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Leaf Characterizations of IRR 400 Series, BPM 24, and RRIC 100 Rubber (*Hevea brasiliensis* Muell. Arg.) Clone Using Leafgram Method

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***Abstract*— Leaf characterization of rubber clones needs to be done from the start, especially for plants in the budwood garden. This function is to produce genetically pure and original plants. The purpose of this study was to characterize the IRR series 400, RRIC 100, and BPM 24 clones based on leaf character using the leafgram method. The types of clones that were characterized were IRR 425, IRR 428, IRR 429, IRR 434, IRR 440, RRIC 100, and BPM 24. The leaves characterized were the middle leaves of each clone as many as 50 leaves per clone, divided into three replications. The leaf characters observed were length, width, top fold point, center point, left strand, large, apex angle, vein angle, basal angle, apex kite angle, basal kite angle, ratio length/width, ratio length/top fold point, ratio left strand/large/ ratio left strand top fold point, ratio left strand/center point, ratio top fold point/large, ratio top fold point/center point, ratio apex/basal angle, ratio vein/apex angle, ratio vein/basal angle and ratio apex kite/basal kite angle. Data were analyzed using MINITAB 16 software and Microsoft Office 2010. The characterization results showed that the distinguishing characters that can be used as characteristic of characters are width, left strand, apex angle, basal angle, ratio length /width, ratio left strand/top fold point, ratio left strand ratio/center point, ratio top fold point/large, ratio apex/basal angle, ratio vein/apex angle, and ratio vein/ basal angle.**

***Keywords*— Leaf; rubber; budwood garden; characterization; leaf gram.**

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I. INTRODUCTION

Characterization of rubber clones in the budwood garden is important to be carried out in order to produce genuine and genetically pure spring sources. Generally, the characterization is to obtain uniformity of plant growth when the planting material that has been collected with the scion from the original and pure budwood garden has been carried out. Plant characterization can help provide information about the desired plant properties to increase selectivity in the field of plant selection, one of which is morphological characters [1]. Leaves are one part of the plant that can be characterized to distinguish one plant from another. Leaves are always available compared to other plant parts [2–4].

Characterization of a plant can be carried out on leaves because leaves are a characteristic tool, fundamental importance to plant as the power to photosynthesis and respiration system[5–7]. Each plant leaf was assessed to have a specific shape and size among the same plants when observed. In rubber plants, the characteristics of several observed characters that have been observed from quantitative characters are specific among clones, so that they can be used to identify a rubber clone[8].

The leaves of the rubber plant show similarities to each other and contain the same information such as color, texture, and shape. Unique traits can be used to differentiate between different clones/species [9–10]. The difference in leaf size, leaf area, leaf length and width ratio, leaf angle was assessed as significantly different among different clones so that it could be used as a marker in clone identification [11]. In this research, several improvements have been made in leaf sampling, measurement of leaf samples using the leafgram method, and adding measured leaf samples to improve accuracy in assessing the observed characters. Previously, characterization with digital and leafgram methods had been carried out on several commercially recommended clone rubber leaves, and the results obtained that with the leafgram method leaf measurements can be done easily and can be done by anyone [8][12-13]. So that in this study, research will be carried out on several IRR series 400 hopeful clones for characterization work to identify the leaves which are expected to be used as a database for quantitative data from some of the observed leaf characters.

Heritability value could be interpreted as the proportion between genetic variance and phenotypic variance. The heritability value of a character does not necessarily indicate that the character inherited is affected by genetic factors or environmental conditions [14]. The purpose of this study was to characterize the rubber clones of the IRR series 400, RRIC 100, and BPM 24 using the leafgram method.

II. Materials and Method

A. Study Site

The leaves are taken from trees in the budwood garden of the experimental garden of the Sungei Putih Research Center, Indonesian Rubber Research Institute which is located at an altitude of 80 meters above sea level, Sungei Putih village, Galang sub-district, Deliserdang district, North Sumatra, Indonesia (Fig. 1).

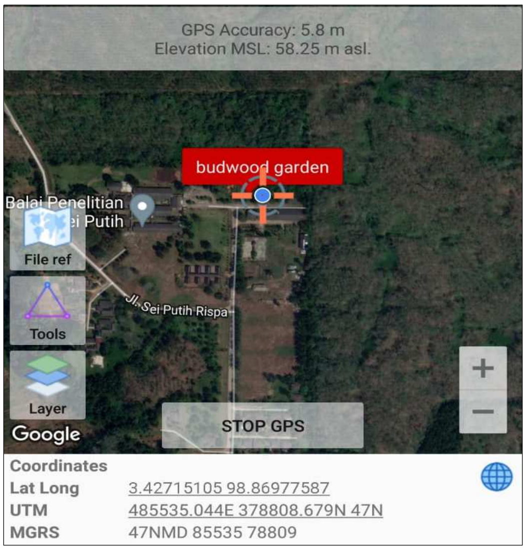

Fig. 1 The location of the research

B. Leaf Sampling

Middle leaf samples were taken from each of the characterized clones, namely IRR 425, IRR 428, IRR 429, IRR 434, IRR 440, RRIC 100, and BPM 24. The leaves used as samples were leaves that were considered normal in terms of plant growth and development (Fig. 2). Leaf characterization was measured using the leaf gram method (Fig.3).

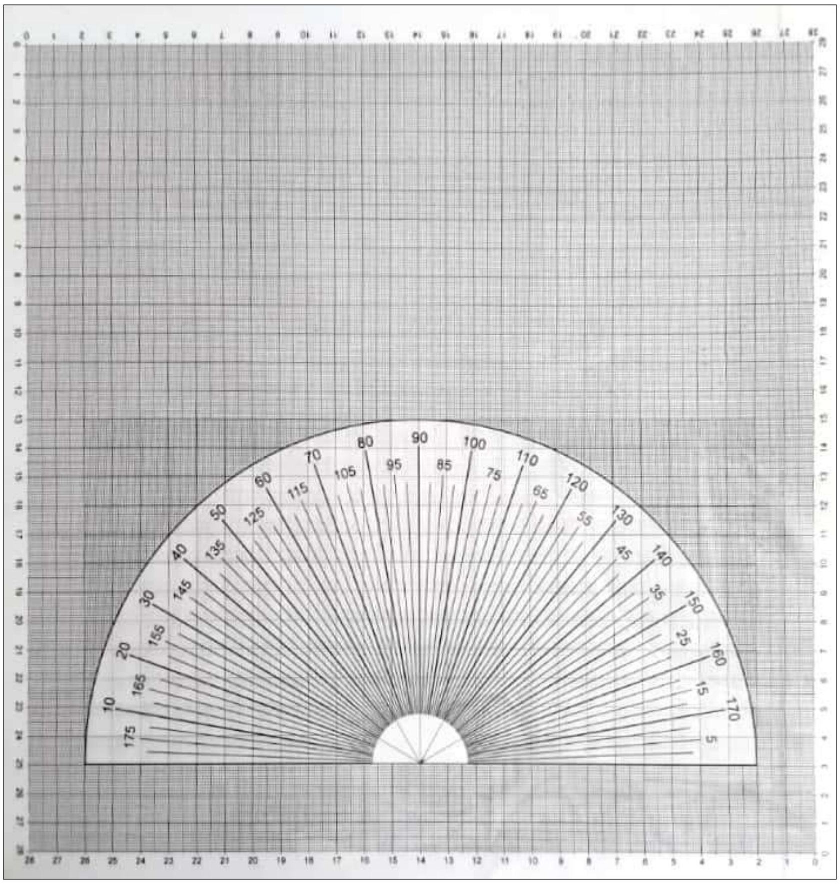

Fig. 2 The instrumental of leaf gram method.

Fig. 3 The middle leaf of each clone were used as samples.

C. Parameters Observed

The parameters measured are several characteristic factors that are considered to be able to differentiate between one clone and another. Characteristics assessed to distinguish each clone are as follows:

1) Size length

- The length of the leaf (L) is measured on a centimeter scale from the bottom of the leaf (base) to the top of the leaf (apex).
- The position of the center of the leaf (CL) is half the length of the leaf.
- The location of the leaf fold (FL) is the length of the leaf bone from the fold point at the maximum leaf width towards the apex.

2) Width

- Leaf width (W) is measured on a centimeter scale at the maximum leaf width.
- The width of the leaf blade to the left of the vein (LW) is measured at the position of the maximum leaf width from the left edge of the leaf to the middle leaf bone.

3) The angle of the leaf

- The natural base angle (B) is measured using an arc at the underside of the leaf following the leaf margin with the arc center point at the base of the leaf blade.
- The natural apex angle (A) is measured in the same way as the arc center point at the tip of the leaf.
- The vein angle (V) is the angle between the veins and the leaf bone measured in one of the veins around the leaf fold point.
- The base angle of the kite (BK) is the angle formed from the base of the leaf blade to the outer side of the leaf at maximum leaf width.
- The kite apex angle (AK) is the angle that forms from the tip of the leaf blade to the outer side of the leaf at maximum leaf width

From the three-leaf characteristics, several other parameters were developed, consisting of:

- Ratio of leaf length and width (L/W).
- Ratio of leaf length to point of a fold (L/ CL).
- Ratio of point fold to center point (CL/CF).
- Ratio of fold point to leaf width (CL/W).
- The ratio of the leaf width of the left side of the vein to the width of the leaf of the right side of the vein on the same leaf blade (SL /SR).
- The ratio of the width strand left to the point of folding (SL/CP).
- The ratio of the blade width of the left side to the center point (CP /FL) The ratio of the natural apex angle to the natural base angle (A/B) The ratio of the angle of the vein to the angle of the natural apex (V/A).
- The angle of the vein to the angle of the natural base (V/B)
- Ratio of the apex's angle to the kite's base angle (AK/BK).

D. Data Analysis

The observed characters were analyzed using MINITAB 16 software to see the variation in the diversity of each clone, if the diversity values obtained were significantly different, it would be followed by the Tukey 5% test. To clarified by looking at the heritability value to see the influence of genetic or environmental factors.

III. Results and Discussion

A. Leaf size characteristics

A total of six-leaf size characters were observed in this study, namely leaves length (cm), width (cm), left strand (cm), left large (cm^2), top fold point (cm), and center point (cm) (Fig. 4). The results of the statistical analysis of these characters showed that three characters had significant differences between the IRR series 400, RRIC 100 and BPM 24 clones were observed, namely the width (cm), left strand (cm), and large (cm^2). The clone with the widest, left strand the widest, and largest is the IRR 440 clone (7.71 cm; 3.96 cm; 146.51 cm^2) and the narrowest clone was the BPM 24 clone. This result looks quite different from previous research [8], from several clones that were characterized, all of the characters were judged to have real differences so that all of these characters could be used as characteristics. It is suspected that in this study the clones characterized had almost the same leaf size and shape and could only be distinguished from the three-leaf size characters. Visually (Fig. 3), most of the leaf shapes look relatively the same, that is, they are oval.

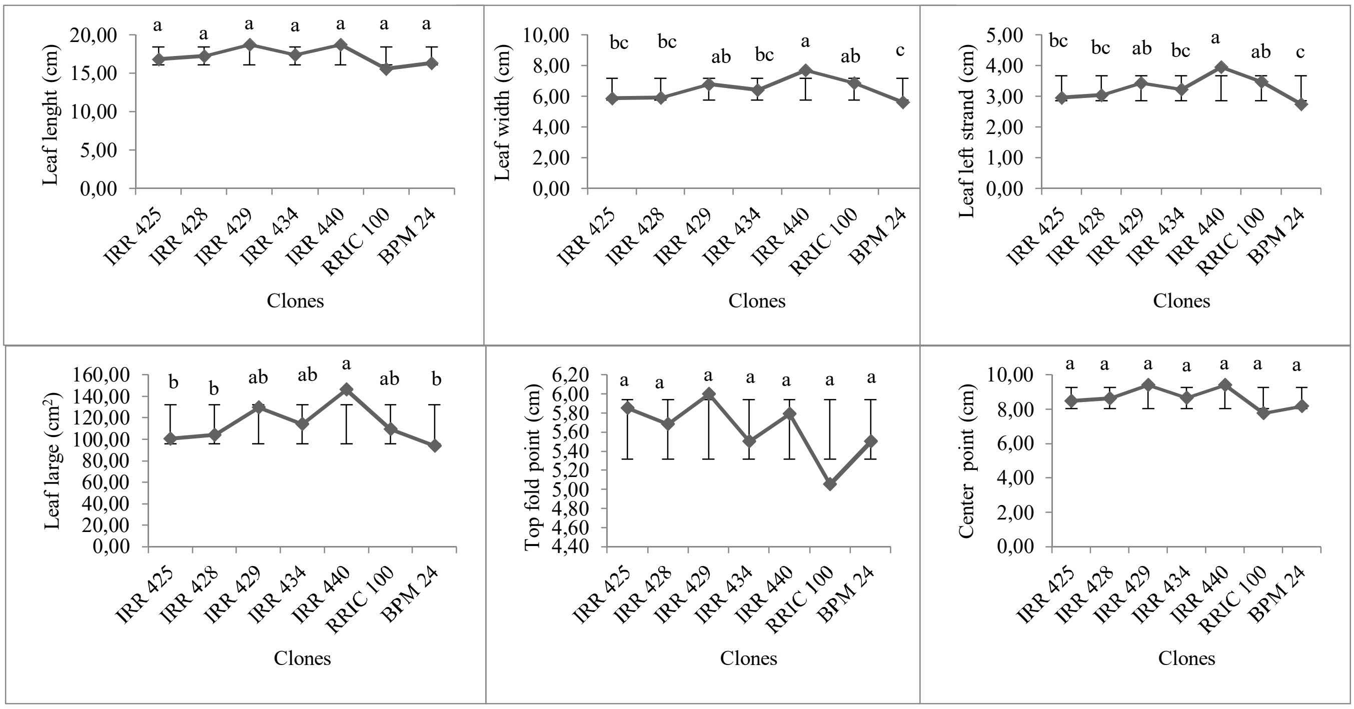

Fig. 4 The pattern of leaf length (cm), width (cm[2]), left strand (cm), large (cm[2]), top fold point (cm) and center point (cm) of IRR 400 series clones, RRIC 100, BPM 24. Data are represented mean ± SD. Means with the same letter are not significantly different for each other ($P < 0.05$) using the Tukey test.

The same thing is also found in some plants, where some morphological characters can be used as distinguishing characters between one clone or plant species with other plants, especially for the selection of plant breeding programs. In cacao plants, it is known that there are significant differences in leaf length and leaf area on the characterization of 6 cocoa clones [15], flavia & Swedish leaf [16], chili pepper accesions[17], Okra accessions [18], pamelo leaves [19], corchorus accession [20], local durio [21], taro [22], xylem zanthoxyloides [23], kenyan leaves [24], Sansevieria genus [25], Philippine leaf [26], Hopea and Shorea species[27] .

B. Leaf Angle Characters

The leaf angle characters observed were five characters, namely the angle of the apex (°), basal (°), vein (°), apex kite (°), and basal kite (°) (Fig. 5).

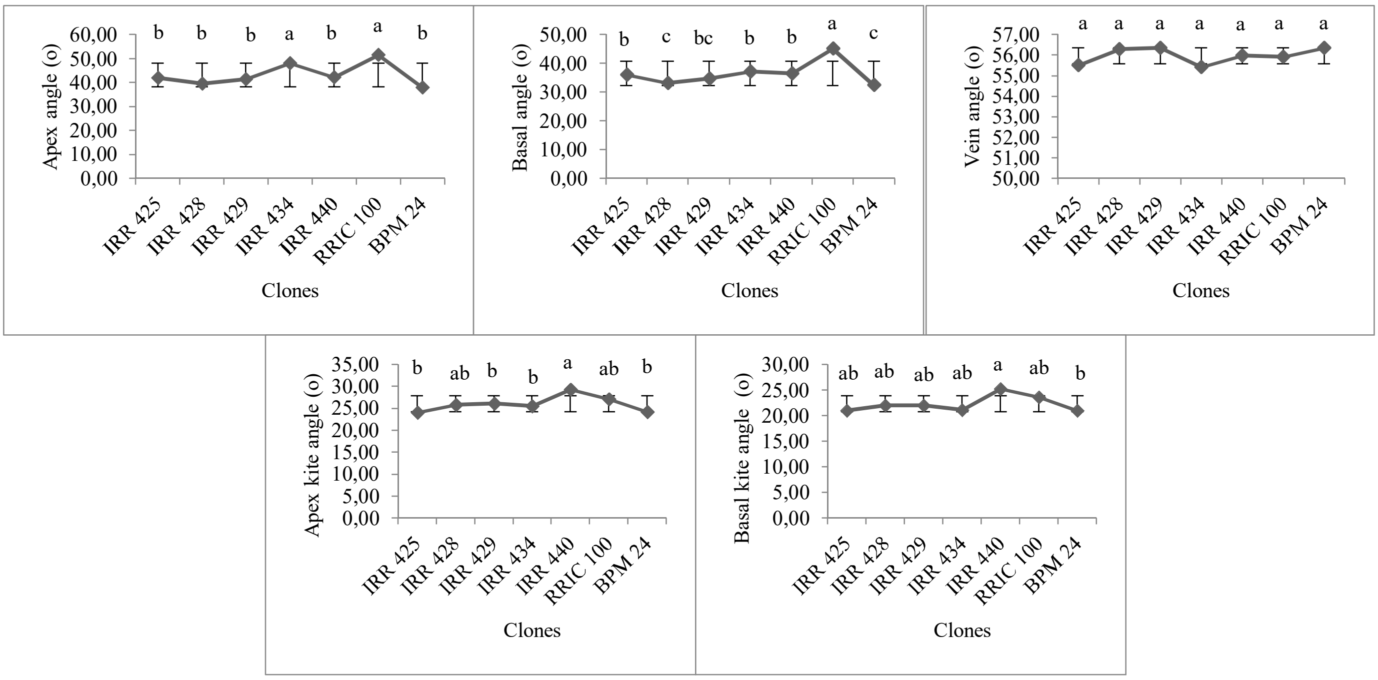

Fig. 5 The pattern of apex angle (°), basal angle (°), vein angle (°), apex kite angle (°), and (basal kite angle (°) of IRR 400 series clones, RRIC 100, BPM 24. Data are represented mean ± SD. Means with the same letter are not significantly different for each other ($P < 0.05$) using the Tukey test.

The results of statistical analysis showed that four characters showed significant differences, namely the angle of the apex (º), basal (º), apex kite (º), and basal kite (º). The apex angle of the characterized leaves showed that two clones had the greatest apex and basal angles, namely the RRIC 100 clone (apex: 51.56º; basal: 45.10º). The largest angle of the apex kite and the basal kite was shown by the same clone, namely the IRR 440 clone (apex kite: 29.32º, basal kite: 25.31º). This indicates that this angular character will show the different shapes of the leaf apex and basal. The results of this characterization indicated that the RRIC 100 clones which had the four greatest angular characters were judged to form a cuspidate apex (Fig. 6).

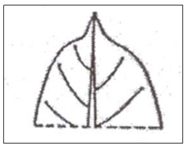

Fig. 6 The shape of a pointed leaf tip (cuspidate)

Other morphological characters that are also proven to be used as distinguishing characters are the apex, vein, and basal. Leaf morphological characteristics of some plants showed that the measured apex, vein, and basal leaves showed significant differences, namely 35 species of dipterocarfaceae[28], local durian of Banten Province[29], vitex negundo [30], a vein of cinnamon cebuense [27], apex, basal, and vein jujube [31], philippine apex [26], Hopea and Shorea species [27].

C. Ratio among characters

A total of eleven character leaf size ratios were observed to characterize the observed clones. These are the ratio length /width, length/top fold point, leaves left strand/ large, leaves left strand/top fold point, leaves left strand/ central point, leaves top fold point/large, leaves top fold point/central point, apex angel/basal angle, vein angle/apex angle, vein angle/basal angle and apex kite angle/basal kite angle (Fig. 7). The results of the analysis on all leaf size ratio characters were significantly different except for two characters, namely the leaves left strand/large and apex kite angle/basal kite angle. The significant difference between the many characters from the apparent leaf character ratio can be used as a marker for each clone analyzed. Data from each of these ratios can be collected into a database which can eventually be compiled to become a data bank that, if possible, is used as digital software.

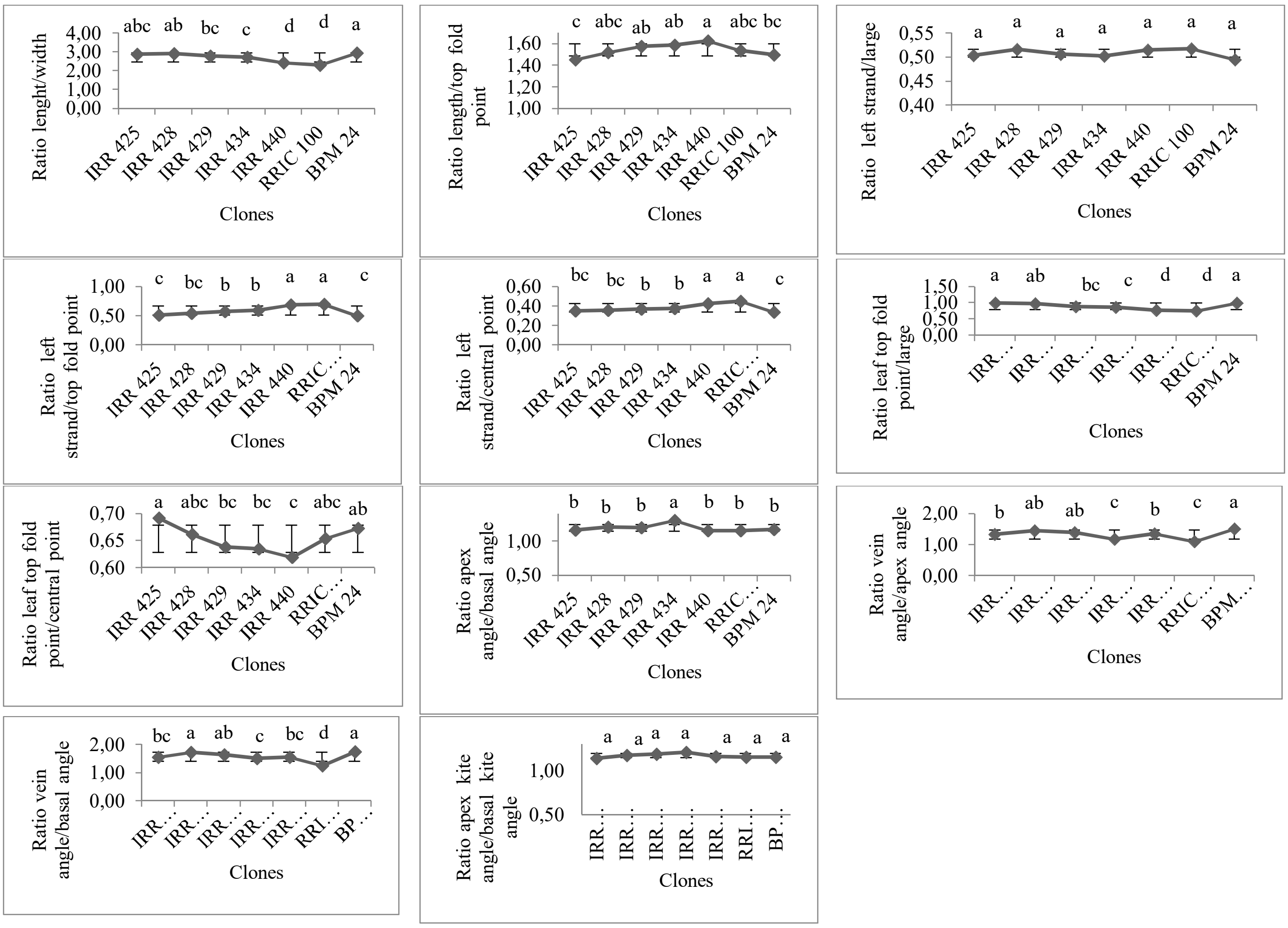

Fig. 7 The pattern of ratio lenght/width, lenght/top fold point, leaves left strand/large, leaves left strand/top fold point, leaves left strand/central point, leaves top fold point/large, leaves top fold point/central point, apex angle/basal angle, vein angle/apex angle, vein angle/basal angle, apex kite angle/basal kite angle of IRR 400 series clones, RRIC 100, BPM 24. Data are represented mean ± SD. Means with the same letter are not significantly different for each other (P < 0.05) using the Tukey test.

Significantly different ratio characters are thought to be less influenced by growing environmental factors. Environmental factors that affect leaf shape are light, humidity, and temperature. Plants that grow with strong sunlight have thick leaves with palisade tissue and thick sponges [8]. The character of the leaf size ratio is also a distinguishing character in Philippine plants [26], Hopea, and Shorea species [27].

D. Heritability

The heritability value of each observed leaf character can be used as an additional character to further validate the certainty of the characters being assessed. For characters that show significant differences in broad outline also have a high heritability value (>0.5). The characters that have a heritability value > 0.5 are width (0.58), left strand (0.65), apex angle (0.83), basal angle (0.90), ratio length/width (0.86), ratio left strand/top fold point (0.83), ratio left strand/center point (0.90), ratio top fold point/large (0.82), ratio apex/basal angle (0.59), ratio vein/apex angle (0.77), and ratio vein/basal angle (0.85). In more detail, the heritability value can be seen in Table 1.

In-plant breeding, this heritability value is calculated as a measure of the precision of a study in knowing a plant's response to a certain environment, especially for new plants that are the result of selection work [32]. High heritability values can be used as markers for other characters in the same plant species or as selection criteria [14]. In addition, it also has an important meaning in obtaining new superior genotypes that have superior traits when identifying important traits that will be maintained [33]. Another important thing in calculating the value of heritability is that it can mainly be done on long-lived plants so that at the beginning it can be done on a limited scale what important things can be assessed regarding genetic variation on plant growth [34]. Especially for this characterization, the existence of high heritability values of several characters assessed will provide something that can be maintained in conditions wherever the plant is grown will provide the same information as the information or assessment obtained in current conditions.

TABLE I
HERITABILITY VALUE OF LEAF CHARACTERS OF IRR 400 SERIES, RRIC 100 AND BPM 24 FROM BUDWOOD GARDEN IN SUNGEI PUTIH RESEARCH CENTER, INDONESIA RUBBER RESEARCH INSTITUTE (IRRI)

Leaf Characters	**Variance components**				**Remarks**
	Genotype variance (δ^2g)	**Environment variance (δ^2e)**	**Phenotype variance (δ^2p)**	**Heritability (h^2)**	
Length (cm)	0.43	1.56	1.99	0.21	Medium
Width (cm)	0.23	0.17	0.40	0.58	High
Top fold point (cm)	0.02	0.15	0.18	0.13	Low
Center point (cm)	0.12	0.38	0.50	0.23	Medium
Left strand (cm)	0.07	0.04	0.11	0.65	High
Large (cm^2)	129.83	223.00	352.83	0.37	Medium
Apex angle (°)	11.42	2.33	13.75	0.83	High
Vein angle (°)	0.02	0.55	0.53	0.03	Low
Basal angle (°)	8.60	0.92	9.51	0.90	High
Apex kite angle (°)	1.37	1.63	3.00	0.46	Medium
Basal kite angle (°)	1.74	6.22	7.96	0.22	Medium
Ratio length/width	0.03	0.005	0.003	0.86	High
Ratio length/top fold point	0.001	0.002	0.003	0.46	Medium
Ratio left strand/large	0.00005	0.000167	0.000172	0.03	Low
Ratio left strand/top fold point	0.00287	0.00058	0.00345	0.83	High
Ratio left strand/centre point	0.00082	0.00010	0.00092	0.90	High
Ratio top fold point/large	0.00515	0.00113	0.00628	0.82	High
Ratio top fold point/center point	0.00026	0.00029	0.00055	0.47	Medium
Ratio apex/basal angle	0.00125	0.00087	0.00213	0.59	High
Ratio vein/apex angle	0.01014	0.00298	0.01312	0.77	High
Ratio vein/basal angle	0.01325	0.00229	0.01554	0.85	High
Ratio apex kite/basal kite angle	0.0002	0.0007	0.0009	0.18	Low

Description: High heritability (H≥50% or H≥0.5), medium heritability (20%<H<50% or 0.2<H<0.5), low heritability (H≤20% or H≤0.2).

IV. CONCLUSION

The results of the analysis of the leaf characters showed that eleven characters could be used as distinguishing characters between one clone and another, namely width, left strand, apex angle, basal angle, ratio left strand/top fold point, ratio left strand/center point, ratio top fold point/large, ratio apex/basal angle, ratio vein/apex angle, and ratio vein/basal angle and these characters also have heritability values > 0.5.

NOMENCLATURE

IRR Indonesia Rubber Research
RRIC Rubber Research Institute of Ceylon
BPM Balai Penelitian Medan

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