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GENETIC VARIATION IN CITRUS CULTIVAR SIAM (CITRUS NOBILIS LOUR. VAR MICROCARPA HASSK) FROM GUNUANG OMEH PRODUCTION CENTRE, WEST SUMATERA, INDONESIA

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ABSTRACT

Fruit characteristics and genetic variation of has been conducted to know source variations of fruit quality product now and to support breeding program. Analysis genetic variation with Random Amplified Polymorphic DNA (RAPD) technique. The test result of 17 primers showed that three primers can amplified DNA Siam citrus of Gunuang Omeh are OPA-02, OPA-13 and OPB-10. Amplification result with three primers displayed 26 bands with average 8.6 bands per primer, OPA-02 showed at most with 11 bands. The proportion of polymorphic loci obtained 100%. Genetic variation value after seed intensification is higher than before intensification group with genetic diversity value (H= 0.3198) and Shannon Diversity Index (I= 0.4809). Heterozygosity value intra-subpopulation (Hs= 0.2874) higher than heterozygosity value inter-subpopulation (Dst= 0.0112) while differentiation value among two population is low (Gst= 0.0378).

KEYWORDS: Citrus nobilis Var. microcarpa Hassk, RAPD, genetic variation.

INTRODUCTION

Citrus (Citrus nobilis Lour. var microcarpa Hassk) cultivar Siam is one of the commodities which have important economic value in Indonesia, because 70-80% of cultivated citrus in Indonesia are Siam citrus.[1] Citrus cultivar Siam popularly cultivated because has distinctive aroma, sweet taste and high productivity so on its very adaptive compared to other citrus[2] so on its very adaptive compared to other citrus[3] The popular citrus cultivar Siam was found some provinces in Indonesia, one of them in West Sumatra, it is called the citrus of "Gunuang Omeh"[4]

The citrus of "Gunuang Omeh" has been attended by the governments since 2008 through an intensification program using the certified seedling from a single parent tree in self-production center of Omeh mountain[5] However, before the intensification program from 1983 to 2008, the farmer still use seedlings from various sources whose quality is not uniform.

Based on the result of field observation that the quality of fruit from production center of citrus "Gunuang Omeh" shows high variation, only about 20% of total fruit production including prime quality and super quality, and the rest is under excellent quality and standard quality. This condition resulted in the production center has not been able to penetrate the modern markets, so it is still dominant to sell in traditional market. The low quality of the fruit may be due to the seedling sources before the intensification programs are still, and after one which still has not used a single parent tree. Therefore, genetic variation has analyzed by Random Amplified Polymorphic DNA (RAPD) markers.

Several studies using RAPD technique to determine the genetic variation of C reticulata[6] and C. sinensis in India.[7] C. reticulate, C. sinensis and C. grandis in Indonesia.[8] Genetic mapping of C. reticulata and C. sinensis,[9,10] histogenic identification of chymera results of Citrus spp.[11] and phylogenetic studies of Citrus spp.[12]

MATERIALS DAN METHODS

RAPD analysis

Young leaf samples from the plants collected from Gunuang Omeh production centre were use as materials. Young leaf were stored in plastics and added silica gel to prevent moisture.

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DNA Isolation

DNA extraction was performed using a CTAB (hexadecyltrimethyl ammonium bromide) method based on the modified Doyle and Doyle (1990) [13] method. The leaf sample was mashed using mortar and pestle in liquid nitrogen. Leaf powder is put into a 2 ml centrifuge tube up to 500 µl volume limit. Each leaf sample was extracted with 750 µl extract buffer (1% CTAB, 50mM Tris-HCl pH 8.0, 1.4 M NaCl and 10 mM Na2EDTA). The suspension is mixed uniformly and incubated in water with a temperature of 65°C for 30-60 minutes, every 10 minutes of suspension homogenized by turn centrifuge tube back and forth. DNA extraction was performed by adding chloroform: isoamil alcohol (24:1) solution in a 1: 1 volume ratio. After that it was centrifuged at a speed of 12000 rpm for 10 minutes with a temperature of 27°C. The precipitation of DNA was done by adding isopropanol into the supernatant. The DNA precipitation is separated from the solution with the aid of a centrifuge at a rate of 12000 rpm for 10 minutes at a temperature of 4° C. The precipitate (pellets) of DNA was purified with 70% cold ethanol and centrifuge at a speed of 12000 rpm for 5 min with a temperature of 4°C then purified again with 70% cold ethanol at a speed of 12000 rpm for 2 min with a temperature of 4°C. After that the precipitated DNA was dried during 1-2 hours and re-melted in TE buffer solution (10 mM Tris-HCl, 1mM EDTA, pH 8.0) of 50 µl. DNA quality and quantity test was performed on agarose electrophoresis gel 1.6%.

DNA Amplification

Tabel 1: Primer was used to analyze RAPD.

No.	Primer	Urutan Nukleotida (5'→3)	Sumber
1	OPA-01	CAG GCC CTT C	Pal et al,2013
1	OPA-02	TGC CGA GCT G	Pal et al,2013, Karsinah et al, 2002
1	OPA-03	AGT CAG CCA C	Sankar et al, 2014
1	OPA-04	AAT GGG GCT G	Pal et al,2013, Al anbari et al, 2014
1	OPA-05	AGG GGT CTT G	Sankar et al, 2014
1	OPA-06	GGT CCC TGA C	Sankar et al, 2014
7	OPA-07	GAA ACG GGT G	Pal et al,2013
1	OPA-08	GTG ACG TAG G	Pal et al,2013
1	OPA-09	GGG TAA CGC C	Maya et al, 2012, Sankar et al, 2014
1	OPA-10	GTG ATG GCA G	Maya et al, 2012
1	OPA-11	CAA TCG CCG T	Pal et al,2013, Sankar et al, 2014
1	OPA-12	TCG GCG ATA G	Al anbari et al, 2014
13	OPA-13	CAG CAC CCA C	Pal et al,2013, Karsinah et al, 2002
14	OPA-16	AGC CAG CGA A	
15	OPAC-12	GGC GAG TGT G	
16	OPAC-15	TGC CGT GAG A	
17	OPB-10	CTG CTG GGA C	Cevik and Gloria, 2007

The DNA samples were amplified on a PCR machine with a 1 degree temperature denaturation profile of 95°C (2 minutes), followed by 45 temperature denaturation cycles of 95° C (1 minute), Annealing temperature 35.8° C (1 minute) and a temperature extension of 72° C (2 minutes). The PCR cycle ends with a cycle end extension of 72° C (7 minutes). Each sample was mixed with 12.5 µL My Taq ™ Red Mix Bioline as PCR reagent, 4 µL DNA isolate, 6.5 µL Nuclease Free Water and 2 µL Primer, using 17 primers (table 1.). The separation of the band of amplified bands was carried out by electrophoresis method on 2% agarose gel in 10 X TBE solution for 2 hours at 60 volt, 150 Ma and 20 Watt.

Data Analysis

DNA bands formed from PCR amplification results are considered 1 character of a DNA locus. All DNA bands with the same migration rate are assumed to be homologous loci. The DNA profile data is then translated into binary data with the provision of zero (0) values for no DNA bands and one (1) for the presence of DNA bands on the same portion. The binary data matrix results were analyzed using POPGENE version 32 software.[14] Genetic variation parameter observed included genetic diversity (H), percentage of polymorphic locus (PLP), Shannon diversity index (I), subpopulation heterozygosity (H_S), total heterozygosity (H_T) and genetic differentiation coefficient (G_{ST}).

RESULTS AND DISCUSSION

Results

The results of primer selection showed that only three primers (OPA-02, OPA-13 and OPB-10) of seventeen primers were used capable amplifying DNA and displaying polymorphic bands (Figures 1, 2 and 3). The number of bands amplified by OPA-02, OPA-13 and OPB-10 are 11, 7 and 8, respectively, with the percentage of the polymorphic bands being 100% (Table2).

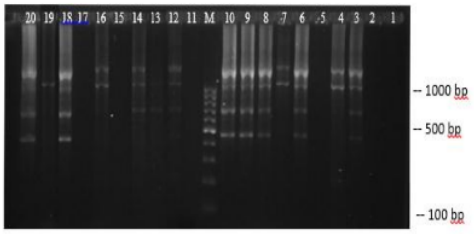

Figure 1: DNA amplification results Orange Citrus Siam Gunuang Omeh with OPA-02.

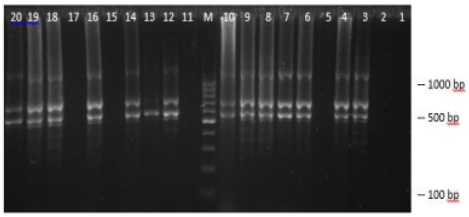

Figure 2: DNA amplification results Citrus Siam Gunuang Omeh primary with OPA-13.

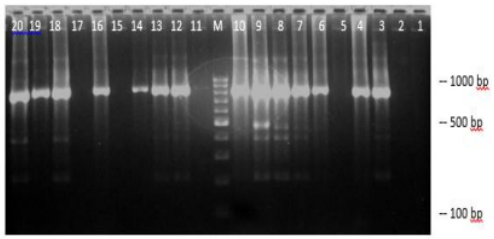

Figure 3: DNA amplification results Citrus Siam Gunuang Omeh with primary OPB-10.

The genetic variation of subpopulation after intensification was higher than the subpopulations before intensification (Table 3). The value of genetic diversity (H), shanon index (I), percentage of polymorphic loci (PLP) from subpopulation after intensification respectively 0.3198, 0.4809 and 96.15%, while in subpopulation before intensification 0.2549, 0.3920 and 80.77%. The heterozygosity value in the group (H_S) was 0.2874 higher than the heterozygosity among subpopulations (D_{ST}) of 0.0112 (Table 4).

Table 2: Total of DNA Band from Citrus nobilis Lour var. microcarpa wich used 3 primer.

No.	Primer	Jumlah pita total	Jumlah pita monomorfik	Jumlah pita polimorfik	Presentase pita polimorfik (%)
1	OPA-02	11	0	11	100,00
2	OPA-13	7	0	7	100,00
3	OPB-10	8	0	8	100,00
Total		26	0	26	300,00
average		8,6	0	8,6	100,00

Table 3: Genetic variation intra groups of Citrus nobilis var. microcarpa.

Group	Total individu	Na	Ne	H	I	PLP(%)
A	10	1,9615	1,5450	0,3198	0,4809	96,15
B	10	1,8077	1,4176	0,2549	0,3920	80,77

Description: A: group after the intensification; B: group before intesifikasi; Na: the observed allele; Ne: effective alleles; H: genetic diversity Nei; I: index diversity Shannon; PLP: percentace loci of polymorphic.

Table 4: Genetic variation inter groups Citrus nobilis Lour. var. microcarpa.

Jumlah sampel	H_T	H_S	D_{ST}	G_{ST}
20	0,2986	0,2874	0,0112	0,0378

Description: H_T: heterozygosity is total; H_S: heterozygosity is in subpopulations; D_{ST}: the value of heterozygosity is between subpopulations; G_{ST}: genetic differentiation between subpopulations

DISCUSSION

The primer (OPA-02, OPA-13 and OPB-10) (OPA-02, OPA-13 and OPB-10) is reported to amplify the other citrus genus OPA-02 in Citrus sinensis (L.) Osb.[7] OPA-13 in C. reticulata[6] and OPB-10 in C. grandis (L.) Osb, C. reticulata Blanco and C. sinensis (L.) Osb.[10] OPA-02, OPA-13 and OPB-10 in that plant respectively display 3, 19 and 2 polymorphic bands. 14 unused primers cannot display DNA bands. However, OPA-09 and OPA-12 can amplify DNA C. aurantifolia, C. delicosa, C. grandia, C. japonica, C. latifolia, C. limette, C. limon, C. medica and C. paradise[15] OPA-03, OPA-05 and OPA-07 can amplify C. sinensis (L.) Osb.[7] OPA-01, OPA-04, OPA-06, OPA-08, OPA-10 and OPA-11 can amplify DNA C. reticulate.[9] This shows a high degree of polymorphism (> 50% polymorphic band), so the three primers can be used for the analysis of genetic variation of Siam citrus of Gunuang Omeh.

The high genetic variation after intensification is caused by not all propagation of seeds using single parent tree and also bring seeds from various sources. Based on the value of genetic diversity (H) for both subpopulations it can be stated that genetic diversity of production center

of Gunuang Omeh is high. The value is supported by the high percentage of polymorphic locus that is 80.77% for the group before the intensification of the superior seeds and 96.15%. For groups after intensification of superior seeds. According to[16] the average value of dicot plant genetic diversity is 0.191 and the long-lived plant is 0.242. According to[17] genetic diversity in the population is caused when there is polymorphism or heterozygosity at a particular locus, whereas genetic diversity between populations occurs when there is a difference in allele frequencies and genotypes between populations. This indicates that the target of the intensification program in 2008 has not been achieved to improve the quality fruit. Therefore, to improve the quality of fruit still need selection of superior parent tree as a source of uniform seed propagation.

The low value of genetic variation among subpopulations in accordance with field interview results, that farmers use some seedlings from plant propagation before intensification and partly from several sources. The low genotype variation between the two groups was supported by the differentiation value (G_{ST}) which was also low at 0.0378. The value of genetic differentiation provides information on genetic differences between the two subpopulations not much different. According to[18] genetic differentiation is low with values ranging from 0 to 0.05. This is related to the propagation of seeds by grafting or grafting buds although the source varies but the differentiation is still low. According to[19] plants with the grafting system have a low genetic differentiation.

CONCLUSION

Genetic variation in citrus siam from production centre Gunuang Omeh after intensification is higher (H= 0, 3198, I= 0, 4809, PLP=96, 15%) than before intensification (H= 0, 2549, I= 0, 3920, PLP= 80, 77%). Siam citrus of gunuang omeh have genetic difference intrapopulation is low with heterozygosity value intrapopulation (Hs= 0, 2986) is higher than heterozygosity value interpopulation (Gst =0, 0378)

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