Microsatellite and SNAP markers used for evaluating pollen dispersal on Pati tall coconuts and Xenia effect on the production of ‘Kopyor’ fruits

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Abstract Kopyor coconut is one of the many unique coconut types existed in Indonesia. To overcome the problem of low kopyor fruit yield, it is necessary to study xenia effect on the fruit yield of this coconut. The combination of kopyor coconut and normal coconut population selected at infarmers’ coconut plantations consisted of 33 normal coconut trees, and 9 kopyor coconut trees. All adult trees surrounding the 9 kopyor heterozygous (Kk) palms were evaluated as potential male candidate parents (pollen donors). All samples genotypes were determined using four SNAP markers and six microsatellite marker loci, parentage analysis using CERVUS software version 2.0. Results of the analysis indicate that xenia effect reduced kopyor fruit yields. Kopyor heterozygous (Kk) female parents produced low number of kopyor fruits when they were surrounded by many normal homozygous (KK) pollen donors. Out of 99 harvested progeny arrays from the kopyor heterozygous (Kk) female parents, none exhibited kopyor phenotype. The results also indicate that the pollen dispersal from normal homozygous (KK) donor palms range from 0 m (self pollination) to 54 m (outcrossing). The occurrence of outcrossing frequency was at least 95% and the selfing frequency is 5%.

1. Introduction
Kopyor coconut is one of the many exotic coconut types existing in Indonesia [1, 2]. The fruit was presumed from coconut plantation which is genetic mutation naturally. As a result of natural mutation, the number of kopyor coconut trees were very slight compared to the normal coconut. Accordingly, this mutant coconut is not develop, but it turns out these coconuts were found in several centers of coconut production in Indonesia. Kopyor properties were controlled by a mutant gene at the locus of K. The fruit couldn’t germinate naturally while homozygous (KK) or heterozygous (Kk) normal fruit could germinate and produce coconut seedlings. One of the problems farmers faced is the production of kopyor fruit has not been optimum. Kopyor coconut plant in Pati could produce as much amount of kopyor fruit type about 1-4 grains per cluster of the total number of of 7-15 pieces of grains [1]. Theoretically, based on the pattern of segregation Mendel's Law, then the chances kopyor fruit formed should reach 25% of the amount of fruit harvested.
One of efforts to overcome the problem of low kopyor fruit yield is to study the xenia effect on the kopyor fruit yield. Xenia is a genetic phenomenon in the form of a direct effect of pollen to the phenotype of fruits yielded by the female parents. In the study of heredity, the expression of genes who carried from the male parent and a female parent are expressed in subsequent generations. With the xenia effect, gene expression brought from the male parent can be expressed in female parents (fruit) [3]. The phenomenon of xenia on coconut trees has been known to have influence on fruit size, weight copra and obstinacy of hybrid trees [4]. According to [5], the xenia effect in cross-pollinated plants not only influences the fruit set, but also determines the shape, maturity, size, color, flavor, and composition of fruits, and research of [6] to further understand xenia phenomenon, was the investigation of the xenia effect in *Castanea henryi* using the cultivars of ‘Huali 1’, ‘Huali 2’, ‘Huali 3’, and ‘Huangzhen’ as materials.

Xenia related to phenotypes are controlled by a recessive gene. The formation of the kopyor coconut depends on the genotype of pollen to pollinate the female flowers of kopyor coconut plantations, so it is an example of xenia. This study to evaluate the effect of xenia on the kopyor coconut in Pati and determine the size range of self-pollination and cross-pollination that occurs in a population Coconut Pati using microsatellite and SNAP marker.

2. Materials And Methods

Samples were collected in kopyor coconut plantation in Dukuhseti Village, Pati Regency, Central Java with GPS location S6 27.649 E111 02.747. The research activities were conducted at the Laboratory of Plant Molecular Biology (PMB Lab) Department of Agronomy and Horticulture Faculty of Agriculture, Bogor. The population is a combination of kopyor heterozygous (Kk) coconut trees with normal coconut tree (KK) or coconut trees that never produce kopyor fruit with a total of 42 mature trees. Coconut trees in the planting area consists of coconut trees as much as 33 normal fruiting trees and 9 kopyor fruiting trees. Selection of the trees used as the female parent was done using purposive random sampling method. The female parents used were 7 palm kopyor trees. From each female parent trees, three palm fruit bunches (progeny) were harvested with a range the amount of progeny per female parent ranged from 2 to 12 pieces.

Total plant DNA sample was isolated with CTAB method [7] with modification [8]. SSR primer pairs were selected from 36 SSR loci [9] and the most polymorphic six primer pairs [10] were used for genotyping parents and progeny were evaluated. Four loci markers were developed based on the SNAP gene nucleotide diversity SUS and WRKY also used genotyping parent and progeny. The development of gene based markers SNAP SUS and WRKY performed following phases of activities that have been conducted on various plants [11, 12]. Gene SUS and WRKY can be used as polymorphic markers for coconut [13, 14].

PCR amplification was conducted using KAPA Bio-PCR kit protocol. Visualization of PCR amplification products for each locus SSR markers was carried out by 6% polyacrylamide gel electrophoresis using Buffer SB [15] and staining gel with silver nitrate. Phases of staining gel with silver nitrate was carried out based on the modified method of [16, 17]. Visualization of PCR amplification products for each locus markers SNAP was carried out using agarose gel electrophoresis (1%) with TBE buffer solution. Visualization of DNA was conducted using red staining gel.

3. Data Analysis

3.1. Identification of the Candidate Males Parents

Identification of pollen donor parent conducted by analyzing the genotype of each progeny and comparing it with all of the mature plant genotypes that were evaluated, potentially as pollen donors. Genotype data of all progeny and mature plants were used to determine the male parents candidates sought. Determination of parents male candidates elected for each progeny was carried out with paternity analysis with software of Cervus version 2.0 [18].
3.2. Pattern Analysis of Pollen Dispersal

Known female parent and assigned males parents identified as pollen donor from any progeny were mapped for the position based on mature plant dispersal maps that have been done previously. The distance between each parent and the pollen donor parent who identified calculated using Map Source software. Self-pollination (selfing) was defined when the male parents who identified closely with their parent. Otherwise, outcrossing is occurred when a female parent and males parents shortly become donors pollen consists of two distinct adult plants. The frequency of occurrence of selfing, outcrossing for various categories of identity crosses are determined by the female parent and the male parents of the results of paternity analysis.

4. Result and Discussions

PIC value of the loci were using SSR markers indicate a higher value than the loci with SNP markers (Table 1). Degree of variation decreased with the level of polymorphism. This was due to the amount of loci on SSR markers that being used more than the amount of loci, on SNP markers. PIC value is a measure of polymorphism between genotypes in a locus which used information on the number of alleles [19]. Total population of homozygous loci using SSR markers was relatively lower compared to markers SNP loci. Similarly, He value of the locus using SSR markers was also higher than that of SNP markers. Heterozygous alleles often found in SSR markers cause the value (He) is higher than the marker SNP. From the Cervus analysis, percentage of normal donor and Kopyor donor, which pollinate the Kopyor female tree could also be obtained which is shown in Table 2.

| Name of loci  | Number of alleles | Total | Heterozygosity | PIC |
|---------------|-------------------|-------|----------------|-----|
| CnCir_87      | 2                 | 64    | 0.444          | 0.372 |
| CnCir_86      | 4                 | 89    | 0.622          | 0.635 |
| CnZ-18        | 4                 | 116   | 0.806          | 0.691 |
| CnZ_51        | 5                 | 89    | 0.618          | 0.66  |
| CnCir_B12     | 5                 | 107   | 0.743          | 0.699 |
| CnCir_56      | 5                 | 81    | 0.563          | 0.647 |
| CnSus1#14     | 2                 | 39    | 0.281          | 0.22  |
| CnSus1#3      | 2                 | 96    | 0.691          | 0.365 |
| WRKY19#1      | 2                 | 116   | 0.829          | 0.374 |
| WRKY 6#3      | 2                 | 123   | 0.854          | 0.371 |

Note: PIC= Polymorphic Information Content. Ho= Observed heterozygosity. He= Expected

| Scheme of pollination | Type of pollination | Number of occurrences | Percentage |
|-----------------------|---------------------|-----------------------|------------|
| Kopyor x Kopyor       | Self                | 3                     | 3%         |
| Kopyor x Kopyor       | Outcross            | 25                    | 25%        |
| Kopyor x Normal       | Outcross            | 74                    | 73%        |
| Total of progeny      |                     | 102                   | 100.00%    |
This study also shows that none of kopyor fruit could be harvested by farmers. It indicates that heterozygous (Kk) kopyor coconuts were pollinated by normal (KK) coconuts, thus no He of kopyor coconut was observed in this study and the trees only produced normal fruits having homozygous KK (50%) and heterozygous Kk (50%).

The existence of normal tree (KK) around the heterozygous (Kk) kopyor tree could directly influence the phenotype of the fruits harvested thus categorized as a xenia effect. Xenia effect could also be studied by pollen analysis was identified by the best parents pollen donors to increased fruit production. This was also done by [20] with an indication of xenia effect found on cucumber fruits and seeds.

Kopyor trait was controlled by a single locus with the recessive allele (k). Meanwhile, normal trait was controlled by allele (K). Coconut trees kopyor of the study sites have the heterozygous genotype (Kk), if it was pollinated by trees kopyor heterozygous (Kk) others, which would produce the expected value of 25% of fruits kopyor (genotype zygotic embryos (kk)) and 75% of normal fruits [21]. Figure 1 shows the class with the shortest distance pollen distance 0 m (selfing) and furthest <60 m, is 54 m. The highest frequencies were at a distance <20 m by 30 times pollination occurred. In Diospyros celebica Bakh indicated that seeds were produced predominantly by outcrossing and pollen dispersal reached up to 166 m [22].

Effect of the position and distances of the assigned male to the female parent recipient on the occurrence of kopyor fruits is presented in figure 1. Xenia negative influence was seen in the productivity of kopyor fruit where the percentage of all the fruits harvested became normal fruit. It is caused by normal palm tree population (KK) is greater in those populations. In mixed cropping conditions with a normal ratio of coconut trees only produce fruit kopyor average as much as 15.8% [23]. So in the research recommendations of [21], in a project Hi Links that most logging in normal coconut (KK) in between the coconuts of Pati’s Genjah Kopyor (Kk) caused an increase in the percentage of kopyor harvested up to 10%. Increasing the percentage of kopyor harvested allegedly associated with the decreasing influence of xenia in cropping due to the number of normal trees (KK), which lesser at the sites.

In a population of coconut plantations, which tend also seen 7 times occur crosses (self pollination) based on the results of the analysis of Cervus. This possibility could occur because coconut having open morphological flower type. Xenia is a symptom of a genetic form of direct effect on the phenotype of pollen seeds and fruits was generated by the female parent. In the study of heredity, expression of genes that carried the male parent and a female parent was assumed, then expressed on the next generation.
With the xenia, the expression of genes that carried the male parent early has been expressed in female parents organs (fruits) embryo or endosperm. Symptoms xenia didn't only affect the color but also the form of sugar content, oil content, form and timing of ripening fruit. Research [24] about Xenia in kopyor coconut explain if homozygous KK normal is replaced with homozygous kk Kopyor coconuts in the field, negative xenia effects on kopyor fruit yield could probably be reversed. Mix coconut plantation consisting of heterozygous Kk and homozygous kk kopyor coconut provenances results in increased kopyor fruit yield because the homozygous kk kopyor coconut could become pollen contributors donating the k allele.

![Figure 2. Dispersal pattern of kopyor coconut pollen Dukuhseti with female parent number R1 from parentage analysis. The symbol identified the position (△) the female parent in kopyor, □ assigned male normal (pollen donor), ◆ the assigned male kopyor and ○ the assigned male in genjah kopyor.](image)

Xenia was not deviation from Mendelian inheritance, but a direct consequence of the multiple fertilization (double fertilization) occurring at flowering, plant embryo development process until the maturity of the seeds. At this stage of embryonic development, a number of genes in the embryo and endosperm expression affect the appearance of seeds, grains or fruits [25]. Unlike the xenia effect, metaxenia is inexplicable with the elements of heredity (chromosomes) carried in the pollen because unlike the chromosomes found in tissues that showed the direct influence of the pollen parent [26]. Metaxenia explained the effect of pollen on the network such as the pericarp fruit of maternal origin of, and other fruit components was not affected by pollen. On the other hand, xenia explained the effect of pollen in the tissues that contains at least one unit genes from the male parents of the embryo and endosperm [27].

5. Conclusion
Coconut plantation in Dukuhseti, Pati, tended to have negative xenia effect with the presence of normal coconuts surrounded by more kopyor fruits. The distance of dispersal pollen range from 0 m (self pollination) to 54 m (the furthest). The frequency of distance the dispersal pollen was highest at a distance <20 m with a total of 30 times pollination. The occurrence of outcrossing frequency was at least 95% and the selfing frequency is 5%.
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