data of those 10 cultivars 6 cultivars showed polymorphism varied between 75 to 100%which located in various length between 250bp and 2,700bp, though they were having different numbers and intensity, Weeden *et al.* (1992) stated some compounds like polysaccharides and/or phenol even in low concentration might caused thin amplification of the fragment. A particular fragment might also be amplified several times but another in few times only because there is such a competition between the sequence of the primer to the template.

Table 4. Polymorphisms of the 10 scabies infested sweet potato cultivars

Primers	Number of loci	Polymorphic	Monomoprhic	Total bands
OPA-1	9	8	1	42
OPA-2	9	9	0	44
OPA-3	8	8	0	48
OPA-4	5	5	0	25
OPA-9	7	7	0	34
OPA 11	12	12	0	35
OPA-13	8	7	1	43
OPA-15	4	3	1	20
OPA-16	8	7	1	38
OPA-18	6	6	0	30
Jumlah	76	72	4	

Following to the amplification, current study showed positive results by means all DNA templates were amplified, indicating gene (s) alteration between the cultivars were tested. Here, 60% of the PCR primers produced polymorphisms, 197 bands which spreaded in 46 loci at various distance from 250bp to 1,700 bp. and 40% monomorphic pattern. Nayak *et al.* (2003); Subositi and Widiyastuti (2013) stated that polymorphisms meant as genome complexity lead to genetic variability among the samples. However, 3 of those 10 primers were selectednot able to produce single fragment on Antin, Beta, and Cilembu cultivars; which might be caused by unsuitable PCR program or uncomplementary of DNA sequence between template and primers. Within the polymorphic group, some cultivars had thicker bands than others (Ut, So, Bo, An, and Be cultivars) which might due to the nitrogen base of the primer could not stick on the correct sequence of the DNA template or low quality DNA template.

Polymorhic groups: The OPA-2 primer produced 38 clear bands which produced 100% polymorphic patterns spreaded in 12loci with various length from 250bp to 1,770bp. At 500 bp, the OPA-2 primer produce some thicker fragments than the longer one, but produced blank columnon Antin cultivar because of poor quality of genomic DNA (λ260/λ2801.87) which blockage the primer to stick on any locus (Sunandar and Imron, 2010). All alleles appeared in loci ranged from 250 to 2,000bp, indicating strong genetic variation of the samples thus no more similar performance for particular phenotypic character. If this primer was able to produce band (s), it will show monomorphic pattern (s). The blank column, however, did not parallel to DNA sequence unavailability, but more related with some human errors (PCR setting condition, or pip petting). Since others however, produced 1 to 6 alleles, as also noted by Winarsih *et al.* (2017) OPA-2 primer did not produce any fragment of cytochrome B coding gene of swine inside the instant noodles. OPA-4 primer in producing 24 visible bands spreaded in 5 lociat 250bp to 1,200bp in a polymorphic pattern. But it also showed zero band on Cilembu which probably more related with some human errors. At 650bp locus, other primers were able to produce at least 1 allele.

OPA-9 primer produced polymorphism with 32 clear bands which were spreaded in 6 loci with various length from 250bp to 850 bp, indicating high genetic variation of the samples. Some DNA fragments, however, were thicker than others. Short size and good quality and quantity of DNA template allowed the sequence of OPA-11 primer produced 41 fragments clear which spreaded on 250bp to 1,500 bp, indicating the well run of PCR during the annealing phase within the cycles. Two samples (Cilembu and Borobudur), however, produced single fragment each at almost similar length (250bp) indicating at a particular locus for amplification there was different nucleotide sequences between the template and the primer. OPA-13 primer produced 45 polymorphic pattern which spreaded in 9 loci from 375bp to 2,700 bp, indicating high genetic variation among the 10 scabies infested sweet potato cultivars. The fragments, however, were not visible clearly and even showed as thick fragments at 350 and 800 bp, indicating at that locus few genes located. The Sutuh cultivar which produced only single thin fragment showed if the nucleotide did not fit to the primer's. OPA-18 primer produced 100% polyorphisms with 25 bands in 5 loci spreaded in 250bp to 1,550bp. A single clear allele pattern were shown in the Utuh and Borobudur cultivars at very closed distance, indicating if these cultivars performed similarly to that particular gene.

Monomoprhic groups: Monomorphic pattern, by means there is no allele variation appeared shown by Figure B7-10, where 182 bands in quite clear bands which spreaded in 30 loci at from 250 to 1,300bp. The data also showed average number of band 45.5 with 66.66%, 85%, 87%, and 91.66% monomorphisms for OPA-1, OPA-15, OPA-3, and OPA-16 consecutively. However, the OPA-3 produced also in consistent bands of thin band on Beta cultivar indicating low DNA concentration; and thick alleles on others when, two or more genes were present at the same locus coincidentally. OPA-1 primer produced 52 fragments which spreaded at 250 to 1,500bp. This was the second highest numbers but they were having inconsistent size. The primer also produced 1 monomorphic pattern at about 400 bps. OPA-3 primer produced 50 fragments in 9 loci spreaded from 350bp to 1,700bp. Interestingly, the primer produced three groups of fragments at 250 bp, 45-750bp, and >750bp. The most crowded performance seen on 500 to 750 bp where 9 cultivars produced thick fragments indicating two or more genes were located closely, but produced light and thin fragment on Beta cultivar at 500bp only. Indicating the sequence of template's nucleotide was incomplement to the primer.

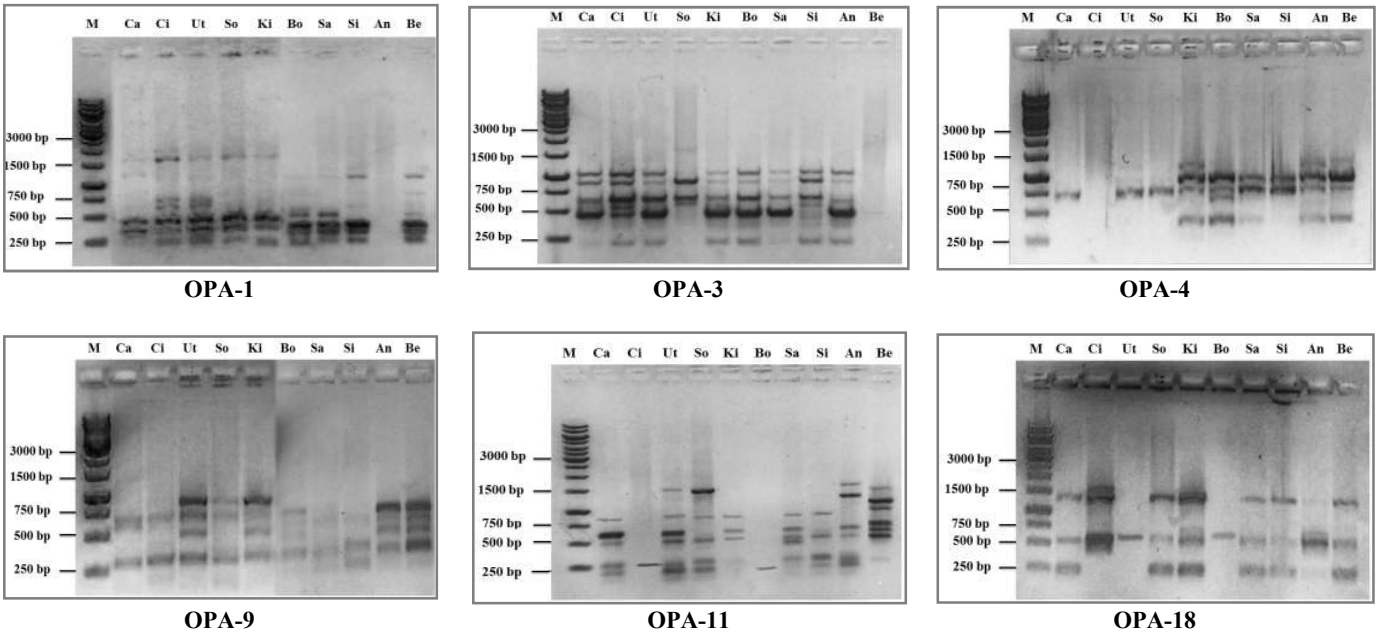

Figure 1. Polymorphisms group of of PCR-RAPD markers using OPA-2, OPA-3, OPA-4, OPA-9, OPA-11,and OPA-18 of the 10 scabies-infested sweet potat

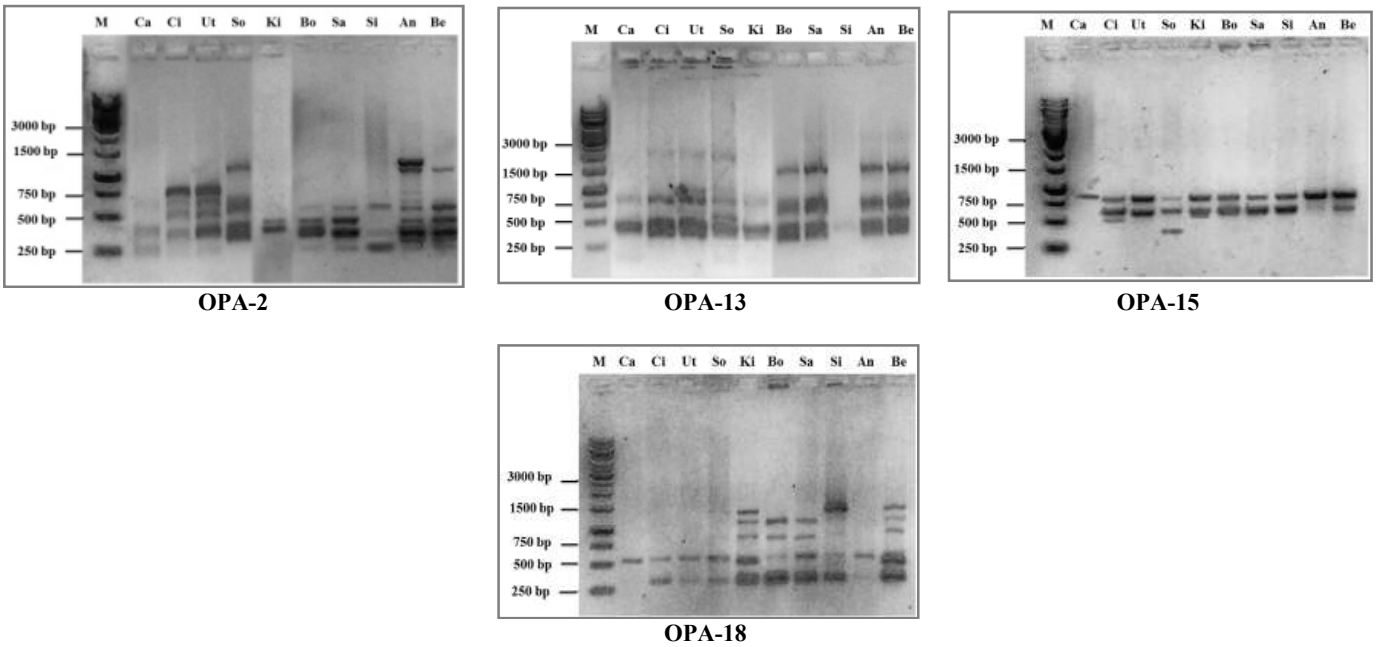

Figure 2. Monomorphisms goupsof of PCR-RAPD markers with one monomorphic pattern at OPA-1; OPA-13, OPA-15; and OPA-16 the scabies-infested sweet potato cultivars

But some thick fragments still appeared on 4 cultivars (Kidang, Borobudur, Solosa kuning, and Beta) which indicating the similar location of two or more genes coincidentally. When the polymorphisms occured, meant the plants had either deletion or insertion on their nucleotides. The primer's sequence, therefore, did not able to stick on the 3' of the DNA sequence of some cultivars and produced blank columns (William *et al.,* 1992; and Semagn *et al.,* 2006); and so caused polymorphisms on the DNAs sequence. Moreover, William et. al. (1992) stated the characterisitc of RAPD was able to detect allel per locus, thus the mutant alleles might not be able to be detected. Genes flow among the mitants might also be detected between populations (Morjan and Riesenberg, 2004); and so increasing genetic variation in the population (Frankham *et al.,* 2002). The longest allele was shown by OPA-13 primer where 70% of the genomic DNAs sample had alleles of 2,300bp while the shortest allele was 250 bp (Figure 1). However, the OPA-16 primer showed also interesting figure since the shortest allele was only 350bp while others was 250 bp. Current data were then agreed to the previous information that the RAPD genome located between 0.1. and 3 kb.

Scoring the RAPD polymorphisms: Scoring the polymorphism was started by assuming the band produced by the primers as putative locus. The DNA bands are in polymorphic patterns when the appearance in the particular locus all samples' template are ≤ 95% and vice versa when they appear on >95% are called as monomorphic. Harkingto (2007), stated the polymorphisms appeared when the amplification show to have different pattern of DNA bands among individuals tested, but when the bands appear of all samples present on the particular locus indicating the monomorphisme existed.

Table 5. Genetic distance within the ten scabies infested sweet potato cultivars

Cultivars	Cangkuang	Cilembu	Ungu t	Solosa k	Kidal	Borobudur	Sari	Sutuh	Antin	Beta
Cangkuang	****									
Cilembu	0.597	******								
Ungu tua	0.506	0.226	*****							
Solosa Kuning	0.550	0.506	0.429	******						
Kidal	1.197	0.997	0.776	0.506	*****					
Borobudur	0.394	0.597	0.709	0.945	0.853	*****				
Sari	0.394	0.853	0.597	0.945	0.853	0.181	***			
Sutuh	0.550	0.853	0.709	0.997	1.197	0.550	0.332	****		
Antin	0.776	0.932	0.709	0.997	1.197	0.550	0.650	0.466	*****	
Beta	1.386	0.932	1.064	0.945	0.303	0.945	0.997	0.945	0.997	****

6 primers (OPA-2, OPA-3, OPA-4, OPA-9, OPA-11, and OPA-8) produced 97.4% polymorphic patterns (Table 4) and so called performed high polymorphisms. As stated by Poerba and Martanti (2008) highly polymorphics of the primers tested were ≥50%. Monomorphisms however, were detected from the rest 4 primers with 143 DNA bands spreaded in 4 loci (Table 4). Performance of OPA-2, OPA-3, and OPA-4 primers, however, showed no amplification on Antin, Beta and Cilembu cultivars were similar to Karsinah *et al.* (2002), who reported 5 primers tested for polymorphisms of the 30 citrus accessions did not amplified at all. But not to OPA-13 primer which was slightly different Karsinah *et al.* (2002). Currently, it showed 87.5% polymorphisms and monomorphic 12.5%, whereas, Karsinah *et al.* (2002) showed 100% polymorphisms. However, in compared with other related studies, this study showed a higher polymorphism ratethan Herawati *et al.* (2018) on local Banyumas thorny plants (*Sallacca sp.*) which was only 80.6% *Kinship relationship within the 10 scabies infested sweet potato cultivars.* The genetic information was analysed according to their p distance done by Mega 7.02 stattistical program (Figure 3). The data were taken to trace for their kinship (Table 5), which ranged from 0.181 to 1.380, where Borobudur and Sari cultivars were closely related in their genetic kinship; but Cangkukang and Beta were opositedly. Nei and Li (1979) stated genetic distance paradox to genetic similarity.

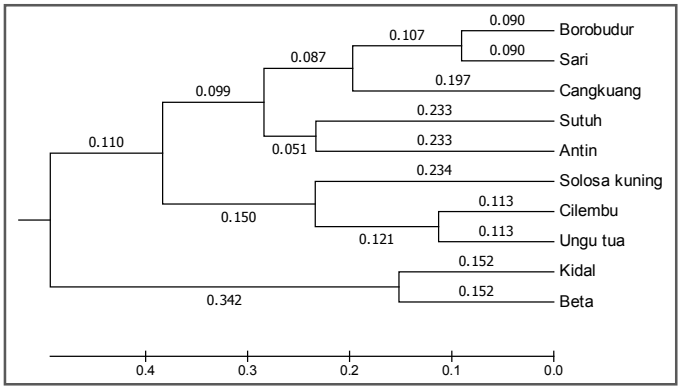

Figure 3. Phenogram often scabies infested sweet potato cultivars analysized by MEGA 7.02

The figure reflected molecular characteristics of those ten scabies infested sweet potato cultivars, which might be grouped4 large groups. However, if a hypothetical vertical line is made on 40% coefficient it would show Ia sub-group which consist of Borobudur and Sari cultivars, while sub-group Ib was Cangkuang; group II were Sutuh and Antin cultivars; and group IIIa was Solosa kuning and IIIb were Cilembu and Ungu tua cultivars; the last group IV consisted of Kidal and beta cultivars. The sub group Ia and IIIb were located at short distance of <15% coefficient. This grouping, however, did not always show a strong genetic relationship among the samples as also moted by Herawati *et al.* (2018), who concluded if the Duku fruit cultivars tent to be more affected by geographic dispersal than RAPD markers. The figure showed also those ten scabies infested sweet potato cultivars were grouped randomly. However, the dendrogram showed the Borobudur and Sari cultivars were closely related (9% coefficient) followed by Cilembu and Ungu tua cultivars (11.3%). Indicating expression of the same locus of particular gene (s).

Conclusion

Of those 10 RAPD primers used to amplify genomic DNA of 10 scabies infested sweet potato cultivars 6 primers showed polymorphic patterns and the rest 4 produced monomorphic ones.

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