Inheritance pattern of endosperm quantity and Kopyor coconut (Cocos nucifera L.) fruit variations

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Abstract. Although genetic resources of Kopyor coconut exists in Agom Jaya district, South Lampung regency, information about their fruit characteristic variations are limited. Hence, genetic inheritance patterns for endosperm quantity characters is not exist. Fruit characteristic variations and endosperm quantity inheritance patterns are important for Kopyor coconut improvement. We reported the Kopyor coconut fruit variations, fruit character correlations and endosperm quantity genetic control. Endosperm quantity was determined using standard scoring, ranged from 1 (low quantity, the same endosperm as normal coconut) to 9 (high quantity). Out of the 254 Kopyor fruits evaluated, the ones having endosperm quantity score 1, 2, 3 and 4 were 51.0%, 18.7%, 13.5%, and 7.6% while those having score 5, 6, 7, 8 and 9 were 4.4%, 3.6%, 0.4%, 0.4%, and 0.4%, respectively. Fruit characters having the highest coefficient of variation (CV) was fruit sizes (CV = 75.2) while the lowest was the shell weight (CV = 18%). A positive correlation was observed between score of endosperm quantity and weight of endosperm. On the other hand, the score of endosperm quantity was negatively correlated with the volume of coconut water. The endosperm quantity was genetically control by a duplicate dominant epistasis.

Keywords: coconut genetic inheritance, duplicate dominant epistasis, tall-Kopyor coconut, abnormal endosperm, natural mutant

1. Introduction
Coconut is a multi-purpose tropical tree. Copra and coconut oil is the two most important economic value products derived from coconut. Among However, there is a mutant coconut having abnormal endosperm phenotype, known as Kopyor coconut [1]. The abnormal phenotype of Kopyor coconut endosperm include: soft tissues, scramble and peeled off from the coconut shell [2]. The abnormal endosperm phenotype is probably associated with a single recessive mutant gene [2,3]. The commercial price of Kopyor coconuts are 5 – 10 times as high as that of the normal coconuts [4]. Therefore, we have previously studied this unique coconut mutant extensively [5-8]

Naturally, coconut trees capable of producing Kopyor nuts usually scatter among 3.7 million hectares of coconut plantations producing Normal nuts existing in Indonesia [9]. However, the centers of commercial Kopyor coconut plantation exist: in various location, such as: South Lampung district (Lampung); Sumenep and Jember (East Java); Pati (Central Java) and Tangerang (Banten) [2,3,8].
The Indonesian government has made efforts to increase Kopyor coconut production. An effort to increase Kopyor coconut production has begun by producing seeds from tissue culture rather than naturally existing seeds, by removing normal coconuts that grow near or around the kopyor coconut [2,3,10,11], expansion of Kopyor coconut plantation area, and crop improvement.

Slow progress in coconut improvement has become major concerns since there is only limited number of new varieties developed through coconut breeding programs. The currently existing Kopyor coconut varieties are either farmer varieties or landraces. Therefore, we need to start conducting breeding using this unique natural mutant to anticipate potential future demand for Kopyor coconut varieties.

However, coconut is a perennial crop and its require at least 4 – 7 years (depending on the varieties) before one can harvest their first fruit. Such characteristics become a major constrain in coconut breeding. Therefore, understanding the genetics controlling certain phenotypic characters will certainly beneficial for devising breeding strategies for the target crops. One of the major character associated with Kopyor coconut is the abnormal endosperm. Therefore, understanding genetic inheritance pattern of Kopyor endosperm will be beneficial for breeding activities of Kopyor coconut in the future.

Moreover, understanding genetic diversity among the available genetic materials will also be useful for designing breeding program. Inbreeding and loss of genetic diversity may hamper the aim of new variety development, especially for naturally outbreeding species [12] such as coconut. Therefore, in addition to understanding genetic inheritance of Kopyor endosperm, we also evaluated phenotypic variability of the Kopyor coconut from the South Lampung district, Lampung Province, Indonesia. Results of this study will give guidelines for the Kopyor coconut breeding by providing information on what character should be improved, how the characters inherit, and what germplasm is available, which are necessary for setting up breeding activities [13].

2. Materials and methods

2.1. Kopyor coconut provenances
Two hundred and fifty-five Kopyor fruits were collected from local farmer plantation at South Lampung, Lampung, Indonesia. The Kopyor fruit was harvested at approximately 10-11 months after pollination by local coconut tree workers. Each of the sampled Kopyor fruit was labeled based on the identity of its female parent. The Kopyor fruit harvest was done during the period of 23 months (January 2015 – November 2016).

The harvested fruit was sent to Plant Molecular Laboratory (PMB Lab.), Department of Agronomy and Horticulture, IPB University for morphological characterization and endosperm quantity measurement. A Photograph was taken for each of the harvested Kopyor fruit for recording. The recorded phenotypes include fruit weight, equatorial and longitudinal fruit length, seed weight, equatorial and longitudinal seed length. Moreover, shell weight and coconut water volume were also measured. The previously developed scoring system [14,15] was used to measure the endosperm quantity of Kopyor coconut. The standard endosperm quantity scoring (Score 1 – 9) used in this experiment was presented in Fig. 1.

2.2. Data analysis
All the collected data were tabulated using MS Excel for Windows. The simple statistic calculation of mean, standard deviation, coefficient of variation, minimum, maximum, and correlation analysis among characters were performed using SAS software version 9.0. Chi-square analysis was performed Microsoft Excel. The expected values were calculated based on the presumed ratio corresponding to the observed values for each character.

2.3. Correlation analysis among the Kopyor fruit characters
Determination of direct and indirect relations among the Kopyor fruit characters is important for identifying selection criteria [17]. Results of correlation analysis showed that there were positive
correlations among fruit weight (FWE) vs seed weight (SWE), longitudinal seed length (LSL), coconut water volume (CWV), endosperm weight (EWE), and shell weight (SHW). Positive correlations were also seen among SWE vs. LSL, CWV, EWE, and SHW; among ESL vs. LSL and SHW; among LSL vs. CWV and EWE; among CWV vs. EWE, SHW, and EQS; and among ENW vs SHW and EQS (Table 2). A significant positive correlation indicates that the correlated variables have a similar direction while a negative one indicates the variables have opposite direction.

3. Results and discussion

3.1. Characteristics of Kopyor fruits

Variation of the Kopyor fruit characters was assessed using 243 Kopyor coconut fruit samples, harvested from 150 female parents. The endosperm abnormality is genetically controlled by a single locus (K) and the abnormal Kopyor endosperm occurs when the endosperm genotype was kkk. Since all female parents producing Kopyor fruits were genetically heterozygous (Kk), only 25% of the total harvested fruits was Kopyor. Validation of the Kopyor phenotypes was done by splitting the fruit and taking the photograph of the endosperm for later used.

![Figure 1. Scoring system used for rapid identification of the Kopyor endosperm quantity. The scoring system was developed by Maskromo et al. [14,15].](image)

Result of the assessment presented in Table 2 showed that variation among the observed characters was high. Fruit character having the highest standard deviation was seed weight (518.62 g) and the lowest was the score of endosperm quantity (0.89). The fruit character with the widest value was the seed weight (3692 g) while the lowest was the score of endosperm quantity (8). Trait variation may be estimated by its standard deviation and range value. However, we cannot only rely on standard deviation and range value statistics to compare variation among different traits since they have a different unit [16]. Coefficient of Variation which is formulated as the standard deviation divided by its mean value is a unit-less statistic. Coefficient of variation (CV) is a measure of the relative variability of a given population [16]. This statistic, therefore, can be used to depict the most variable among the observed characters. Based on the calculated CV value, the equatorial fruit width (CV = 75.14) was the most variable character while the shell weight (CV = 18.13) as the least (Table 1).
Table 1. Mean value, standard deviation, minimum, maximum, and coefficient of variation of the evaluated Kopyor coconut fruit characters.

| Observed variable                  | N  | X    | STD   | Min | Max  | CV |
|------------------------------------|----|------|-------|-----|------|----|
| Fruit weight (FEW, g)              | 243| 2348.00 | 518.62 | 208 | 3900 | 22.09 |
| Equatorial fruit length (EFL, cm)  | 243| 514.24 | 386.38 | 45  | 566.7 | 75.14 |
| Longitudinal fruit length (LFL, cm)| 243| 486.96 | 221.98 | 48  | 785  | 45.58 |
| Seed weight (SWE, g)               | 243| 968.93 | 234.62 | 200 | 1950 | 24.21 |
| Equatorial seed length (ESL, cm)   | 243| 328.46 | 133.05 | 31  | 484  | 40.51 |
| Longitudinal seed length (LSL, cm) | 243| 301.64 | 151.89 | 26  | 465  | 50.35 |
| Coconut water volume (CWV, ml)     | 242| 358.83 | 138.77 | 50  | 800  | 38.67 |
| Endosperm weight (EWE, g)          | 243| 306.28 | 71.51  | 100 | 510  | 23.35 |
| Shell weight (SHW, g)              | 277| 291.59 | 52.87  | 150 | 430  | 18.13 |
| Endosperm quantity score (EQS)     | 175| 2.06  | 0.89   | 1   | 9    | 43.20 |

Notes: N = number of evaluated samples; x = mean value; STD = standard deviation; Min = minimum value; max = maximum value; CV = Coefficient of variation.

Since endosperm quantity is the major fruit characters in the Kopyor coconut, we focus our discussion on the correlation between the EQS vs other traits. Table 2 shows that the EQS was positively correlated to the CWV and ENW. This result was not unexpected as the score of endosperm is the reflection of the quantity of endosperm and both quantity endosperm and water coconut (liquid endosperm) fill central cavity inside the coconut shell. This result also indicates that selection for endosperm quantity could be done indirectly through the fruit weight.

3.2. The genetic inheritance pattern of endosperm quantity

Knowledge of the genetic inheritance pattern of a certain trait could provide guidelines for setting up a crop improvement program. Ideally, the genetic inheritance pattern can be obtained by crossing parental plants and evaluate its progeny. This method was quite difficult to do for tall coconut since it is open pollinated and the provenance may be more than 30 m. Therefore, poly-cross approach could be more appropriate method for studying the genetic inheritance pattern of Kopyor coconut endosperm quantity.

Kopyor character was affected by xenia effect (10,11) and controlled by a single locus (K). Therefore, the phenotypic ratio of normal vs. kopyor fruits harvested from a heterozygous Kopyor tree is 3 : 1. The number of normal fruits are three times higher than the number of kopyor fruits. In this study, we harvested 251 kopyor fruit. To meet the 3 : 1 ratio of Normal vs. Kopyor fruits we have to add 753 normal fruits having the endosperm quantity score = 1). All the data score endosperm quantity of Kopyor (251 data) and normal fruit (753 data) were used for Chi-square analysis. The frequency of endosperm quantity score among the harvested Kopyor fruits were presented in Fig. 2.

The average, min and max value of EQS of Kopyor fruits was 2.12 ± 1.49, 1 and 9 respectively. The frequency distribution of EQS data was not normally distributed and they skewed to the left (Fig. 2). The Fig. 2 also indicated that the EQS trait is considered under the control of qualitative trait loci (a qualitative trait).

Chi-square analysis for the EQS frequencies based on all segregation ratios of three phenotype class models showed significant deviation, indication the EQS segregation ratios did not fit to the three class models. However, results of Chi-square analysis for the EQS frequencies based on all segregation ratios of two phenotype class models showed non-significant results for the 15 : 1 segregation model. The ratio of low EQS class frequency (combine frequencies of score 1 and 2) vs. high EQS class (combine frequencies of score 3 – 9) meet the expected ratio of 15 : 1, indicating the inheritance pattern of the EQS fits to the duplicate dominant epistasis model (Table 3). The frequency of low EQS class vs. high EQS class was presented in Fig. 3.
Table 2. Coefficient Correlation among the observed fruit characters

| Traits | FEW | EFW | LFW | SWE  | ESL | LSL | CWV | ENW | SHW | EQS |
|--------|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|
| FEW    | -   |     |     |      |     |     |     |     |     |     |
| EFW    | 0.02| -   |     |      |     |     |     |     |     |     |
| LFW    | 0.14| 0.04| -   |      |     |     |     |     |     |     |
| SWE    | 0.63**| 0.01| 0.05| -   |     |     |     |     |     |     |
| ESL    | 0.07| 0.01| 0.10| -0.14| - |     |     |     |     |     |
| LSL    | 0.09| 0.01| 0.14| 0.24**| 0.15*| - |     |     |     |     |
| CWV    | 0.60**| 0.02| 0.05| 0.79**| 0.11| 0.21**| - |     |     |     |
| ENW    | 0.43**| -0.04| 0.13| 0.63**| 0.11| 0.13*| 0.34**| - |     |     |
| SHW    | 0.50**| -0.03| 0.11| 0.64**| 0.15*| 0.10| 0.46**| 0.51**| - |     |
| EQS    | -0.03| -0.03| 0.07| -0.076| -0.06| -0.10| 0.38**| 0.43**| 0.01| - |

Notes: FEW = fruit weight; EFL = equatorial fruit length; LFL = longitudinal fruit length; SWE = seed weight; ESL = equatorial seed length; LSL = longitudinal seed length; CWV = coconut water volume; ENW = endosperm weight; SHW = shell weight; EQS = endosperm quantity score; ** = significant at α = 1%; * = significant at α = 5%.

Figure 2. Frequency of the endosperm quantity scores (EQS) from a total of 1004 coconut fruits with either normal or abnormal (Kopyor) endosperm.

*Total number of coconut fruits with normal endosperm with EQS = 1 were 753 while Kopyor fruits with EQS = 1 were 128. All fruits having EQS > 1 were from Kopyor coconuts.

Therefore, the pattern of EQS genetic inheritance can be evaluated by using Chi-square distribution or Mendelian genetic approach. For Mendelian genetic analysis, the frequency of EQSs were grouped into either two or three phenotype classes and Chi-square analysis was later performed based on the EQS frequencies (Table 3).

The total number of low EQS fruits were 982. It consisted of 753 having Normal endosperm and 175 having Kopyor endosperm. If the K locus determine the Kopyor phenotype and the Q locus is the endosperm quantity, using the duplicate dominant epistasis model then the low EQS fruits with normal endosperm were predicted to have a $K^{-}/Q^{-}$ genotype while those with Kopyor endosperm were $kkk/Q^{-}$. Meanwhile, the total number of high EQS fruits was 76, and based on the duplicate dominant epistasis model their endosperm was predicted to have $kkk/qqq$ genotype.
Therefore, the predicted phenotype for coconut fruits having a K--/qqq endosperm would be Normal.
and low EQS while that of kkk/Qqq would be Kopyor and low EQS (Fig. 4). The genetic control of the Kopyor and the endosperm quantity in coconut fruits was like those reported for rice awn trait [18], which is also a duplicate dominant epistasis. Miko [19] describes that in the duplicate dominant epistasis ratio, both loci are complete dominant.

**Figure 3.** Frequency of endosperm quantity. Note: Kopyor fruit: LQ Kopyor fruit (kkkQ--; 175 fruits) and HQ Kopyor fruit (kkkqqq; 76 fruits), Normal fruits: LQ Normal fruits (K--Q--; and K--qqq; 753 fruits) HQ Normal fruits (K--qqq = 0 fruit), Mix: LQ Kopyor + LQ Normal fruits (928 fruits) and HQ Kopyor + HQ Normal fruits (76 fruits).

Fig. 5. showed the complete Punnet diagram of the predicted endosperm genotype segregations and their phenotypes in an F2 generation. The Punnet diagram showed that the proportion of individuals with the K--/Q-- genotypes was 9/16, the K--/qqq was 3/16 and kkk/Q-- was 3/16. The phenotype of coconut fruits having either the K--/Q--, the K--/qqq or the kkk/Q-- genotypes is low endosperm quantity. However, coconut fruits having both the K--/Q-- and K--/qqq genotype have Normal endosperm while that of kkk/Q-- has Kopyor endosperm. The high endosperm quantity is only expressed if the endosperm has a kkk/qqq endosperm genotype and the proportion of this was only 1/16 of the total harvested coconut fruits.
**Figure 4.** Hypothetic model of possible mutations at both the K and the Q loci controlling Kopyor and endosperm quantity in coconut. (A) Mutation and genetic constitution of the zygotic embryos (diploid). (B) Mutation and genetic constitution of the endosperm (triploid). The K locus is associated with Kopyor trait of the endosperm while the Q locus is associated with the quantity of the endosperm.

**Figure 5.** Hypothetic model of possible segregating progenies derived from hybridization of two heterozygous parents having double mutations at both the K and the Q loci controlling Kopyor and endosperm quantity in coconut. (A) Segregation of the zygotic embryos (diploid). (B) Segregation of the endosperm (triploid). The K locus is associated with Kopyor trait of the endosperm while the Q locus is associated with the quantity of the endosperm. Light green cells stand for segregating coconut fruits having Kopyor and low endosperm quantity (3/16) while dark green is one having Kopyor and high endosperm quantity (1/16). White cells stand for segregating coconut fruits having Normal and low quantity endosperm.

### 4. Conclusion

There is a high variation for most of the observed coconut fruit characters in the studied Kopyor coconut population. Positive correlation was presence among the endosperm quantity score (EQS) and the endosperm weight and coconut water content. Interaction between the locus controlling Kopyor character (K) and that controlling the endosperm quantity (Q) determine the final quantity of coconut fruit endosperm quantity. The inheritance pattern of coconut fruit endosperm quantity is most probably a duplicate recessive epistasis and the phenotypic segregation ratios between low vs. high endosperm quantity was 15: 1. The endosperm genotype of the high endosperm quantity fruits is predicted as $kk/kq$ while their zygotic embryos is $kk/qk$. The endosperm genotype of the low endosperm quantity fruits is predicted as either $kkk/Q-$ (for Kopyor endosperm) or $K- /Q-$ (for Normal endosperm).
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