

## Genetic of Salak Pondoh, Gading Varieties and Its Hybrids Based on RAPD Markers

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### Abstract

A molecular marker of parent and offspring is used to find fast and accurate markers influenced by DNA isolation and amplification. This research aims to find the most suitable DNA isolation and DNA amplification methods. This study used four DNA isolation methods; namely IM01, IM02, IM03, and IM04. DNA amplification used ten protocols (AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010). The results of the research showed that the most suitable DNA isolation method for salak was IM0, and the most suitable DNA amplification for salak was AP04 that produces the highest value of DNA bands.

**Keywords:** DNA isolation; DNA amplification; hybrids

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### Introduction

Salak (*Salacca zalacca* (Gaertner) Voss) has excellent potential to be planted in Indonesia; the tropical climate supports the growth of salak. Thirty varieties of zalacca are distributed in Indonesia (1), including Ivory and Pondoh. Pondoh zalacca is the most famous zalacca compared to other zalacca varieties. Pondoh Salak has a sweet taste even though it is not yet ripe and dark brown or blackish-brown skin color. Salak Gading also considered as a well-known zalacca variety, which is unique to its yellow skin and the flesh is thicker than Pondoh zalacca, but has a mild taste. This research aimed to develop salak, which has a sweet taste, yellow skin, and thick flesh.

Zalacca breeding through crossing is objected to increase zalacca potential (2). Pondoh and Ivory zalacca crossing is expected to produce offspring with sweet taste, yellow skin, and thick flesh. According to Anna Meyer (3), all aspects of how living things look, function, and behave are determined by DNA. DNA analysis was carried out in this study to obtain accurate data in a short time.

There are two stages of DNA analysis, namely DNA isolation and amplification. According to Thomas and Dominic (2013), DNA isolation aims to separate DNA in the cell nucleus from materials and separate DNA in the cell nucleus from other materials and molecules. DNA amplification aims to double the target DNA

This research modifies the existing isolation method and DNA amplification protocol, then analyzes each difference in it.. The main objective is to find superior salak varieties through studying the best and most appropriate methods in DNA isolation and amplification.

### Materials and Methods

This study was conducted from March to August 2017 in the Genetics and Plant Breeding Laboratory of Faculty of Biology, Universitas Gadjah Mada. This research was conducted in three stages. First, male Pondoh and female Ivory were crossed in the Salak Nusantara Collection Garden, Bangunkerto Turi Temple, Sleman, Yogyakarta. Second, the results of the crossing were planted on Pucang Sawit Street RT 3 RW 2 Jebres Surakarta.

Third, DNA analysis was carried out at the Genetics and Breeding Laboratory of the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

This study used 4 DNA isolation methods, namely IM01, IM02, IM03, and IM04. IM01 uses DNA Nandariyah isolation method (4). IM02 used the Doyle and Doyle (5) method modified by Borges et al. (6). IM03 used the DNA isolation method that has been listed in the manual of the GE Healthcare Nucleon Phytopure Genomic DNA Extraction Kits RPN 8511. IM04 used the Nandariyah DNA isolation method (4) with modification of the extraction buffer.

DNA amplification used ten different protocols (AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010). The 10 DNA amplification protocols were distinguished by DNA concentration, primary type, primary concentration, cycle, temperature, and pre denaturation time to post elongation. The difference in migration of DNA bands in agarose gel was the difference in migration of individual allele (7). The samples used were male Pondoh (P), female Gading salak (G), results of crossing Pondoh and Gading salak (F), and other salak varieties (K). The number of repetitions was adjusted to the needs. The salak material for analysis used young leaves.

## Results and Discussion

### DNA isolation

DNA isolation aims to obtain DNA with high concentration and good purity. DNA isolation consists of three stages, first destruction of cell walls (lysis), second, separation of DNA from other solid materials, and finally, DNA purification (8). The method of DNA isolation used depends on the type of plant or plant tissue (9). According to Syafaruddin et al. (8), the DNA isolation method is needed in plants containing high secondary metabolite compounds to get good quantity and quality of DNA, such as in salak leaves. Differences in the composition, dosage, time, repetition, etc., affect the DNA produced. This study uses 4 DNA isolation methods (IM01, IM02, IM03, and IM04). The difference between the four methods lies in the addition of PVP, extraction buffer, chloroform, isoamyl alcohol, sodium acetate, incubation at

a specific temperature, and washing of DNA pellets.

IM01, IM02, and IM04 protocols used PVP to facilitate the grinding of salacca leaves while also using extraction buffers with the composition of CTAB, NaCl, EDTA, Tris-HCl and  $\beta$ -mercaptoethanol. According to Syafaruddin et al. (8), the composition can eliminate polysaccharides and polyphenols. IM01 mixed the entire extraction buffer composition into one, while IM02 and IM04 separated  $\beta$ -mercaptoethanol from other ingredients.  $\beta$ -mercaptoethanol had an unpleasant odor that disrupts DNA isolation activity (10). IM01 and IM04 added sodium acetate to keep the pH stable during the DNA isolation process. IM01, IM03, and IM04 washed DNA pellets using 70% ethanol three times. Ethanol was easily lost by evaporating. IM02 washed DNA pellets using absolute alcohol and 70% alcohol. The absolute alcohol, 70% alcohol, and ethanol have almost the same properties.

### A230, A260, A280 and A320

The absorbance value at 230 nm represented polysaccharide contaminants or other contaminants. Nucleic acids such as RNA, DNA, and free nucleotides have a strong absorbance at 260 nm. Protein had a strong absorbance at 280 nm (11). The absorbance value at 320 nm provided a general measurement of the turbidity of the sample (12).

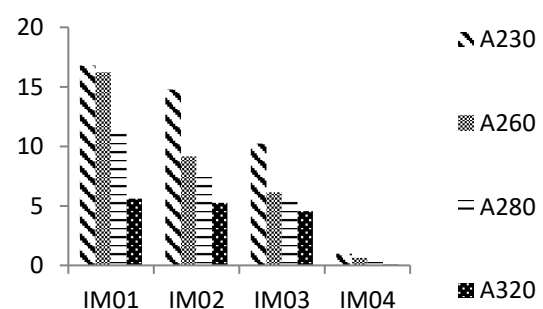


Figure 1. Average values of A230, A260, A280, and A320 of the DNA isolation method used

The results showed that the highest A230 was IM01 with a value of 16,800 and the lowest was IM04 with a value of 0.990. The highest A260 was IM01 with a value of 16.220 and the lowest was IM04 with a value of 0.623. The highest A280 was IM01 with a value of 11,300 and the lowest was IM04 with

a value of 0.336. The highest A320 was IM01 with a value of 5.600 and the lowest was IM04 with a value of 0.079 (Figure 1).

#### DNA purity

DNA purity compares pure DNA and impurities (protein) (13). A260 divided by A280 can be used to estimate the DNA purity. DNA is said to be pure if it has a purity value of 1.8-2.0. The value of DNA purity lower than 1.8 means that there was protein contamination; while a value higher than 2.0 means that there was a phenol contamination.

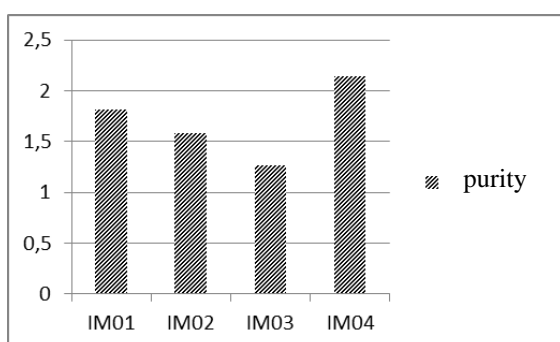


Figure 2. Average DNA purity value from the DNA isolation method used

Based on the results of the research, the purest DNA was IM01, with a value of 1,810. The DNA purity value of IM02 was 1.579, indicating that DNA was contaminated with a protein. The DNA purity value of IM03 was 1,261, indicating that DNA was contaminated with a protein. The DNA purity value of IM04 was 2,143, indicating that it was phenol contaminated (Figure 2).

The comparison of chloroform and isoamyl alcohol used in IM01, IM02, and IM04 was 3: 1: 3, while IM03 only used chloroform without the addition of isoamyl alcohol according to the manufacturer's manual. Chloroform and isoamyl alcohol (CIA) are significant for binding proteins and cell membrane lipids and dissolve them to form deposits (14). The dose of chloroform and isoamyl alcohol IM01 was enough to produce DNA with good purity, while IM02 was not enough, so there were still protein contaminants.

IM01 and IM04 used the same dose of chloroform and isoamyl alcohol but produced very different DNA purity, and this was because the extraction buffer composition were different. IM01 extraction buffer composition was 2% CTAB 20 ml, 100 mM

Tris pH 9.0 10 ml; 1.4 M NaCl 28 ml; 20 mM EDTA 4 ml; H<sub>2</sub>O 38 ml; and  $\beta$ -mercaptoethanol 0.1 ml; while the IM04 extraction buffer composition was CTAB 10 gr; 10 mM Tris-HCl 5 ml; 1.4 M 140 ml; 20 mM EDTA pH 8 20 ml; H<sub>2</sub>O 335 ml; and addition of 75  $\mu$ -mercaptoethanol separately. According to Maftuchah et al. (15), the composition of extraction buffer and pH is very important in the optimization strategy of DNA isolation, and extraction buffer is the most important compound to prevent DNA from being degraded. DNA and RNA have insoluble properties in organic solvents (8). The addition of chloroform will not lose DNA and RNA.

IM03 used GE Healthcare Nucleon Phytopure Genomic DNA Extraction Kits RPN 8511. According to manual from the factory, the extraction kit can isolate the DNA of *Cocos nucifera*, which in plain view has almost the same properties as the salacca leaves, but research on salak leaves yields less satisfactory purity. This can be caused by the salak leaves that have been used for too long.

#### DNA concentration

DNA concentration is used as a reference to improve the success of DNA amplification. DNA concentration was calculated by A260 times 50 times dilution factor. The higher the concentration of DNA, the easier it is for researchers because it can be diluted as needed and as a DNA stock



Note: G: Salak Gading; P: Salak Pondoh; F: Crossing of Salak Pondoh and Gading

Figure 3. DNA genome electrophoresis IM04

IM02 and IM03 did not produce DNA bands at all. Protein contaminants in IM02 and IM03 caused inaccurate estimates of DNA concentration. According to Hoy MA (16), chaotropic salt contaminants, RNA, and proteins such as EDTA and tris buffering lead to inaccurate estimates of DNA concentration. The improper storage of IM02 and IM03 caused DNA damage. DNA is readily damaged

at inappropriate temperatures. The quality of DNA will be maintained at low temperatures.

IM04 produces DNA bands that are thin, faint, and smear because of phenol contamination (Figure 3). The presence of phenolic compounds can affect DNA quality. The more phenolic compounds the DNA quality is getting worse (8). Genomic DNA electrophoresis produced from the four isolation methods, only two showed DNA bands, namely IM01 and IM04. There were differences between IM01 and IM04; IM01 had good DNA purity while IM04 was contaminated with phenols. Insufficient DNA purity may not necessarily produce genomic DNA bands.

#### *DNA amplification*

DNA amplification in this study used the PCR-RAPD method. RAPD (Random Amplified Polymorphic DNA) is a PCR-based DNA marker. RAPD is widely used in identifying interspecies and interspecies diversity (17). RAPD markers are widely used because they are faster, cheaper, more accessible, do not require special skills, do not use radioisotopes, do not require DNA sequence information, and DNA samples are needed (18). PCR optimization is essential to find the most suitable DNA amplification protocol. The trick is to try various DNA concentrations, primary types, temperatures, cycles, and pre denaturation times to post elongation (19).

DNA amplification protocols used in this study were AP01, AP02, AP03, AP04, AP05, AP06, AP07, AP08, AP09, and AP010. The ten DNA amplification protocols were distinguished by DNA concentration, primary type, primary concentration, cycle, temperature, and pre denaturation time to post elongation. Based on research, only AP04 and AP010 succeeded in producing DNA bands.

AP04 and AP10 have differences and similarities. The difference lies in the purity of DNA. AP04 uses IM01 DNA (good purity), while AP10 uses DNA IM04 (phenol contaminated). Maftuchah et al. (15) stated that PCR be done with moderate quality DNA, as in IM04. The AP04 and AP010 equations lied in the primers (OPA-16 [50]), pre denaturation (95.0°C for 3 minutes), denaturation (95.0°C for 15 seconds), annealing (36.0°C, 37.6°C, 38.5°C and 40.0°C for 15 seconds), extension

(72.0°C for 1 minute), post Elongation (72.0°C for 5 minutes) and cycle (45 times).

Unsuccessful DNA amplification protocols (AP01, AP02, AP03, AP05, AP06, AP07, AP08, and AP09) used almost the same procedures have succeeded (AP04 and AP10), but there are some differences. AP01 used different annealing temperatures, annealing times, and PCR cycles. AP02 used annealing temperatures as well as different PCR cycles. AP03 and AP06 used different primary concentrations. AP05, AP07, and AP09 used damaged DNA. AP08 used denaturation temperature, denaturation time, annealing temperature, annealing time, extension time, and different PCR cycles. The quality of DNA used by AP08 also has declined due to improper storage.

The success of PCR is influenced by the concentration of printed DNA, primers, MgCl<sub>2</sub>, and the number of cycles. A few that are not appropriate can affect the success of PCR (20). Unclear and faint DNA bands can be caused by the distribution of primary attachment sites to DNA, competition for primary attachment that causes specific fragments to be amplified in large quantities, and DNA concentrations that are too small (19). High DNA concentrations will increase the concentration of contaminants. Large concentrations of contaminants can inhibit the primary attachment to the printed DNA. Different brands of Thermocycler PCR devices can also affect whether DNA bands appear because different tools require different optimizations.

#### *Genetic analysis*

Efforts to increase the salak diversity can be made through the crossing. Crosses need different parental varieties to produce superior varieties (18). This study used male Pondoh salak and female Gading as parents. Pondoh Salak has sweet taste, brown skin, and medium size. Salak Gading has the properties of astringent taste, yellow skin, and large size. The expected phenotypes of the crossing are sweetness, yellow color, and large size. Crossovers between genotype can only be known after the plant is 4 years old when the plant bears fruits. The weakness can be overcome by using molecular markers Random amplified polymorphic DNA.

The two parents are not pure strains, (even tend to be hybrid varieties) so they are considered to have heterozygous genes.

Mendel's Law 2 states that two heterozygous parents who are crossed will freely produce offspring with a combination of genes. Pondoh and Gading salak cross will produce offspring with a combination of random parents (dominant genes in superior traits cover recessive genes in traits that are not pure strains), so they are considered to have heterozygous genes. Pondoh and Gading cross will produce offspring with a combination of random genes. One of the results of the crossing will inherit the superior traits of the two parents (dominant genes in superior traits cover recessive genes in traits that are not superior) to create a superior variety. These superior varieties are expected to be found in this study. The next stage, propagation, was done vegetatively in order to maintain its superior nature.

The results showed that F had a DNA band parallel to G, but none parallel to P. P gave DNA bands with sizes of 300, 450, and 700 base pairs. G raises DNA bands with sizes 300, 350, 400, 500, and 600 base pairs. F raises the DNA band with a size of 600 base pairs. F and G both gave DNA band on the size of 600 base pairs.

This study uses RAPD markers, so that homozygous dominant and heterozygous genes will both produce DNA bands. It cannot be distinguished which are homozygous dominant and which are heterozygous. Recessive homozygous genes do not produce DNA bands (Novalina and Sagala 2011). This can be interpreted that only dominant genes can produce DNA bands. Dominant genes usually describe superior traits, while recessive genes describe superior traits.

P and G are the parents of F, so P and G must have passed down their genes to F. Figure 6 shows the absence of DNA bands that are parallel between P and F. This indicates that P passed down the recessive gene to F, but P did not pass the dominant gene to F. Figure 6 shows the presence of DNA bands parallel to G and F. This indicates that G passed recessive genes or not to F.

Based on observations showing that F was not a superior variety as expected in this study. The expected superior varieties would get superior traits from both ancestors (get dominant genes from both ancestors). F only gets superior traits from G alone (F only gets dominant genes from G). It is assumed that the actual superior variety was one of the samples

that has not been successfully observed in this study because the new recessive superiority might be seen in F<sub>2</sub>.

This research still has many limitations. It is hoped that at least it can contribute valuable ideas. Further research is needed to obtain superior varieties of salacca plants.

### Conclusion

The most suitable method of DNA isolation for salak is IM0 that is the extraction buffer composition used of 2% CTAB 20 ml; 100 mM tris pH 9.0 10 ml; 1.4 M NaCl 28 ml; 20 mM EDTA 4 ml;  $\beta$ -mercaptoethanol 0.1 ml; and H<sub>2</sub>O 38 ml. Giving 500  $\mu$ l CIA three times. Addition of sodium acetate 1:10 from reagent volume. Washing DNA pellets using 70% ethanol. The most suitable DNA amplification for salak was AP04 that was using OPA-16 primers [50]; pre-denaturation 95.0°C 3 minutes; denaturation of 95.0°C 15 seconds; annealing 38.5°C 15 seconds; 72.0°C extension for 1 minute; post elongation 72.0°C 5 minutes; and 45 cycles.

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### Conflict of Interest

All authors declare no conflicts of interest in this section.

### References

1. Lestari R. Growth and physiological responses of salak cultivars (*Salacca zalacca* (Gaertn.) Voss) to different growing media. *J Agric Sci.* 2011;3(4):261–71.
2. Budiyanti T, Hadiati S, Prihatini, Sobir S. Genetic diversity of Indonesian snake fruits as food diversification resources. *J Int J Adv Sci Eng Inf Technol.* 2015;5(3):41–4.
3. Meyer A. Hunting the double helix: how DNA is solving puzzles of the past. *Crows Nest: Allen & Unwin*; 2005.
4. Nandariyah. Study genetic variability of cultivar salak based on morphology and RAPD. Yogyakarta: University Gadjah Mada; 2007.
5. Doyle J, Doyle J. A rapid DNA isolation procedure for small quantities of fresh leaf

- tissue. *Phytochem Bull.* 1987;9:11–5.
6. Borges A, Rosa M, Recchia G, Queiroz-Silva J, Bressan E, Veasey E. CTAB methods for DNA extraction of sweetpotato for microsatellite analysis. *Sci Agric.* 2009;66(4):529–34.
  7. Sinaga A, Putri L, Siregar L. Analisis Keragaman genetik andaliman (*Zanthoxylum acanthopodium* DC.) Sumatera Utara menggunakan marka RAPD (Random Amplified Polymorphic DNA). *J Online Agroteknologi.* 2015;3(1):350–8.
  8. Syafaruddin, Randriani E, Santoso T. Efektivitas dan efisiensi isolasi dan purifikasi DNA jambu mete. *J Bul Ristri.* 2011;2(2):151–60.
  9. Jose J, Usha R. Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). *J Plant Mol Biol Rep.* 2000;18:349–55.
  10. Hermanson G. Bioconjugate techniques. Chennai: Academic Press; 1996.
  11. Conn P. Laboratory methods in cell biology: biochemistry and cell culture. Chennai: Academic Press; 2012.
  12. MIT Open Course Ware Massachusetts Institute of Technology. UV spectrophotometric measurement of DNA concentration and purity [Internet]. 2008. Available from: <https://www.ocw.mit.edu>
  13. Thoyibi, Arifin M, Abraha K. Purity test DNA melon (*Cucumis melo* L.) cultivar "Gama Melon Basket" with Plasmon Resonance (SPR) based nanoparticle silver. *Indones J Appl Phys.* 2015;
  14. Das S, Dash H. Microbial biotechnology a laboratory manual for bacterial systems. London: Springer; 2015.
  15. Maftuchah, Winaya A, Zainudin A. Teknik Fundamental Analysis Technique of molecular Biology. Yogyakarta: Deepublish; 2015.
  16. Hoy M, Yu F, Meyer J, Tarazona O, Jeyaprakash A, Wu K. Transcriptome sequencing and annotation of the predatory mite *Metaseiulus occidentalis* (Acari: *Phytoseiidae*): a cautionary tale about possible contamination by prey sequences. *Exp Appl Acarol.* 2013;59(3):283–96.
  17. Siregar U, Olivia R. Genetic variability populasi sengon population (*Paraserianthes falcataria* (L) Nielsen) of community forest in Java based on RAPD. Bogor: Silviculture Department Faculty Forestry IPB; 2012.
  18. Rosmaina, Zulfahmi, Handoyo D. Genetic relationship tanaman jambu bol (*Syzygium malaccense* (L.) Merr. & Perry) based on RAPD (Random Amplified Polymorphic DNA). *J Agrotek Trop.* 2013;2(1):6–10.
  19. Langga I, Restu M, Kuswinanti T. Optimalisation temperature and long incubation in DNA extraction of bitti (*Vitex cofassus* Reinw) and genetic variability analysis by RAPD-PCR. *J Sains Teknol.* 2012;12(3):265–76.
  20. Uslan, Pharmawati M. Optimization concentration DNA and MgCl<sub>2</sub> reaction of Polymerase Chain Reaction Random Amplified Polymorphic DNA for analysis genetic diversity faloak (*Sterculia quadrifida* R.Br). *J Bioslogos.* 2015;5(1):26.

