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Original Paper

Identification of Kinship and Characterization of Yellow Bean Local Robusta Coffee

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***Abstract*— Curahpoh Village, Bondowoso Regency, has the potential to become a center for producing Robusta coffee. The uniqueness is found in several Robusta coffee outliers which produce yellow fruit resembling yellow cattura and yellow bourbon which are only found in Arabica species. A new clone of "yellow robusta coffee" found in Curahpoh Village needs to be characterized and identified for its kinship with existing robusta coffees on the market. It is hoped that the results of this study will become the basis for claims for the first superior new yellow coffee clone in Robusta coffee. This study used several morphological, physiological parameters to characterize these superior clones and identification of kinship with several other Robusta clones using RAPD (Random Amplified Polymorphic DNA). Other robusta clones as a comparison were imported from several community plantations or state-owned enterprises in Bondowoso district and its surroundings. The interim results in this study were that Robusta coffee in Curahpoh village was morphologically identical to BP 44, BP 42, BP 534 and BP358. This hypothesis is supported by the appearance of the leaves which are known to have waves that are quite strong compared to other coffee varieties collected in the trial greenhouse. Morphologically, Robusta coffee in Curahpoh village has similarities/identical with BP 44, BP 42, BP 534 and BP358 on the parameters of leaf number, leaf shape, leaf morphology and fruit color on the plant. Clones BP409, BP42, Sinasense, BP358, BP939, and Propelegitu are coffee clones that are molecularly highly related.**

Keywords*— morphology, RAPD, yellow coffee

I. INTRODUCTION

Curahpoh Village, Bondowoso Regency, has the potential to become a center for producing Robusta coffee [1]. Robusta coffee planting area is more than 100 ha with a productivity of 90 tons/year [2]. Robusta coffee is coffee that is commonly cultivated by smallholder plantations [3]–[5]. The conditions for growing at an altitude below 1000 meters above sea level make Robusta coffee more flexible and popular with farmers [6], [7]. Besides that, Robusta is known as a type of coffee that is resistant to biotic and abiotic stresses [8]. Meanwhile, Arabica coffee, even though it is more desirable in the market for its aroma, is more susceptible to pests, especially when grown below 1000 meters above sea level [9]. The difference between Robusta coffee and Arabica coffee is found in the number of chromosomes in the two types [10]. Robusta has diploid chromosomes (2n 2X) [11] while Arabica has allotetraploid (2n 4X) [12]. From this it can be seen that it is impossible to cross the two because of the difference in the number of chromosomes [13]. Visible differences can also be seen from the shape of the leaves. Arabica coffee usually has smaller leaves and the tips of the leaves are relatively more pointed [14]. Robusta coffee generally has physiological ripe fruit that is red and green to yellow when it is young [15]. The uniqueness is found in several Robusta coffee outliers which produce yellow fruit resembling yellow cattura and yellow bourbon which are only found in Arabica species. In this new plant, initially the fruit is green and then changes color to yellow and lasts until it is physiologically ripe. A new clone of "yellow robusta coffee" found in Curahpoh Village needs to be characterized and identified for its kinship with existing robusta coffees on the market. It is hoped that the results of this study will become the basis for claims for the first superior new yellow coffee clone in Robusta coffee. The contribution of universities in the development of superior coffee has seeds with yellow fruit skin which are important for farmers. This research is in line with the vision of the University of Jember in developing environmentally sound industrial agricultural products. In the Jember University Research Master Plan where the development of community coffee is a leading research with specific theme number 1, namely Development of community coffee towards an organic system to improve people's welfare. This research is also in line with the study program's research and service roadmap where the development of yellow coffee is the focus of activities with coffee farmer partners in Curahpoh as a fostered village. The purpose of this study was to confirm Robusta coffee with yellow fruit skin and to identify the kinship of this new superior clone based on molecular markers when compared to similar types of coffee grown around Bondowoso.

II. MATERIALS AND METHODS

The research began with a field visit to Curahpoh Village to see the potential for robusta coffee with yellow skin. Visits were also made to several other robusta coffee plantations to obtain robusta coffee buds as planting material.

A. *Description of Experimental Location*

Coffee inventory was carried out by collecting Robusta coffee around Bondowoso to compare its diversity. Several garden locations around Bondowoso, namely Jember, Banyuwangi and Situbondo. From the visited gardens, superior seeds were taken as plasmanutfah.

B. *Planting Material Production*

Robusta coffee seedlings with several types of clones were planted in a greenhouse using rootstock imported from the Cocoa Coffee Research Center. Stalks from several locations of superior robusta coffee plantations were planted using grafting techniques. Seedlings are planted with a minimum of 5 replications and cultivated for 6 months to produce seeds that are ready for planting.

C. *Agronomic Character Observation*

Agronomic characters were observed by looking at the growth in each experimental unit. This study used several morphological and physiological parameters to characterize these superior clones. Some of the parameters observed were growth rate, number of leaves, leaf shape, leaf morphology, fruit color on the parent plant, annual production potential and leaf chlorophyll content.

D. *Identification of Robusta Coffee Kinship*

Identification of kinship was carried out by looking at the genetic similarity between Robusta coffee with yellow skin compared to several Robusta clones that have been inventoried. The method used is RAPD (Random Amplified Polymorphic DNA) [16]. The primers used in the identification of molecular markers using the RAPD method according to [3] are as follows:

TABLE I. LIST OF PRIMERS TO BE USED FOR RAPD

Primers	Sequences	Tm (°C)
OPI 07	5' CAG CGA CAA G 3'	33.5
OPJ 19	5' GGA GAC CAC T 3'	33.4
OPY 15	5' AGT CGC CCT T 3'	36.9
OPI 20	5' AAA GTG CGG G 3'	36.2
OPX 16	5' CTC TGT TCG G 3'	31.6
OPL 18	5' ACC ACC CAC C 3'	38.7
OPX 20	5' CCC AGC TAG A 3'	31.8
OPY 10	5' CAA ACG TGG G 3'	33.7
OPN 18	5' GGT GAG GTC A 3'	32.9
OPM 04	5' GGC GGT TGT C 3'	38.6

III. RESULTS AND DISCUSSION

A. *Characterization of Local Robusta Coffee in Curahpoh Village*

Fig 1. Morphology of the 8th leaf germplasm of yellow robusta coffee in Curahpoh Village

The first activity of this research was a field visit, where the aim of this activity was to find out the main plant for planting coffee with yellow fruit skin. Looking at the morphology in (Figure 1), it can be seen that there is genetic variation in the form of differences in leaf characters. Plants 2, 3, 6 and 8 have leaf blades with more visible waves, when compared to coffee 1, 4, 5 and 7. In addition, variations in the size of the length and width of the leaves are also quite large, so further observations are needed to find out the causes of the differences. Physiology and morphology observed from field observations. For further observations it is also necessary to group them based on the results of the initial observations that have been made. Plants with elliptic and ovate leaf shapes need to be grouped and then made more detailed observations to find out whether they need to be separated or not in carrying out the next research stage. This is done to reduce data bias in observations at a later stage.

Based on the observation of leaf shape, there are several characteristic differences in the observed samples. There are several samples that show elliptic leaf shapes, but there are some that are ovate. Apart from that, from observing the shape of the plants, there are also many significant differences, namely some plants have a cylindrical shape and some plants have an inverted cone shape. Data on plant height and canopy diameter also vary quite a lot. Between 1.7 m and 2.5 m for plant height and between 1.9 m and 3.2 m for plant canopy diameter. Therefore there is a need for deeper identification and analysis regarding the variations in observations of coffee plants in the field. Is the difference due to differences in the growing environment or differences due to its genetic nature. This is because this coffee plant grows in Perhutani forest areas which have different shade cover and some plants grow in steep contour areas. However, several other characteristics have different ranges that do not vary much.

An inventory of robusta coffee species from various sources has also been carried out, most of which were obtained from the Coffee and Cocoa Research Center. There are also some coffees

obtained from private plantation companies in Kab. Jember. The coffee seedlings obtained were planted in the experimental garden of the Faculty of Agriculture for further observation. The next plan is to molecularly test the kinship of Robusta coffee from the Curahpoh plantation compared to the several Robusta varieties that have been collected.

Robusta coffee comes from various sources. There are several varieties including BP358 Sehasence, BP 308, BP 42, BP 44, BP 534, Propelegitim, BP 237, BP 939, Sintaro 2 and SA 237. Plasmanutfah coffee in Curahpoh is then compared with coffee with identified names and specific characteristics. Based on the results of observations, it is known that robusta coffee in Curahpoh village is morphologically identical to BP 44, BP 42, BP 534 and BP358. This hypothesis is supported by the appearance of the leaves which are known to have waves that are quite strong compared to other coffee varieties collected in the trial greenhouse. So it is necessary to test more deeply from physiological observations and then proceed to molecular observations

Fig 2. Robusta coffee that has been identified from coffee and cocoa research centers and from private plantations in Jember Regency

B. Test for Diversity Using RAPD

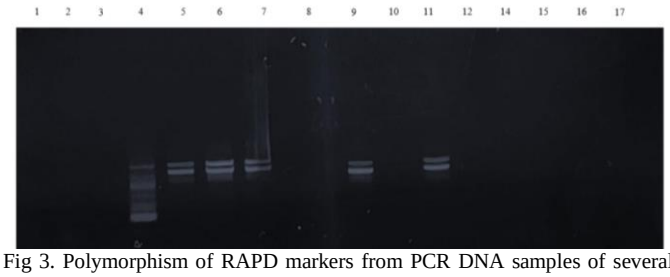

Fig 3. Polymorphism of RAPD markers from PCR DNA samples of several coffee plants with primers OPA02, OPA-03 and OPA-05 (Keterangan M=Marker, 1=BP936, 2=BP534. 3=Simtaro2, 4=BP409, 5=BP42, 6=Sinasense, 7=BP358, 8=BP308, 9=BP939, 10=BP237, 11=Propelegitu)

The diversity of several types of coffee that have been inventoried by the team is proven by molecular analysis. The method used is RAPD with 11 samples. The choice of primers in the RAPD analysis affects the polymorphism of the resulting bands because each primer has its own attachment site, as a result the polymorphic DNA bands produced by each primer differ, both in terms of the number of base pairs and the number of DNA bands [17]. The intensity of the amplified DNA bands in each primer is strongly influenced by the purity and concentration of the DNA template [18], [19]. DNA templates containing compounds such as polysaccharides and phenolic compounds, and concentrations of template DNA that are too small often result in dim or unclear amplified DNA bands [20], [21]. We found this unclear condition in RAPD marker polymorphism from PCR results of DNA samples using the primer OPA-01. The use of primer OPA-01 is less effective. It can be seen that only clone BP939 can be amplified from this primer, even though it is vague that clone BP358 is also able to generate OPA-01 primer. The opposite is seen in the use of other primers that look better. It can be seen that the use of primers OPA-02, OPA-03 and OPA-06 produces a more clearly visible band. Although optimization is needed to generalize it.

The results of molecular identification using RAPD showed a kinship relationship between coffee and Simtaro2, BP42, Sinasense, BP358, BP939 and Propelegitu clones. This is reflected in the results of polymorphism readings using the OPA-02 marker. While using the OPA 03 primer, it can be seen that Simtaro2, BP409, BP42, Sinasense, BP358, BP939, BP237 and Propelegitu clones. The use of the OPA-05 primer made it even more convincing that the BP409, BP42, Sinasense, BP358, BP939, and Propelegitu clones were coffee clones with a high affinity for traits/relationships (Figure 3).

IV. CONCLUSIONS

The conclusions that can be drawn from the identification of the diversity of several Robusta coffee clones based on morphological and molecular observations are as follows: Morphologically the Robusta coffee in Curahpoh village has similarities/identical with BP 44, BP 42, BP 534 and BP358 on the parameters of number of leaves, leaf shape, Leaf morphology and fruit color in plants. Clones BP409, BP42, Sinasense, BP358, BP939, and Propelegitu are coffee clones that are molecularly highly related.

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