

RESEARCH

Morphology and RAPD (random amplification of polymorphic DNA) based classification of genetic variability of *Java Salacca* (*Salacca zalacca* Gaertner. Voss)

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Abstract

The research aimed to role out the genetic variability and the classification of *Java Salacca* based on its morphological and molecular characters and to find out the genetic relationship among salacca cultivars that can be selected as parental material for breeding program. The salacca from Manonjaya (West Java), Banjarnegara, Bejalen, Lawu and Saratan (Central Java), *Super Pondoh*, *Black Pondoh*, *Gading*, *Kembangarum*, *Madu* and *Manggala* (Sleman-Yogyakarta) and *Suwaru* (East Java) were used in our study. Morphological characters and classification analysis by RAPD method with six random primers (OPA-11, OPA-16, OPA-17, OPA-18, OPX-15 and OPX-17) were used as classification variable. The genetic variability among cultivars of *Java Salacca* was presented by the similarity matrixes and dendogram. Based on the morphological classification, the twelve salacca cultivars was divided at four clusters: 1) *Manonjaya*, *Manggala*, *Suwaru* and *Kembangarum*, 2) *Super Pondoh* and *Black Pondoh* 3) *Banjarnegara*, *Saratan* and *Bejalen*, 4) *Gading*, *Madu* and *Lawu*. Based on the molecular-RAPD method, the twelve salacca cultivars was also divided into four clusters but difference member of cultivars: 1) *Super Pondoh*, *Banjarnegara*, *Black Pondoh*, *Gading*, *Kembangarum* and *Suwaru*, 2) *Bejalen*, *Saratan* and *Lawu*, 3) *Madu* and *Manggala* and 4) *Manonjaya*. Based on the two classification system used in the study I found the close relationship of *Saratan Salacca* and *Bejalen Salacca* from Central Java origin also *Super Pondoh Salacca* and *Black Pondoh Salacca* from Sleman-Yogyakarta origin.

Key words: Morphology, Classification, Genetic variability, RAPD

INTRODUCTION

Salacca plant (*Salacca zalacca* Gaertner. Voss) is an originally tropical plant of Indonesia, belong to *Palmae* group, divisio of *Spermatophyta*, class of *Monocotyledoneae*, ordo of *Spadicifloreae*, genus of *Salacca*, species of *Salacca edulis* ⁽¹⁾. Ashari ⁽²⁾ proposed the possibility of salacca plant was originated from Indonesia, since its wild type still could be found in West Java region until South of Sumatera and in Kalimantan forests.

The *Salacca* genus consist of 21 species spread naturally in regions of Malesiana, from Myanmar, Thailand, Malaysia, Filipina, and

Indonesia specially in Kalimantan, South of Sumatera and West Java. Three species of salacca plant have been cultivated: the *Sumatrana Salacca* in Padangsidempuan and its near regions, the *Salacca zalacca* in Java, Madura, Bali, Sulawesi and Ambon, and the *Salacca wallichiana* in Thailand ⁽¹⁾. For cultivated salacca it has different names in different places. Salacca in Thailand named as

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Sala; as *Yingan* in Birma and as *Snake Fruit* in England⁽³⁾.

Java Island has regions for salacca cultivation center and also we could find a different name of salacca based on their region name. For example, *Condet Salacca* is a salacca name cultivated in Condet of Jakarta. *Manonjaya Salacca* cultivated in district Manonjaya Tasikmalaya, *Banjarnegara Salacca* in Banjarnegara regency, *Kembangarum Salacca* in Kembangarum village, *Nglumut Salacca* in Nglumut village (Magelang), and *Suwaru Salacca* in Suwaru Malang regency⁽⁴⁾. Salacca also sometime named by its fruit skin colour or fruit flesh taste, like the *Gading Salacca* by its yellow fruit skin⁽⁵⁾.

Plant breeding program as an effort to find a new superior varieties is not common in Indonesia. For the best of my knowledge, usually the new varieties released by Indonesian government derived from local varieties, not as result from a breeding program. In point of the breeding program, it needs information about variability and its classification which can be used to show the level and relationship among cultivars for selection based.

MATERIALS AND METHODS

Morphological Characterization

The research was done at salacca cultivation centers in Java. The data of plant morphological characters were measured on the field while the physical and chemical nature data of the salacca fruit and RAPD analysis was done in Agriculture Faculty Sebelas Maret University laboratory.

In total of 19 variables were observed on the field: height, attached leaf end width (cm), longest torn length (cm), torn numbers per-25 cm of sheat, numbers of leaflets per sheat, leaf top surface color, torn color, fruit skin color and fruit meat color, weight of each fruit (gram), thickness of fruit meat (mm), weight of seed (gram), fruit tannin level (%), leaf tannin level (%), fruit sugar level (°Brix), any existence of torn at the blade abaxial, rolled leaf end, the arrangement of fruit skin, and any existence of honey-like fruit liquid.

RAPD (Random Amplification of Polymorphic DNA)

RAPD was done through stages: DNA extraction, DNA quality and quantity tests, primer selection, DNA amplification (PCR RAPD process), electrophoresis, visualization of DNA result amplification and result analysis. DNA extraction was done based on CTAB method⁽⁶⁾ modified by Nandariyah⁽⁷⁾ with time reducing and centrifugation speed modification for minimizing the DNA damages. DNA amplification was performed by early denaturizing for 3 minutes at 95 °C, continued with 40 cycles of amplification with denaturizing at 94 °C for 1 minute, annealing at 37 °C for 45 seconds, extension at 72 °C for 1.5 minutes; ended with final extension stage at 72 °C for 5 minutes. Primers used for DNA amplification (RAPD) were OPA-11, OPA-16, OPA-17, OPA-18, OPX-15, and OPX-17. DNA quantity was assayed by spectrophotometer and DNA quality was checked by electrophoresis. Visualization of amplification products was performed by standard gel electrophoresis.

Salacca Plants Classification

Salacca plants classification based on morphology and RAPD was done by counting the correlation coefficient rate among cultivars based on the whole morphological character and data of DNA bands numbers obtained from RAPD amplification result. Pearson's correlation was used for similarity matrix arrangement⁽⁸⁾. Cultivar groupings were made based on correlation coefficient rate in dendrogram based on grouping method according to Davis⁽⁹⁾.

RESULTS AND DISCUSSION

The Varieties of Java Salacca (*Salacca zalacca Gaertner. Voss*) and RAPD Profiles

There are two kind of classification: phenotypic classification, a method of classification based on morphological characters, and phylogenetic classification which based on genetic relationship. Plant classification based on morphological characters using visual characters which can

be measured (phenotype) as a result from combination between genotype and environment factors. The influence of environment and the growing phase of plant usually result in classification by phenetic clustering method. Phylogenetic classification is a method of classification based on the genotypic without the influence of environment. RAPD technique is one of method to perform the phylogenetic classification. This technique has advantage since its simplicity, easy to practice, accurate result and a relative not so time consumed to identify germ plasm variation ⁽¹⁰⁾. RAPD technique analysis has been used in many kind of genetic variability among plant genotypes, plant population in plant breeding program and germ plasm collection ⁽¹¹⁾.

Madu Salacca is cultivated and originated from kecamatan Turi, Sleman DIY. Its specialty is its very sweet taste; crisp without sour. When we ripening the fruit there will coming out a honey-like liquid. *Banjarnegara Salacca*, the salacca cultivated in Banjarnegara becomes rarely found at present since the farmers decide to change it with *Pondoh Salacca* from Sleman (Figure 1).



Figure 1. *Madu Salacca* plantation and its fruits in Regency of Sleman.

DNA amplification obtained from *Madu Salacca* with OPA-11 primer yielded 1-6 bands consisted of 1 monomorphic band and 5 polymorphic bands with 3 pattern bands. Polymorphic bands were detected at size of 250, 450, 750, 1.000 and 1.500 base pair (bp). DNA amplification of *Banjarnegara Salacca* yielded 1-4 bands consisted of 1 monomorphic band and 3 polymorphic bands with 3 pattern bands detected at size of 250, 450 and 1.000 bp. The relationship between *Madu* and *Banjarnegara* cultivar was shown by the size of pattern bands and polymorphic bands in

each cultivar. *Madu Salacca* is salacca cultivated from the result of vegetative multiplication, while *Banjarnegara Salacca* from the result of generative multiplication. Genetic difference between those two cultivars was supported from identification result based on morphological signifier which was located both of them in different group.

DNA amplification from *Manonjaya Salacca* using OPA-11 primer yielded 7 bands with 3 patterns of 2 polymorphic bands at 450 and 150 bp, and 5 monomorphic bands detected with 250, 500, 750, 1.000 and 1.500 bp. DNA amplification from *Suwaru Salacca* yielded 1-7 bands consisted of 2 monomorphic bands, 6 polymorphic bands, and 5 patterns bands. From the DNA bands profiles it was showed that *Manonjaya Salacca* had smaller varieties compared to that of *Suwaru Salacca*. *Suwaru Salacca* yielded 250, 450, 1.000, 1.100 and 1.500 bp. The natural cross for years may cause the higher genetic variety improvement of *Suwaru Salacca* (Figure 2).

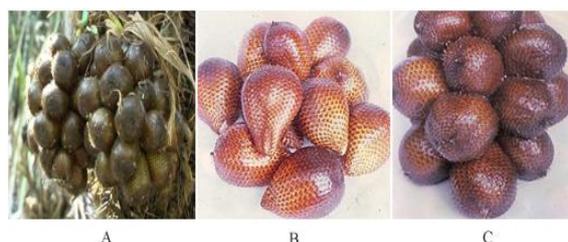


Figure 2. The *Manonjaya Salacca* cultivar origin from Ciamis Regency West Java (A), *Banjarnegara Salacca* from Banjarnegara Central of Java (B) and *Super Pondoh* origin from Sleman Regency (C).

Lawu cultivars, named as *Lawu Salacca*, yielded 2-4 bands, with 2 monomorphic bands, 2 polimorphic bands with 3 patterns bands. *Lawu Salacca* and *Kembangarum Salacca* had genetic closeness based on their RAPD profiles. This result was concordant with grouping result based on morphology signifier. *Lawu* and *Kembangarum* cultivars are plants cultivated through generative multiplication (seeding). *Lawu Salacca* had almost similar morphology characteristics and taste to that of *Kembangarum Salacca*. However, these two salacca are not interesting for the consumers because their taste is still less sweet than *Pondoh Salacca*. DNA bands

profiles of *Bejalen Salacca* and *Saratan Salacca* amplified by OPA-11 primer showed similarity on bands numbers and patterns.

Black Pondoh Salacca and *Gading Salacca* were originated from Sleman regency, in Turi district. *Black Pondoh Salacca* had a delicious taste like "Pondoh" (a name of palm bud). The fruit was relative small. The characteristic of *Gading Salacca* was a yellow fruit skin, 215.30 cm in plant height, with rarely thorns (197.30 in average), 66.50 gram of fruit weight and had a sugar content of 20.2 °Brix. The color of leaflets was light green. Morphological characters of *Manggala* leaflets were the rolled of top leaf and the strip line fruit skin that could not be gained from *Pondoh* cultivars so that it was predicted that 2000 bp band was the signifier for *Manggala Salacca* (Figure 3 and 4).

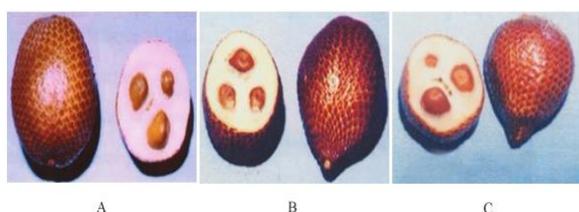


Figure 3. The *Suwaru Salacca* from Malang, East of Java (A), *Kembangarum Salacca* from Turi District of Sleman Regency (B), and *Saratan Salacca* from Magelang, Central of Java (C).



Figure 4. The *Black Pondoh Salacca* (A), *Gading* (B) and *Manggala* (C) cultivars origin from Sleman.

Dendogram of Salacca Cultivars Classification Based on Morphology Nature of The Plants

The number of similarity rate of morphology nature among cultivars pictured in dendogram reflects how similar or closeness in the relationship among the

cultivars. *Salacca* cultivar classification based on morphology similarity resulted in four groups consisted of group I (*Manonjaya*, *Manggala*, *Suwaru*, and *Kembangarum*), group II (*Super Pondoh* and *Black Pondoh*), group III (*Banjarnegara*, *Saratan* and *Bejalen*), and group IV (*Gading*, *Madu* and *Lawu*) cultivars (Figure 5).

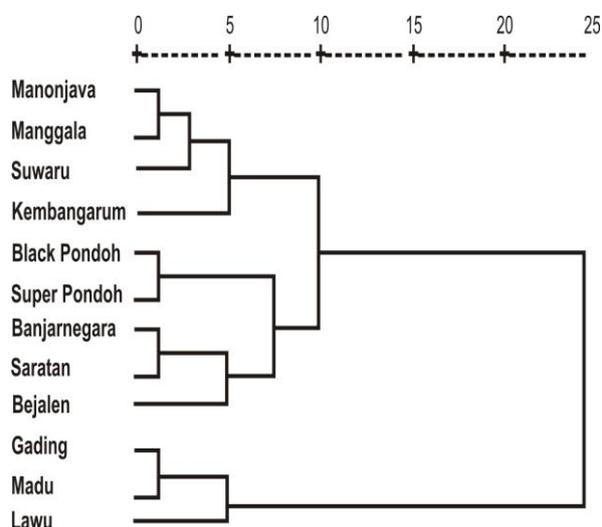


Figure 5. Dendogram of *Java Salacca* based on the morphological characters.

The characters similarity supported by the distance closeness in the group resulted a hypothesis that those members of the group have the same ancestor. Furtado ⁽¹²⁾ reported the existence of salacca plant (*Salacca zalacca*) in the northwest of Java (around Manonjaya and Tasikmalaya area).

The polymorphic band produced were three to six bands on each primer used in this study. OPA-11 and OPA-18 primer resulted in high inter-cultivar polymorphism DNA band (85.71 %), OPA-17 and OPX-17 resulted in 66.66 % polymorphic bands and lowest polymorphism bands by primary OPA-16 and OPX-15 (50 %). The length of the polymorphic bands were around 250-3000 bp.

Dendogram Salacca Cultivars Classification Based on RAPD

The classification of salacca based on the RAPD results separated *Manonjaya Salacca* cultivars from the others. According to its origin, this salacca cultivated in Java came from the southern part of West Java so it is

possible that *Manonjaya Salacca* is one of salacca cultivars that had been previously developed before the development of salacca cultivation in other regions in Java Island (2). The RAPD-based salacca cultivar groupings also separates *Pondoh Salacca* from Sleman's into two groups that were *Black Pondoh Salacca* and *Super Pondoh Salacca* on group I while *Madu* and *Manggala* cultivars on group IV (Figure 6).

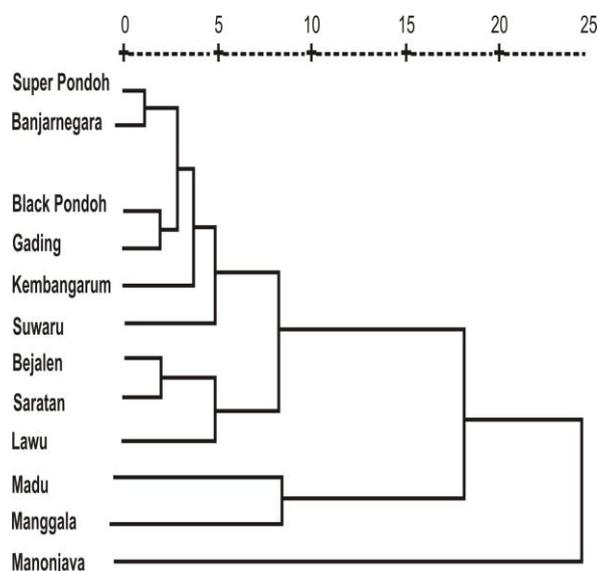


Figure 6. Dendrogram of *Java Salacca* based on the RAPD results

Equivalence of the grouping results based on morphology and RAPD signifiers was shown on several cultivars placed within the same group and had close relationship like *Bejalen*, *Saratan* and *Banjarnegara* cultivars. Geographical location of those three cultivars was close enough that it strengthens the hypothesis that they were identical. *Super Pondoh Salacca* and *Black Pondoh Salacca* based on morphological and RAPD were in the same group, so that it was estimated that both of the cultivars came from the same cultivar. *Madu* and *Manggala* cultivars based on morphological classification were in distant relation, but placed in the same group based on RAPD results, so it was considered that the morphological difference both of them was more influenced by the environment. For further analysis it is necessary to plant them on the same environment.

Finally, I concluded that there are genetic variability and relationship among salacca

cultivated in Java. Environment influences the classification result of salacca plant based on the morphological indicator. Salacca plant classification based on RAPD is more difficult and money-consuming but it has strengths in plant taxonomy system that is more useful in determining the genetic relationship among plant species. Salacca plant cultivars classification system based on RAPD can be used in efforts to construct a better result than the morphological-based classification system (Table 1).

Table 1. The RAPD results

Primer	Sequence (5' - 3')	Number of Polymorphic band	Number of Monomorphic band	Percent (%)
OPA-11	CAATCGC CGT	6	1	85.71
OPA-16	AGCCAGC GAA	3	3	50
OPA-17	GACCGCT TGT	4	2	66.66
OPA-18	AGGTGAC GGT	6	1	85.71
OPX-15	CAGACAA GCC	3	3	50
OPX-17	GACACGG ACC	4	2	66.66
Total		26	12	

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