SOMACLONAL VARIATION ON THE PINEAPPLE IN VITRO CULTURE AS DETECTED BY MOLECULAR MARKERS

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ABSTRACT

Pineapple is the third of world important fruit, thus the research pertaining of this fruit had been conducted rapidly, including its *in vitro* aspect. The raising concern of *in vitro* methods was the somaclonal variation on the plantlets. This research was conducted in order to determine the molecular profile of the pineapple plantlets derived from terminal shoots and crown explant (6 samples each) on the 4th sub culture (using MS media with 2.0 mg ml^{-1} BA). Five RAPD primers (OPA02, OPA03, OPA07, OPA13, and OPA19) were used to produce bands which were then scored manually, the Dice-Sorensen coefficient similarity were calculated on the NTSYSpc 2.10x. The results suggested that either plantlets derived from terminal shoot or crown explants demonstrated genetic variation. The crown explant plantlets showed highest (0.9697) and the lowest (0.6000) similarity coefficient. On the average, the similarity coefficient of crown explants plantlets (0.8647) were the highest among other plantlets, which was suggested that the crown explant produce more genetically uniform plantlets than other explants. Furthermore, it was revealed that the use of low BA on the 4th sub culture of pineapple *in vitro* culture induced somaclonal variation. To produce *in vitro* clonal pineapple the frequency of subculture and concentration of BA used must be reduced.

Keywords: *Ananas* sp., coefficient similarity, RAPD

INTRODUCTION

Pineapple is the third world's most important fruit and cultivated in always every topical region. Since its high economical value, the research regarding to this fruit had been conducted intensely, including the *in vitro* and molecular aspects. In the present, the pineapple *in vitro* technique was also applied as tools in plant breeding. Indonesian Tropical Fruit Research Institute has been conducted a pineapple breeding program which produced many hybrids. However, these hybrids were still unreleased to to the lack on an adequate seeds. The pineapple conservative propagation method only produced a limited number of seeds, thus the *in vitro* culture was one of the alternatives.

The first accomplishment pineapple *in vitro* culture was reported by Wakasa *et al.* (1978) who used varied explant (crown, slip, and syncarp) to induce multiple shoots. Although Wakasa (1979) successfully planted the plantlets to the field, he found some variation in leaves and spine morphology. Since then, the pineapple *in vitro* studies was expanded, some even reported a production of thousand of shoots from single explants (Kiss *et al.*, 1995; Be & Debergh 2005; Hamad & Taha 2008; Al-saif *et al.*, 2011; Farahani 2014). Separately, some research on the pineapple molecular aspect were conducted to

detect the somaclonal variation on the *in vitro* plantlets using isozyme and RAPD (Rapid Amplified DNA Polymorphism) markers (Feuser *et al*., 2003; Santos *et al*., 2008; Roostika *et al.,* 2015). Other factors that might induce somaclonal variation on *in vitro* plantlets were type of explant, frequency of subculture, and the use of plant growth hormone (Bairu *et al*., 2011).

The unpublished research had reported somaclonal variation in all pineapple plants derived from *in vitro* propagation. Thus, the detection of somaclonal variation on the plant early stage had to be conducted to prevent severe loss by the using the unappropriate seeds. The research was done in order to examine the genetic profiling of the 4th sub culture of pineapple *in vitro* plantlets.

MATERIAL AND METHOD

Plantlets of *in vitro* plantlets of pineapple hybrids 18×5(10) derived from four different explants, namely hapas, crown, sucker, and aerial suckers which were grew on MS media (Murashige & Skoog, 1962) modified with 2 mg ml^{-1} BA. The 4th sub culture plantlets were chosen as the lowest sub culture frequency which the plantlets were usually brought to the acclimatization process.

DNA of 6 plantlets each from the same explants were extracted using CTAB method (Doyle & Doyle, 1987). The DNA extract were then used as templates on RAPD reaction with 5 primers as recommended by Roostika *et al.* (2015) (Table 1). RAPD PCR reaction was performed with Taq PCR reaction mixture 4.25 µl (KAPPA) with 1 µl of RAPD, 1 µl (10 ng) of sample DNA, and ddH_2O to a final volume of 12.5 µl reaction. PCR was performed by 45 cycles with the following programs: pre denaturation 95 ^{0}C for 3 minutes, denaturation 95 ^{0}C for 15 seconds, annealing 36 ^{0}C for 15 seconds, extension 72 ^{0}C for 5 seconds, and final extension: 72 ^{0}C for 10 minutes.

Table 1. Applied primers to detect somaclonal variation on pineapple *in vitro* plantlets

Primers	Sekuens (5'-3')
OPA02	TGCCGAGCTG
OPA03	AGTCAGCCAC
OPA07	GAAACGGGTG
OPA13	CAGCACCCAC
OPA17	GACCGCTTGT

DNA amplification product was separated by electrophoresis at 50 V for 20 minutes. The band patterns were scored manually and automated using software BioDoc Analyzer. The Dice-Sorensen coefficient of similarity was conducted using the NTSYSpc 2.10x software (Rohlf, 2004).

RESULTS AND DISCUSSION

Somaclonal variation has become a major concern in pineapple micropropagation, thus early detection method to determine the plantlets genetic variability had been developed, included RAPD-PCR (Roostika, 2012). The RAPD reaction on the pineapple plantlets derived from four type of explants, namely

hapas, crown, suckers, and aerial suckers were conducted using five primers. The DNA samples were extracted from each plantlets that were originated from the same explant source. The gel image of those amplifications were presented in Figure 1 and 2.

Overall it was demonstrated that primers used in this study produced 3-10 bands, included some monomorphic and at least one polymorphic bands. Primes OPA2 and OPA3 seems to produce the least polymorphic band on hapas and crown derived plantlets, whereas primer primer OPA07 produce the most polymorphic band on suckers and aerial suckers derived plantlets. All primers used were able to revealed genetic variability among the plantlets.

Table 2. Dice-Sorensen coefficient similarity matrix of 4th subculture pineapple plantlets derived from several type of explants

Samples	H1	H2	H3	H4	H5
H2	0.5806				
H3	0.8824	0.6207			
H4	0.8824	0.6207	0.9375		
H5	0.9143	0.6000	0.9697	0.9091	
H6	0.8824	0.6207	0.9375	0.9375	0.9697

Samples	C1	C2	C3	C4	C5
C2	0.9474				
C3	0.9444	0.9474			
C4	0.8750	0.8235	0.8750		
C5	0.8485	0.8571	0.9091	0.8966	
C6	0.8421	0.8500	0.7895	0.7647	0.8000

Samples	S1	S2	S3	S4	S5
S2	0.6250				
S3	0.6667	0.8089			
S4	0.7143	0.7059	0.6316		
S5	0.6667	0.6667	0.7907	0.8000	
S6	0.6250	0.6842	0.7619	0.8235	0.9231

Samples	C1	C2	C3	C4	C5
C2	0.9474				
C3	0.9444	0.9474			
C4	0.8750	0.8235	0.8750		
C5	0.8485	0.8571	0.9091	0.8966	
C6	0.8421	0.8500	0.7895	0.7647	0.8000

H: Plantlets derived from hapas explant, C: plantlets derived from crown explant, S: plantlets derived from sucker explants, AS: plantlets derived from aerial sucker explants. The highlighted numbers were the lowest and highest value within the same type of explants

The Dice-Sorensen similarity coefficient were calculated for each type of plantlets which was resulted that none of the explants produced 100% genetically uniform plantlets (none 1,000 similarity coefficient found- Table 2). Although, this study did not involve the mother plant (explants source), the result had already detected genetic variability among the explants that came from the same explants source which also confirmed by Roostika (2012). The earlier study also explained that the genetic variability further will resulting a somaclonal variation on pineapple. The genetic and epigenetic changes in plant *in vitro* culture induced somaclonal variation that might be occurred on far beyond rate that expected in nature (Wang & Wang, 2012). Those changes included point mutation, transposition activity of mobile genetic elements, chromosomal rearrangement, or ploidy level changes which induced genetic instability (Azman *et al.,* 2014).

Moreover, it was also revealed the hapas explant derived plantlets showed both the biggest (0.9697) and lowest value (0.6000). The average coefficient similarity from hapas, crown, suckers, and aerial suckers derived plantlets were 0.8177, 0.8647, 0.7263, and 0.7892, respectively. Those values indicated that the crown explants produce more genetically similar plantlets compare to the other explants types as also indicated by Wakasa (1978). It was reported that the genetic fidelity of *in vitro* plants were mostly depend on explant source, the explant tissue can affect the frequency and nature of somaclonal variation (Leva *et al.,* 2012).

The media used in this study was modified MS which supplemented with 2 mg ml^{-1} BAP. In previous research, the application of common cytokines such as

BAP produce many (up to 31 shoots per explant) *in vitro* pineapple shoots (Zuraida *et al.,* 2011). However, it seems that the said level of plant hormone had already induce cytological changes in pineapple. It was had explained that hormonal element of culture medium are powerful agent of variation (Leva *et al,.* 2012). Furthermore, stress condition during *in vitro* culture, such as wounding, exposure to plant hormone and other specific elements on *in vitro* medium caused cytological changes (Azman *et al.,* 2014).

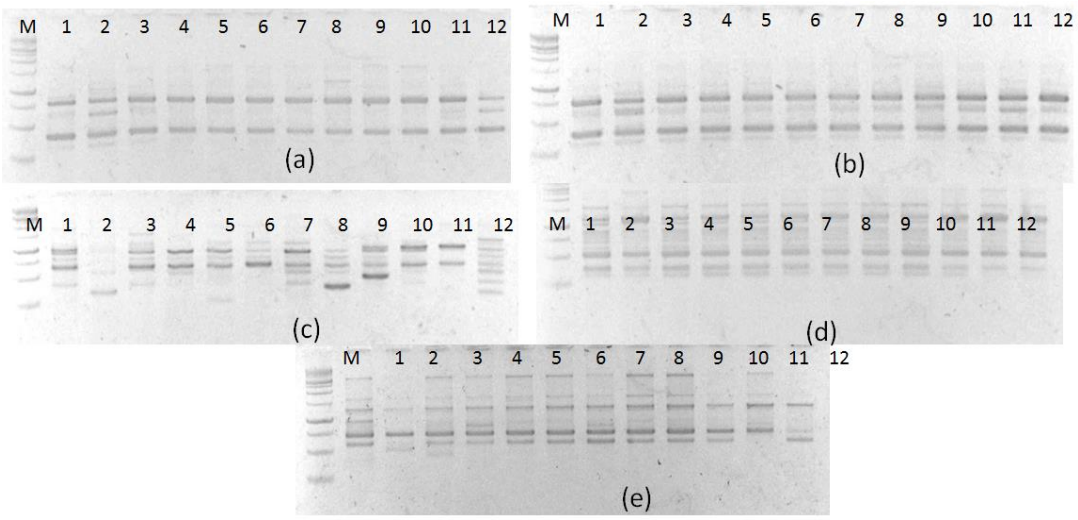

Figure 1. Gel Electrophoresis of RAPD analysis of 4th subculture pineapple *in vitro* culture, with primers: (a) OPA02, (b) OPA03, (c) OPA07, (d) OPA13, and (e) OPA17. (M: 100 kb marker, 1-6: plantlets derived from hapas explants, 7-12: plantlets derived from crown explants).

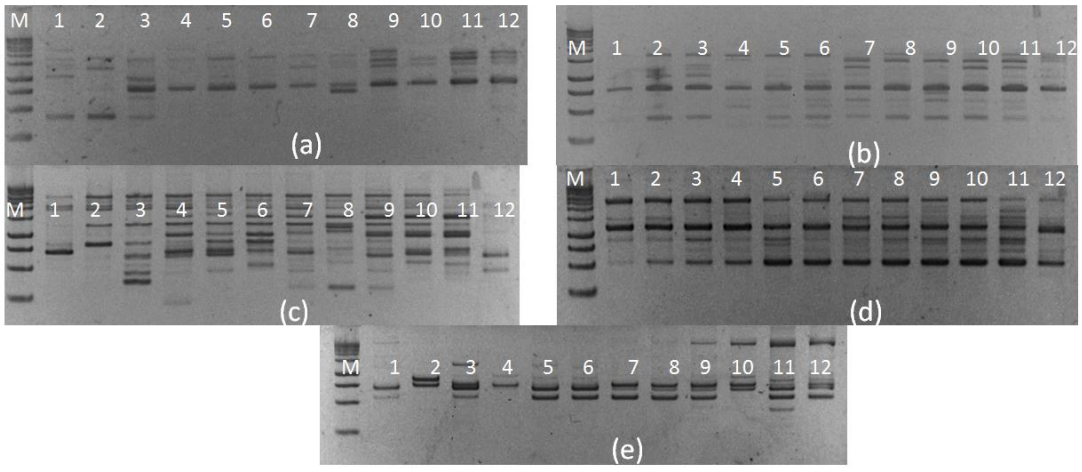

Figure 2. Gel Electrophoresis of RAPD analysis of 4th subculture pineapple *in vitro* culture, with primers: (a) OPA02, (b) OPA03, (c) OPA07, (d) OPA13, and (e) OPA17. (M: 100 kb marker, 1-6: plantlets derived from suckers explants, 7-12: plantlets derived from aerial suckers explants).

The plantlets examined on this study came from the fourth subculture cycle. Usually, *in vitro* culture of pineapple was repeatedly conducted twice or more to induce more shoots. Hamad and Taha (2008) produced total of more than 120,000 shoots per explant within four times subculture and 75 days incubation period. In the present study, the four time subculture seems had already caused variation. The rapid multiplication of culture may affect genetic stability and thus lead to somaclonal variation (Leva *et al.* 2012).

CONCLUSION

The application of 2 mg ml^{-1} BA on MS media and four time subculture on the same media to induce pineapple *in vitro* multiple shoots might caused genetic variation of the plantlets which were grew from the same explants. Crown explant induced lower percentage of genetic variation than hapas, suckers, and aerial suckers explants. Therefore, the use of lower concentration of BA, less sub culture frequency, and the use of crown explants were suggested in order to produce more genetically similar pineapple plantlets.

REFERENCES

Azman, A.S., C. Mhiri, M.A. Grandbastein, S.M. Tan. 2014. Transposable elements and the detection of somaclonal variation in plant tissue culture: A review. Malays. Appl. Biol. 43(1):1-12.

Al-Saif A.M., A.B.M.S. Hossain, R.M. Taha. 2011. Effect of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr.) *in vitro.* African J. Biotec. 10(27): 5291-5295.

Bairu M.W., A.O.Aremu, J.Van Stadden. 2011. Somaclonal variation in plants: causes and detection methods. Plant Growth Regul. 63:147-173.

Be L.V., P.C. Debergh. 2006. Potential low-cost micropropagation of pineapple (*Ananas comosus*). South African J. Bot. 72:191-194.

Doyle J.J., J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11-15.

Farahani F. 2014. Micropropagation and growth of *in vitro* pineapple (*Ananas comosus* L. Merr.) in Iran. Plant Archives 14(1):337-341.

Feuser S., K. Meler, M. Daquinta, M.P. Guerra, R.O. Nodari. 2003. Genetic fidelity of micropropagated pineapple (*Ananas comosus*) plantlets assessed by isozyme and RAPD markers. Plant Cell Org.Tiss.Cult. 72:221-227.

Hamad A.M., R. M. Taha. 2008. Effect of sequential on *in vitro* proliferation capacity and shoot formations pattern of pineapple (*Ananas comosus* L. Merr.) over different incubation periods. Sci. Hortic. 117:329-334.

Kiss E., J. Kiss, G. Gyulai, L.E. Haszky. 1995. A novel method for rapid micropropagation of pineapple. Hort. Sci. 30(1):127-129.

Murashige and Skoog, 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Rohlf F.J. 1998. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1.Applied Biostatics, New York ion and prospect. Buletin AgroBio 6(1): 34-40.

Roostika I., N. Khumaida, S.W. Ardie. 2015. RAPD analysis to detect somaclonal variation of pineapple *in vitro* culture during micropropagation. Biotropia 22(2): 109-119.

Santos M.D.M., G.C.S. Buso, A.C. Torres. 2008. Evaluation of genetic variability in micropropagated propagules of ornamental pineapple [*Ananas comosus* var. *bracteatus* (Lindley) Coppens and Leal] using RAPD markers. *Genet. Mol. Res* 7.4:1097-1105.

Wakasa K., Y. Koga, M. Kudo. 1978. Differentiation from *in vitro* culture of *Ananas comosus.* Japan J. Breed. 28(2):113-121.

Wakasa K. 1979. Variation in the plants differentiated from the tissue culture of pineapple. Japan J. Breed. 29 (1):13-22.

Roostika, I. 2012. Pengembangan metode organogenesis dan embriogenesis somatik pada nanas (*Ananas comosus* L. (Merr.) serta deteks dini untuk mereduksi keragaman somaklonal. Disertasi IPB, Bogor.

A.R. Leva, R. Petrucelli, L.M.R. Rinaldi. 2012. Somaclonal variation in tissue culture: A case study with olive, In: Recent advance in plant *in vitro* culture. Intech. 123-150.

Wang, Q.M., L. Wang. 2012. An evolutionary view of plant tissue culture: somaclonal variation and selection. Plant Cell Rep. 31:1535-1547.