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Frequency Distribution and Potence Ratio of Agronomic Traits in F1 Generation of Intraspecific Crosses of Robusta Coffee

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Abstract

Leaf morphological traits and internode length significantly affect the adaptability and production of coffee plants, respectively. Therefore, genetic studies of these two characters can support the effectiveness of Robusta coffee breeding program for obtaining superior planting materials. This study aimed to determine the diversity and frequency distribution of F1 generation on internode length and leaf size of Robusta coffee crosses. The study involved six groups of F1 progeny and their reciprocals (F1R) resulted from a cross of three parents, namely BP 961, Q 121, and BP 409. Each cross combination was planted with 41–49 progenies singly side by side in Kaliwining Experimental Station, Jember, Indonesia. The observed traits were internode length and leaf size. Analysis results of progeny variation distribution showed that most of the F1 and F1R progenies from intraspecific Robusta crosses had shorter branches than both parents in all combinations of crosses, even in BP 961 × BP 409 crosses. In addition, all F1 and F1R progenies had shorter internodes than both parents. More than 50% of the progeny of Q 121 × BP 409 and BP 961 × BP 409 crosses showed leaf length trait between parents. This indicates the absence of dominance of one parent in the progeny. Less than 20.9% of the progeny had a narrower leaf size than Q 121 parent. Most of the F1 and F1R crosses had a leaf width between both parents. The leaf length and leaf width traits were incomplete dominance or recessive, while the internode length was overdominant.

Keywords: genetic, morphology, characters, trait, Robusta

INTRODUCTION

Robusta coffee remains the backbone of national coffee production in Indonesia. In 2018, its production 87% dominated came from smallholder farmers (Ditjenbun, 2017). The coffee demand increases per year. Between

2011 to 2017, Indonesia's coffee consumption ranks the second largest in the world after Vietnam (Wibowo, 2019). However, the high increase in consumption has not been accompanied by an increase climate change, production decreased in

2019 (Ditjenbun, 2021). In addition, drought stress has been a factor inhibiting the growth and productivity of coffee (Sumirat, 2008; Erdiansyah *et al.*, 2019); it could reduce production by 10% (Syakir & Surmaini,

2017). The use of superior planting materials is one effort to improve coffee production and tackle the problem of seasonal changes (Supriyadi, 2014). As a coffee research institution in Indonesia.

method to create these superior planting materials is by cross breeding between superior Robusta coffee clones considering that cross breeding is common because the process is easy, and there are variations in genotypes in the progeny. With diallelic crosses, breeders can evaluate and select parents or combine them to obtain improvements in a population (Dalimunthe *et al*., 2015). Evaluation can be done by analyzing the phenotypic diversity of the progeny from crosses and conducting quantitative genetic studies. Quantitative genetics can help speed up the selection process, determine the breeding method used, and make the selection objectives more precise (Han *et al*., 2020).

Robusta coffee comes in two groups: the Congolese type originating from the Democratic Republic of Congo and the Conilon type originating from Gabon (Cubry *et al*., 2013; Sumirat, 2016). In general, *Coffea canephora* is divided into the Guinean and the Congolese groups. The Guinean group, originating from West Africa (Guinea and Ivory Coast), has morphological traits of smaller leaf size, small fruits, and smaller plant crown, making it easy to cultivate (Souza *et al*., 2013), lacking vigor, drought-tolerant, susceptible to leaf rust, and low taste quality (Silva *et al*., 2017; Carvalho *et al*., 2019). The Congolese group originates from central Africa and has morphological traits of a taller plant crown, more vigorous, wider leaf size, big fruits, more resistance to leaf rust, drought-intolerant, and better taste quality (Montagnon *et al*., 2003; Alexsandro *et al*., 2017; Carvalho *et al*., 2019). The Congolese type is divided into five subgroups, namely SG1, SG2, B, C, and UW, distinguished by their origin (Herrera & Lambot, 2017; Alexsandro *et al*., 2017; Labouisse *et al*., 2020; Kiwuka *et*

Interspecific crosses are commonly used to obtain resistance genes found in other species, while intraspecific crosses are commonly used to increase production. Genetic diversity is the basis for improving plant traits (Cubry *et al*., 2013; Gimase *et al*., 2015; Kiwuka *et al*., 2021). Robusta coffee breeding development dominantly uses two methods, namely mass selection and selection from crosses based on genetic distance between groups (Herrera & Lambot, 2017), and the focus of development is on increasing production and increasing fruit size (Mishra & Slater, 2012; Giles *et al*., 2018).

We need to select the varied progenies of crosses for superior planting materials. Observing the agronomic character is crucial in supporting the success of a plant breeding program (Akpertey *et al*., 2019). For example, leaf size is related to plant adaptability and photosynthetic efficiency, while internode length can be used to estimate coffee productivity. Plants with small leaf sizes have the potential to have high adaptability to high temperature environments (Wang *et al*., 2019). The internode length of the branch has an indirect influence on determining production (Beksisa *et al*., 2017). Leaves and branches are plant organs that provide initial responses when environmental stress occurs, and both traits are easy to observe (Valencia-Lozano *et al*., 2021). These two organs can be used as selection criteria for Robusta coffee for fruit growth and yield (Ali *et al*., 2015). In addition to morphological observations, knowledge of genetic variability and the degree of dominance of a population is also very that contribute to the inheritance of traits (Pandiarana *et al*., 2015; Haquarsum, 2006). Therefore, the selection of superior elders and estimation of the combined value of the two elders (heterosis) can be more effective (Batte *et al*., 2020). This study aimed to examine the frequency distribution of progeny based on morphological traits of branches and leaves of

MATERIALS AND METHODS

Robusta coffee crosses in this study involved three parents: BP 961 (P1), Q 121 (P2), and BP 409 (P3). The three parents have a high genetic distance, and the genotype of the progeny has been selected for purity based on the results of molecular testing (Sumirat *et al*., 2009). BP 961 is a superior genotype of Robusta coffee with good resistance to parasitic nematodes. Q 121 is a Robusta coffee genotype selected in Bangelan Plantation, Malang (Sumirat *et al*., 2009), has high pro- duction potential, and is a descendant of Conilon, also known as Kouilou. Q 121 belongs to the SG1 subgroup, which has the charac- teristics of the Guinean group. BP 409 is a Robusta coffee clone with high production potential (±2.3 tons ha^{-1}), moderate resistance to parasitic nematodes, and moderate resistance to coffee berry borer. BP 961 and BP 409 are from the Robusta coffee lineage of the Congolese group with the Robusta subgroup.

The experiment used dialelic crosses without selfing the parents so that there were six combinations of crosses. The F1 progeny from each cross combination were sown and planted as many as 41–49 progenies in single rows side by side and grouped following the cross combination with the pollinator genotype BP 42. Planting

(according to Schmidt & Ferguson). The F1

cross and its reciprocal (F1R) of BP 961 × Q 121 were coded A. Code B was for the F1 and F1R progeny of Q 121 × BP 409. Code C was for F1 and F1R progeny of BP 961

× BP 409. The spacing was 2.5 m x 2.5 m with *Leucaena leucocophala* shade (1:2).

Observations of plant morphology were carried out once at the beginning of the dry season when the progeny was four years after planting (the planting year was 2000). Observations were carried out in the vegetative phase, and the measured morphological traits included branch length, leaf length, and leaf width. Observations for each genotype were repeated five times. All observations were made following the IPGRI (1996) guidelines. The frequency distribution of

using the formula of $hp = \frac{\overline{F1}-\overline{MP}}{\overline{HP}-\overline{MP}}$, where hp is the potential value ratio, $\overline{F1}$ is the average of F1 or F1R heredity $\overline{HP}$ is the average values, of the highest $\overline{MP}$ is the middle value of the two parents (Petr, 1959; Haquarsum,

RESULTS AND DISCUSSION

Robusta coffee was crossed between subgroups with different morphological traits on leaves and branches in this study. Crosses between parents with a high genetic distance can cause high heterosis values (Batte *et al*.,

Parent Morphological Characters

Observation of plant morphological traits or characters is the initial stage in evaluating plant growth (Mishra & Slater, coffee is divided into the Guinean type, which is susceptible to leaf rust, and the Congolese type, which is more resistant to leaf rust (Alexsandro *et al.*, 2017; Carvalho *et al.*, 2019). Farmers in Indonesia mostly cultivate the Congolese type—it has four subgroups: SG1, SG2, B, and C (Sumirat, 2016; Carvalho *et al.*, 2019). A code A cross (BP 961 × Q 121) produced 88 genotypes, a code B cross (Q 121 × BP 409) produced 97 progenies, and a code C cross (BP 961 × BP 409) produced 89 progenies. Each genotype resulting from the cross had different morphological variations. Determination of the distribution of morphological diversity was based on the average value of the morphological characters of each observed trait. The mean value of the morphological characters of each parent is presented in Table 1. BP 961 had branch lengths that were not significantly different to Q 121 and BP 409. BP 409 parent had the longest internode, and Q 121 had the shortest internode. BP 409 had significantly longer and wider leaves than parents BP 961 and Q 12 (26.80 cm and 11.70 cm, respectively). Q 121 had the narrowest leaf width according to the morphological characteristics of the Guinean Robusta coffee group. The Q 121 parent was the descendant of the Conilon

Frequency Distribution of Internode Character

BP 409 had the longest internode (7.22 cm) while BP 961 and Q 121 had almost the same internode length (6.68 cm and 6.18 cm, respectively). The BP 961 × Q 121 cross resulted in 58.5% of F1 progeny and 89.3% of F1R progeny with a shorter internode length than the Q 121 parent. As many as 31.7% of F1 progeny and 8.5% of F1R progeny had internode length almost similar to the branch internode length of Q 121. As much 2.4% of F1, and 2.1% of F1R progenies showed branch internode length almost similar with BP 961 parent. As many as 7.3% of F1 progeny had internode length between the both parents, and there were no F1 or F1R progeny with internode length longer than BP 961 parent (Figure 1). Most of F1R progeny of BP 961 × Q 121 cross had shorter internode length than both parents.

In the Q 121 × BP 409 crossing, 89.6% of the F1 progeny and 73.5% of the F1R progeny had shorter branch internode than the Q 121 parent. The progeny distribution that resembled Q 121 was 10.4% for F1 and 20.4% for F1R. As many as 6.1% of F1R progeny had internode length between both parents, and no F1 and F1R progeny had the branch internode length similar to or longer than BP 409

Table 1. The leaf morphological characteristics of parents

Genotype	Internode length (cm)	Leaf length (cm)	Leaf width (cm)
BP 961	6.68 ab	19.40 b	8.20 b
Q 121	6.18 b	18.70 b	6.30 c
BP 409	7.22 a	26.80 a	11.70 a
CV (%)	10.41	9.51	9.86

Notes: The numbers followed by different letters in the same column show a significant difference at the 5% level; cv is coefficient of variance.

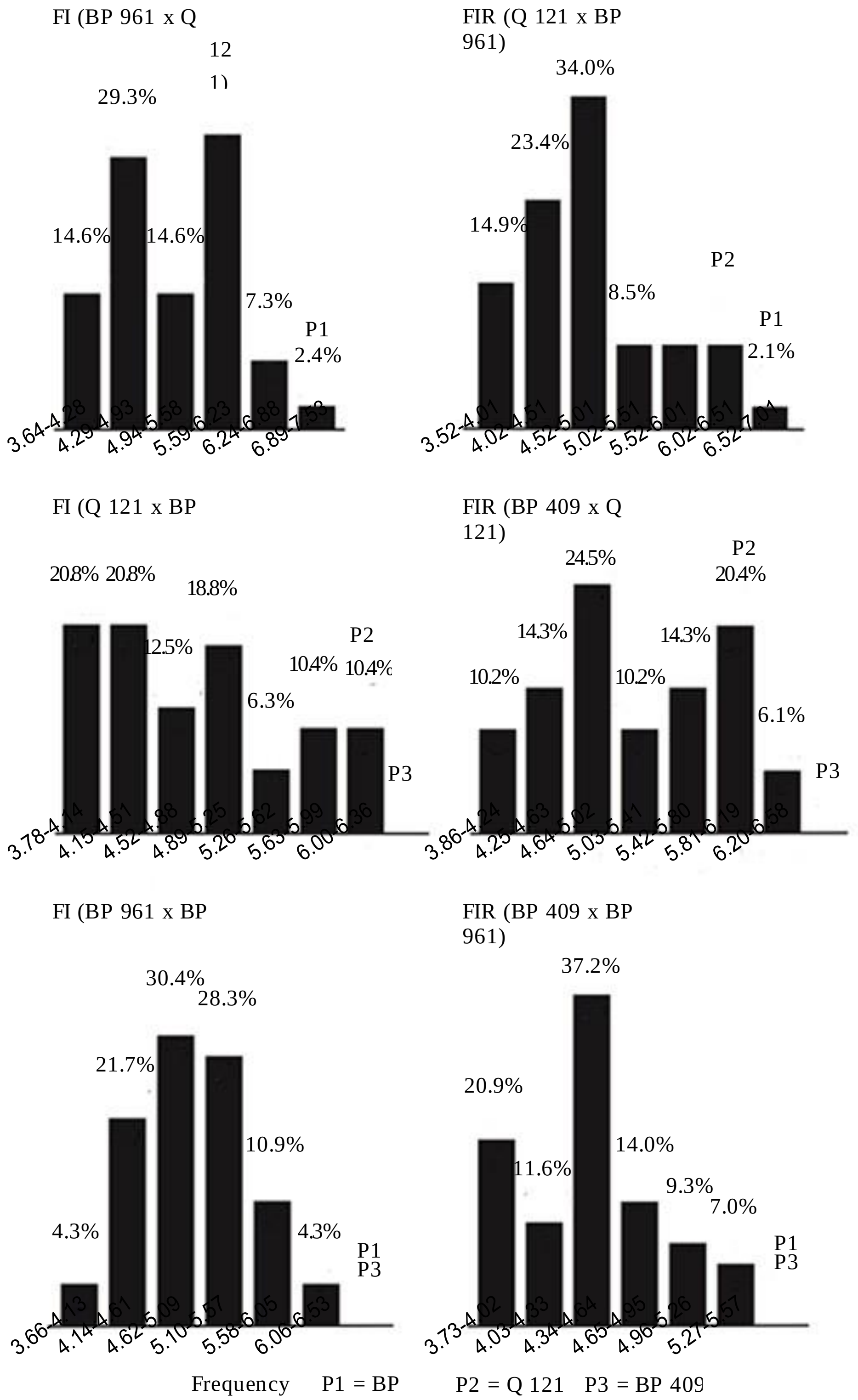

Figure 1. Frequency distribution on internode length (cm) of Robusta coffee progenies. Figures in the histogram are number of plants observed, while figures on histograms are percentage of frequency distribution

In the three combinations of parents, most F1 and F1R populations had shorter branch internodes than the parent with the shortest branch internode, considering that bunches of coffee cherries grow on the branch internode. The shorter internode branch has more bunches of coffee cherries. F1 progeny of Arabica coffee usually have longer branch internodes than their parents (Gimase *et al.*, 2015); however, our study confirmed different results since the progeny of the Robusta coffee crosses had higher proportion of shorter branch internodes. Furthermore, internode length and number were positively correlated with coffee bean production (Beksisa *et al.*, 2017). The length and number of main branch internodes also have a positive effect on the total number of pods of soybean plant (Allen *et al.*, 2018). Arabica is divided into two growing types, where the short-branch, dwarf Arabica, has higher productivity than the tall type (Hulupi, 2016) so that indirectly, the internode length can be used as a selection criterion to predict plant production. Selection could be made on the population of F1 progeny from the Q 121 × BP 961 crossing, which included seven genotypes with an internode length range of 3.52–4.01 cm, to get progeny with a short internode length. The progeny popula- tion of a short internode length can also be selected from the F1 progeny of

Frequency Distribution of Leaf Length Character

BP 409 parent had the longest leaf (26.8 cm), whereas Q 121 parent had the shortest leaf (18.7 cm). BP 961 parent had an average leaf length of 19.40 cm. Meanwhile the cross between BP 961 and Q 121 produced 7.3% F1 and 8.5% F1R progeny, which the Q 121 parent. Total of 14.6% of F1 progeny and 19.2% of F1R progeny had leaves resem- bling the Q 121 parent, and 36.6% of F1 progeny and 23.4% of F1R progeny had leaves resem- bling the BP 961 parent. Proportion of F1 and F1R progeny with leaves longer than BP 961 were 41.5% and 48.9%, respectively.

In the crossing of Q 121 × BP 409, 2.1% of F1 progeny showed shorter leaf morphology than the Q 121 parent. Progeny with leaf length re- sembling the parent BP 961 was only 2.1% of F1 generation and 6.1% of F1R progeny resembling the BP 409 parent. As many as 2.0% of F1R progeny had longer leaves than the BP 409 parent. Most of F1 and F1R progeny had leaf lengths between Q 121 and BP 409 parents, 95.8% and 91.9%, respectively (Figure 2).

Crossing between BP 961×BP 409 resulted in the frequency distribution of leaf length of F1 and F1R progeny, which was shorter than BP 961 by 13.0% and 14.0%, respectively. Leaf length of 21.8% population of F1 progeny and 18.6% of F1R progeny resembled the BP 961 parent, and 8.7% of F1 progeny resembled the BP 409 parent. Like the Q 121 × BP 409 cross, leaf length for most F1 and F1R progeny were also between the BP 961 and BP 409 parents, i.e. 56.5% and 67.4%, respectively. After obtaining variations in leaf size, the following breeding program was to select progeny with small leaf sizes. Leaf morphology is closely correlated with the environment and plant organs responsive to climate change (Li *et al.*, 2012). Narrow-leaved progeny are expected to be more adaptive to drought because they have more efficient energy exchange capacity (Wang *et al.*, 2019). The F1 and F1R

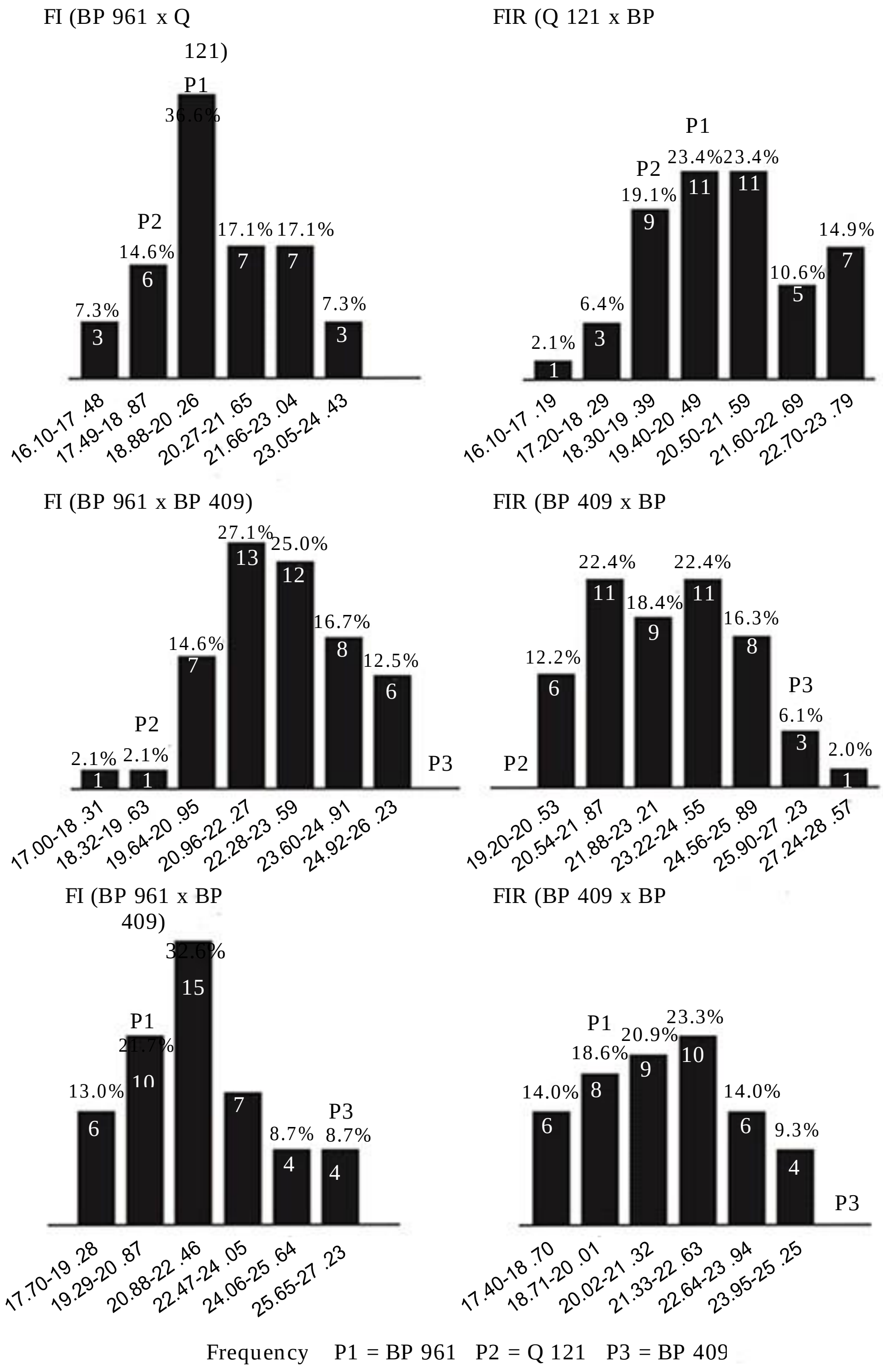

Figure 2. Frequency distribution on leaf length (cm) of Robusta coffee progenies. Figures in histogram are number of plants observed, while figures on histograms are percentage of frequency distribution

Frequency Distribution of Leaf Width Character

BP 409 genotype was the parent with the widest leaf size, which was 11.7 cm, while genotype Q 121 was the parent with the narrowest leaf size, which was 6.30 cm. BP 961 had average leaf width 8.20 cm. The crossing BP 961 × Q 121 resulted 7.3% of F1 progeny and 6.4% of F1R progeny having narrower leaf sizes than the Q 121 parent, while 19.6% of F1 progeny, and 19.1% of F1R progeny having wider leaf sizes than the BP 961 parent. Total of 19.5% of F1 progeny and 6.4% of F1R progeny resembled to the Q 121 parent, and 14.6% of F1 and 25.5% of F1R progeny resembled to the BP 961 parent. The highest proportion of F1 and F1R progeny population had leaf sizes ranging between Q 121 and BP 961 parents, i.e. 39.0% and 42.6%, respectively (Figure 3).

The Q 121 × BP 409 crossing resulted in 93.7% of F1 and 100% of F1R progeny having leaf width between parents Q 121 and BP 409; and only 6.3% of F1 progeny resembled the Q 121 parent. The large proportion of F1 and F1R progeny with characters between the two parents indicated the absence of dominance of one parent. Each parent contributed equally to the inheritance of leaf width characters to their progeny. The proportion of F1 and F1R progeny in the BP 961 × BP 409 cross whose leaf size was narrower than that of the BP 961 parent was 17.4% and 20.9%, and there were no progenies whose leaf size was wider than the BP 409 parent. Total of 21.7% of F1 progeny and 25.6% of F1R progeny had leaf width similar to that

Leaves represent morphological characters that are easy to observe and responsive to environmental conditions (Li *et al.*, 2012). The environment in which plants grow greatly affects the morphology of plant leaves (Royer *et al.*, 2008; Yang *et al.*, 2015). For example, *Acer rubrum* growing in cold areas has larger and wider leaf teeth (serration) than *Quercus kelloggii* growing in warmer areas (Royer *et al.*, 2008). The leaves of *Q. kelloggii* have more number of and narrower serrations—this may be related to inhibiting the evaporation process from the leaf surface. Plants that grow in hot places tend to have smaller and narrower leaves (Royer *et al.*, 2008). Variations in leaf morphology are often associated with the exchange of carbon, water, and energy of plants with their environment (Li *et al.*, 2020). The narrow size of leaves can be used as an indirect selection character in coffee plants to obtain genotypes resistant to drought stress. However, the results must be further proven with more specific tests.

In this experiment, selection of narrow-leaf progeny could be performed on the population of F1 and

Coefficient of Dominance

The dominance value (*hp*) is used to calculate the gene action of each parent. If the degree of dominance is 0 (hp = 0), then the observed character does not have the dominance of one of the parents. The value of hp = 1 or hp = -1 indicates

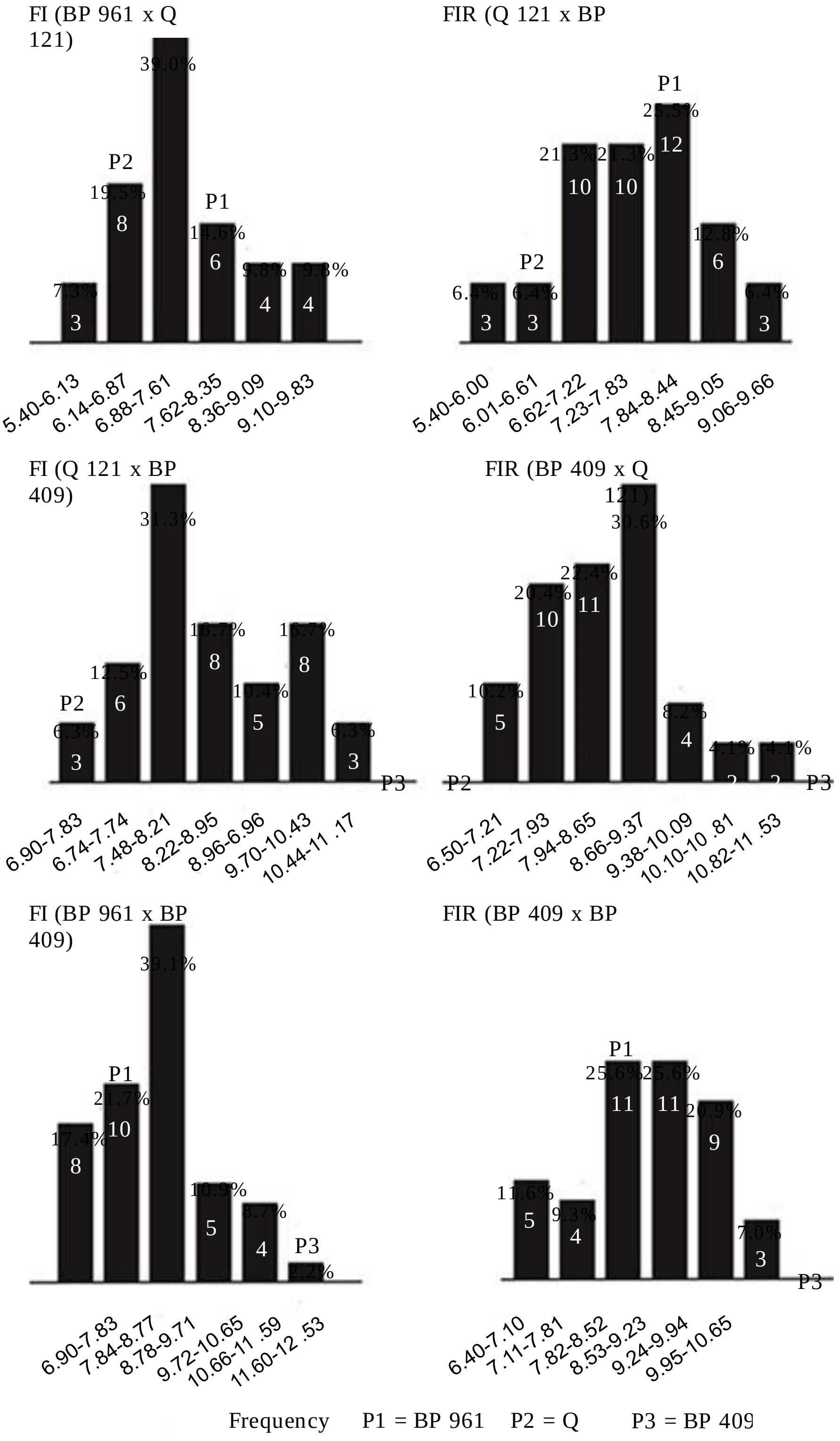

Figure 3. Frequency distribution of leaf width (cm) of Robusta coffee progenies. Figures in histogram are number of plants observed, while figures on histograms are percentage of frequency distribution

of $0<hp<1$ indicates the presence of incomplete dominance; a $-1<hp<0$ indicates incomplete recessive gene action; if the value of $hp>1$ or $hp<-1$ indicates an overdominance (Dalimunthe *et al*., 2015; Pandiarana *et al*., 2015; Haquarsum, 2006). In the BP 961 × Q 121 cross and its reciprocal (F1R), the degree of dominance of each cross combination for the character of internode length and leaf size was <-1 (Table 2). This indicates an overdominance of internode length and leaf size. Negative values for the degree of dominance have also been reported in leaf area and the number of internodes of pumpkin plants (El-Tahawey *et al*., 2015). A positive value in the degree of dominance indicates a variation in the dominance levels (partial to perfect), while a negative value indicates a variation in the recessive level of a character (Solieman *et al*., 2013).

The Q 121 × BP 409 crossing also showed an overdominance for the internode length character ($hp>1$) (Table 2). In leaf length character, the degree of dominance was -0.05 (F1) and 0.05 for reciprocity (F1R). These results indicated the presence of recessive or incomplete dominance for leaf length. Incomplete recessive gene action also occurred in leaf width. In the BP 961 × BP 409 crossing, incomplete dominant gene action occurred in branch internode length and incomplete recessive gene action occurred in leaf size. Incomplete

Dominance analysis is involved in estimating the contribution of parental genes to their progenies (Petr, 1959). The degree of dominance, also known as the potence ratio (Batool *et al*., 2013; Solieman *et al*., 2013; El-Tahawey *et al*., 2015; Kumar *et al*., 2017), is beneficial for determining more effective breeding program strategies. Crossing between two different subgroups in Robusta coffee produces an overdominant effect on internode length characters. The high genetic distance between BP 961 parent (from Robusta subgroup) and Q 121 parent (from Kouilou subgroup) caused an overdominant effect on one of the parents. The population resulting from a cross between the two subgroups is an ideal combination for a reciprocal recurrent selection program in Robusta coffee (Souza *et al*., 2013). The overdominant effect is a combination of two different alleles at the same locus (Batte *et al*., 2020), and one of them has a bigger influence. The internode length character can be used as a selection criterion at the beginning of the Robusta coffee breeding process.

Crossing between parents with high genetic distance is an effective method to obtain genotype variation. Genetic diversity is an essential part of plant breeding programs (Labouisse *et al*., 2020). Progeny evaluation is part of a long conventional breeding process after the parent selection and hybridization have been completed (Mishra & Slater, 2012). In crossbreeds, the

Table 2. Dominance values of Robusta coffee progenies

Parameter	BP 961 × Q 121		Q 121 × BP 409		BP 961 × BP 409	
	F1	F1R	F1	F1R	F1	F1R
Internode length	-5.00	-6.97	3.17	4.23	0.25	0.40
Leaf length	-3.55	-3.02	-0.05	0.05	-0.16	-0.15
Leaf width	-6.64	-8.58	-0.36	-0.51	-0.54	-0.78

Notes: F1 is hybrid filial 1 and F1R is the reciprocal of F1; BP 961 is parent 1 (P1); Q 121 is parent 2 (P2); BP 409 is parent 3 (P3).

the effectiveness of breeding programs to obtain superior offspring. Offspring with advanced selection will be utilized and developed into a gene pool as a breeding live, while other progenies can be utilized to conserve genetic resources (Akpertey *et al.*, 2019). The distribution of progeny frequency analysis aims to group the progeny characters with their crossed parents. These results are substantial for determining the next breeding program. For example, to get progenies with short internodes, selection can be made on the F1R generation of BP 961 × Q 121 and F1R of BP 961 × BP 409 because they have a higher proportion of progenies with short internodes than the other progenies. The internode length character is used as the initial selection criteria for yields because it has a high level of effectiveness (Srinivasan, 1982) and was positively correlated with the number of internodes of main branches (Fufa *et al.*, 2020) and the number of fruits per internode (Muvunyi *et al.*, 2017). This is very important for annual crops, especially coffee, because it can shorten the selection time in the breeding program. Branch internode length can be used as a pre-selection criterion before selection is made on yield. The criterion will be different if the purpose of selection is to get progeny whose growth is vigor. Vigorous plant is identical to the large leaf size, so the selection is made on progenies that are superior to the two parents. The distribution of progenies with longer and wider leaves was only found in the F1 and F1R generations of BP 961 × Q 121 so that the selection of vigor plants could be carried out on both populations.

tion criterion to obtain plant genotypes that are adaptive to hot environments (Wang *et al.*, 2019) and lower in nutrients (Kleiman & Aarssen, 2007). This adaptive nature is due to the higher leaf intensity (Kleiman & Aarssen, 2007).

The objective of Robusta coffee breeding in the future is to support mechanization and adaptation to hot environments. The BP 961 and BP 409 parents, which belonged to the Robusta type subgroup, tended to have wide leaf sizes and short branch internodes, while the Q 121 parent, from the Conilon type subgroup, had the opposite trait. Small leaf size can be a selection criterion for plants that are highly adapted to hot and dry environments (Wang *et al.*, 2019). Intraspecific crosses between the two subgroups are expected to produce genetic variations of progenies that can encourage the formation of new varieties (Silva *et al.*, 2017). The analysis results showed that improvement in traits began to happen in the progenies of crosses between subgroups. Most progenies were dominated by short internode (Figure 1), which was expected to increase fruit production. Pre-selection on the narrow leaf size is expected to produce new superior planting material resistant to hot environments. If the superior progeny of Robusta coffee has been selected, the development of the following planting material can be clonal. This propagation method is more relevant for individual plants that are genetically heterozygous to obtain uniformity of plant morphological characters (Souza *et*

CONCLUSIONS

Intraspecific crosses in Robusta coffee resulted in most progenies (>70%) having shorter branch internodes than the two parents. Selection of the short internode progeny was performed on the F1R progeny from BP 961 × Q 121 and BP 961 × BP 409 crosses. The progeny genotype with a narrow leaf character was produced by the combination of the F1 and F1R crosses of BP 961 × Q 121. The degree of dominance for the internode length character was overdominance, while the leaf size in each cross combination was recessive or incomplete dominance where alleles in one locus were partially expressed. The character of branch internode length and leaf size can be used as pre-selection criteria for high-yielding genotypes that are adaptive to hot

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Author Contributions

Both authors have contributed equally in preparing the manuscript for this publi- cation. All authors have read and agreed to publish this manuscript.

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