Comparative study of leaf stomata profiles among different ploidy levels and genomic groups of bananas (Musa L.)

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Abstract. Stomata are presumable to be a potential taxonomic differentiating character. This study was aimed to describe the leaf stomata characteristics of banana from different genomic groups, to analyze its clustering pattern, and to confirm the potential character of leaf stomata as a taxonomic marker. Plant materials used in this study were twelve banana leaves; representing 6 genomic groups (AAw, AACv, AAA, AAB, ABB, and BBw). Stomata observation was carried out by replica method on both adaxial and abaxial of leaf near to petiole. Results showed that number of stomata in all bananas were higher on abaxial than adaxial. The diploid bananas have rounded shape of stomata while the triploid bananas have elliptical shape. Bananas with B genome tend to have regular stomata arrangement. Ploidy level in bananas is inversely correlated to the number of stomata, but positively correlated to stomata length and size. The diploid bananas were having high number of stomata but smaller stomata length and size, than the triploids. The genome size is influenced to the stomata size. Bananas with A genome tend to have larger stomata size than B genome bananas. Clustering analysis based on stomata profiles resulted two main groups following its ploidy level and genomic groups. Thus, stomata profiles can be used as a complementary character to classify among different genomic groups of banana.

Keywords: banana, characteristic, genome, leaf, ploidy, stomata

1. Introduction

Bananas are large, perennial, monocotyledonous herbaceous plant belonging to the genus Musa of the Musaceae family. The genus is native throughout the Indo-Malaysian region, and is considered as the main center of banana diversity both wild seeded species and seedless cultivated varieties [1, 2]. In Indonesia, there are not less than 200 banana cultivars recognized with many local names and any possible synonyms among and within cultivars [2, 3]. Bananas are widely distributed and cultivated, mostly found growing in backyards and very little as an estate crop if compared with other Indonesian tropical fruits [4, 5, 6].

Musa acuminata Colla (A genome donor, x=11) and Musa balbisiana Colla (B genome donor, x=11) are believed to be the ancestors of major banana cultivars that we know today [1]. Due to very complex evolutionary processes such as natural hybridization between wild species and somatic mutation proceeds for a long time, followed by natural selection, vegetative propagation, and the effect of environments, resulted to high diversity of bananas with various ploidy level and genomic...
composition, such as diploid (AA, BB, AB), triploid (AAA, AAB, ABB), and tetraploid (AAAA, AAAB) [1, 7, 8, 9].

Stomata are small pores or openings in plant tissue that play an important role in regulating water loss (transpiration) and gaseous exchange in and out the atmosphere for photosynthesis and respiration [10]. Stomata is considered as a character evolution [11]. It was associated to the environmental and climate changes [12, 13, 14]. Generally, the stomata are located on green parts of the plant, mostly the leaves. Stomata in most of bananas are regularly arranged and found in adaxial and abaxial parts of the leaf. It is anomocytic type, with two kidney-shaped guard cells, and subsidiary cell number varied 4 to 6 [15, 16, 17, 18].

Previous studies reported that stomata characteristics are varied among genomic group of bananas. Stomata size and density of bananas were influenced by genotype. Stomata size was proportional to ploidy level in bananas while stomata density had the expected complementary relationship [19]. The size of guard cells in triploid cultivars was considered longer than that diploid cultivars [17]. Further, the stomata density among dessert bananas (AA and AAA groups) differed between the abaxial and adaxial surface. The abaxial surface showed more conspicuous and well-organized wax layer and high stomata density [20]. In addition, there are direct proportional relationship between stomata density and length in bananas, where the greater the density, the shorter its length [21]. Thus, stomata profiles are presumable to be a potential taxonomic differentiating character for bananas. This current study was aimed to describe the leaf stomata characteristics of Indonesian banana cultivars at different ploidy levels and genomic groups i.e. AAw, AAcv, AAA, AAB, ABB, and BBw; to analyze its clustering pattern, and to confirm the potential character of leaf stomata as a taxonomic marker. The finding of this study are expected to support the classification of the rich local cultivars diversity of Indonesian bananas.

2. Materials and Methods

2.1. Plant materials and leaf sample preparation

Plant materials used in this study were twelve banana cultivars which representing 6 genomic groups i.e. AAw, AAcv, AAA, AAB, ABB, and BBw (Table 1). The leaves sample were obtained from banana living collection of Purwodadi Botanic Garden – Indonesian Institute of Sciences in Pasuruan, East Java. Each genomic group consists of two cultivars or species which functioning as a replicate. The leave blades were sampled from a mature banana plant, in particular the third leaf from the flag leaf of the inflorescence (at maximum point).

| No. | Location | Coll. number | Local name/ species | Genomic group | Collection locality |
|-----|----------|--------------|---------------------|---------------|-------------------|
| 1   | XXIV. D  | 12-a         | Cici/ M. acuminata var. rutilifes | AAw           | Tuban, East Java  |
| 2   | Glass house | NA          | Monyet/ M. acuminata var. nakaii | AAw           | Pasuruan, East Java |
| 3   | XXIV. E  | 104-abc      | Emas                | AAcv          | Bangkalan, Madura, East Java |
| 4   | XXIV. E  | 9            | Jaran               | AAcv          | Kebumen, Central Java |
| 5   | XXIV. D  | 42           | Billa               | AAA           | Sumbawa, West Nusa Tenggara |
| 6   | XXIV. D  | 5a-c         | Kreas               | AAA           | Tegal, Central Java |
| 7   | XXIV. E  | 14-ab        | Raja Lingi          | AAB           | Yogyakarta        |
| 8   | XXIV. E  | 127-abc      | Raja Sajen          | AAB           | Malang, East Java |
| 9   | XXIV. D  | 29-a         | Sobo Londo          | ABB           | Pasuruan, East Java |
| 10  | XXIV. D  | 17-abc       | Ebung               | ABB           | Pasuruan, East Java |
| 11  | XXIV. B  | 19-ab        | Klutuk Wulung       | BBw           | Kebumen, Central Java |
| 12  | XXIV. D  | 1-abc        | Klutuk Ijo          | BBw           | Pasuruan, East Java |
2.2. Stomata observation

Stomata observation was conducted in Plant Taxonomy Laboratory of Biology Department, Brawijaya University. Stomata profiles were observed using replica method for either stomata of opens profile and closed profile. Stomata with opens profile was taken directly on the field, during the daylight at 9.00 to 11.00 am. Whilst, stomata of closed profile was observed using leaves sample that has been fixed in the alcohol 70%.

The leaf sample was taken at the middle part of the leaf blade near to petiole. The leaf sample surface was coated with a clear nail polish and let it dry. Subsequently, apply the clear tape on it and lift it up gently. The clear tape with stomata replica was then stick on the glass object that has been labeled. Then, it was observed under the microscope CH20 with 400× magnification. Stomata profile was observed on both adaxial/ upper and abaxial/ lower surface of leaf sample, and both qualitative and quantitative (morphometry) characteristics.

2.3. Data analysis

ANOVA oneway test was applied using SPSS 20.0 for Windows to analyze the variance within and between genomic groups subjected to stomata morphometry data. Tukey post hoc statistical significance test was also employed (P < 0.05). Further, clustering and similarity-distance analysis were performed to identify the clustering pattern and similarity-distance among bananas examined based on stomata profiles both qualitative and quantitative data. Qualitative data was scored prior to analysis, while quantitative data was using real nominal data. The analysis was conducted using Paleontological statistics software package (Past3 version 1.0.0.0) based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and Gower similarity index for mixed data type, 100 bootstraps replication [22].

3. Result and Discussion

3.1. General characteristic of stomata in banana

In accordance to previous studies, stomata type in banana is considered anomocytic, where the structure of the subsidiary cells was quite similar to the epidermis around them, either from the shape nor the size [10]. The stomata were present on both the adaxial and abaxial surfaces of the leaf. Stomata of most monocot plants are available both in upper and lower epidermis of leaves or known as an amphistomatic leaves (stomata on both surfaces), whilst on most of dicot plants are only in lower epidermis [23, 24]. Nonetheless, a banana accession (AK4B) from East Kalimantan reported to have stomata only on abaxial [16].

Furthermore, the stomata opening is surrounded by a pair of guard cells in kidney-shaped which controls the opening and closing of the stomata aperture. Those guard cells are in turn surrounded by subsidiary cells which provide a supporting role for the guard cells. The number of subsidiary cells of bananas examined in this study were about 4 to 7 cells (Figure 1).

![Figure 1. A leaf stomata replica of banana (Raja Lingi - AAB): (a) guard cell, (b) stomata opening, and (c) subsidiary cell](image-url)
3.2. Stomata qualitative profiles among different genomic group of bananas

Stomata qualitative profile in bananas have exhibited varying characteristics but showing interesting pattern following its genomic group (Figure 2, Table 2). Bananas of AAw, AAcv, and AAA groups (A genome bananas) tend to have smooth to intermediate corrugation surface of stomata and epidermal layer. Whilst, bananas of AAB, ABB, and BB groups (B genome bananas) tend to have intermediate to rough corrugation. The rough corrugation was represented the position of stomata which deeply embedded in the leaf tissues or known as sunken stomata. The sunken stomata is considered as an adaptation to prevent excess transpiration due to sunlight exposure [25]. In addition, banana plants also show adaptations by having wax layer on leaves surfaces both adaxial and abaxial. The A genome bananas tend to have dull appearance (waxy) at upper surface, whilst B genome bananas have more wax on the lower surface [26], in order to prevent water loss during transpiration of excess sunlight.

The diploid bananas (AAw, AAcv, and BBw) have rounded shape of stomata (at opening profile) while the triploid bananas (AAA, AAB, ABB) have elliptical shape. The stomata shape was affected by the size ratio between its length to width. Ploidy level determines the cells size and the stomata itself. The size of stomata in diploid cultivars was considered shorter than the triploid cultivars [17], thus the stomata shape in diploid is rounded whilst in triploid is elliptical. The stomata of Malaysian bananas AA (Intan, Jari Buaya) and AAA (Novaria, Raja Udang) groups reported to also have an elliptical shape [20].

![Figure 2](image_url)

**Figure 2.** Leaf stomata and epidermis replica of six genomic groups of bananas on abaxial surface at opening profile: a. Cici (AAw), b. Monyet (AAw), c. Emas (AAcv), d. Jaran (AAw), e. Billa (AAA), f. Kreas (AAA), g. Raja Lingi (AAB), h. Raja Sajen (AAB), i. Sobo Londo (ABB), j. Ebung (ABB),
k. Klutuk Wulung (BBw), and l. Klutuk Ijo (BBw). Bar = 50μm.

Table 2. Qualitative profiles of stomata among different genomic groups of bananas

| Local name (Genomic group) | Surface corrugation of stomata & epidermal | Stomata shape | Stomata distribution & arrangement | Subsidiary cell number | Epidermis cell shape (front view) |
|---------------------------|------------------------------------------|--------------|-----------------------------------|-----------------------|----------------------------------|
|                           |                                          |              | adaxial                           | abaxial               |                                  |
| Cici (AAw)                | intermediate                             | rounded      | random                            | irregular             | 4-6                              | elliptical                      |
| Monyet (AAw)              | smooth                                   | rounded      | random                            | regular               | 4-6                              | elliptical                      |
| Emas (AAcv)               | intermediate                             | rounded      | irregular to zig zag              | regular               | 4-6                              | elliptical                      |
| Jaran (AAcv)              | intermediate                             | elliptical   | random                            | irregular             | 4-6                              | elliptical                      |
| Billa (AAA)               | intermediate                             | elliptical   | random                            | irregular             | 4-6                              | elliptical                      |
| Kreas (AAA)               | intermediate                             | elliptical   | random                            | irregular             | 4-6                              | elliptical                      |
| R. Lingi (AAB)            | rough                                    | elliptical   | random                            | irregular             | 4-6                              | rectangular                     |
| R. Sajen (AAB)            | intermediate                             | elliptical   | random                            | regular               | 4-7                              | rectangular                     |
| Sobo Londo (ABB)          | rough                                    | elliptical   | regular                           | regular               | 4-7                              | rectangular                     |
| Ebung (ABB)               | rough                                    | elliptical   | regular                           | regular               | 4-7                              | rectangular                     |
| K. Wulung (BBw)           | intermediate                             | rounded      | random                            | regular               | 4-6                              | rectangular                     |
| K. Ijo (BBw)              | rough                                    | rounded      | random                            | regular               | 4-6                              | rectangular                     |

Leaves of most monocot plants are typically linear with parallel venation and the stomata are usually arranged in regular rows in lamina [11]. However, stomata distribution and arrangement on leaf surfaces of bananas in this study were differs between adaxial and abaxial. On adaxial, the stomata were mostly irregular and randomly distributed, except ABB group bananas (Sobo Londo and Ebung) which have regular arrangement. Whilst, on abaxial mostly well-organized (regularly distributed and arranged) (Table 2). Hence, bananas with B genome tend to have regular stomata arrangement.

In addition, epidermis cell shape of bananas with more than one A genome have elliptical shape meanwhile bananas with at least one B genome have rectangular shape. Interestingly, ABB group bananas have the highest number of subsidiary cells approximately 4 up to 7. Consequently, they have thick and rough leaves compared to other bananas examined. It may also associated to environmental adaptations [12, 14].

3.3. Morphometry stomata among different genomic group of bananas

3.3.1. Number of stomata (per field-of-view)

This study result on stomata morphometry in bananas showed that generally the number of stomata per field-of-view was significantly higher on abaxial (in average 27.40 ± 6.12) than adaxial (in average 5.85 ± 3.65). On adaxial, Klutuk Ijo and Klutuk Wulung (BBw) were considered to have smallest stomata number (1.00 ± 0.00), meanwhile wild banana Monyet (AAw) was the highest (10.50 ± 3.54). On the other hand, Klutuk Ijo and Klutuk Wulung (BBw) were considered to have the highest stomata number on abaxial, i.e. 42.00 ± 2.83 and 35.50 ± 6.36, respectively. In addition, on abaxial wild banana Monyet (AAw) and Cici (AAw) also have high number of stomata but still in lower amount than wild banana Klutuk Ijo (BBw) and Klutuk Wulung (BBw). Triploid hybrids were considered in intermediate amount of stomata (Table 3).

Those results showed that number of stomata in bananas was confirmed inversely correlated to the ploidy level on abaxial surface [19]. The diploid bananas, particularly the wild bananas were having high number of stomata than the triploid bananas. Furthermore, the number of stomata on adaxial of B genome bananas (in average 2.58 ± 1.56) were smaller than A genome bananas (in average 9.12 ± 1.12), but not significantly different for the abaxial. However, B genome bananas were considered higher in stomata number abaxially than A genome bananas. The increase in stomata number associated to the greater photosynthetic rate under high-light conditions and then increase the crop yield [13, 27]. Consequently, indeed B genome bananas are having larger fruit and bunches performances, also higher in yields than A genome bananas [28, 29].
3.3.2. Stomata size at open profile

The size of the stomata is controlled by a pair of guard cells during the daylight. At open profile, stomata width both on abaxial and adaxial surfaces of bananas were not significantly different, ranged about 5.00 µm (Kreas, Raja Lingi) to 13.75 µm (Sobo Londo). However, the stomata length were longer on abaxial about 131.76% in average than adaxial. The stomata length was ranged about 13.75 µm (Klutuk Wulung - BBw, Klutuk Ijo - BBw) to 25 µm (Raja Lingi - AAB) on adaxial and 15.63 µm (Klutuk Ijo - BBw) to 32.50 µm (Sobo Londo - ABB) on abaxial (Table 3). The diploid bananas tend to have shorter length of stomata than triploid bananas. Indeed, the length of stomata is directly related to the ploidy level of bananas [17, 19, 22].

In terms of ratio length to width (L/W) of stomata, triploid bananas (adaxial 2.67; abaxial 3.69) were also tend to have higher ratio than diploid bananas (adaxial 2.58; abaxial 3.27). Ratio L/W is represented the shape of stomata. Therefore, this data support the facts that triploid bananas tend to have a more of an elliptical shape due to the high ratio L/W compared to diploid bananas (rounded) (Figure 1).

Stomata opening size area on abaxial were larger than the adaxial, in average about 212.76 µm and 161.98 µm, respectively. On abaxial, stomata size area of Klutuk Ijo (BBw) was the smallest (117.19 ± 6.63 µm) and Sobo Londo (ABB) was the largest (446.88 ± 57.45 µm). Meanwhile, on adaxial stomata size area of Kreas (AAA) was the smallest (71.88 ± 4.42 µm) and Sobo Londo (ABB) was the largest (275.00 ± 53.03 µm). The triploid bananas also considered have larger stomata size area than the diploid ones, except Kreas (AAA) on adaxial and Ebung (ABB) on abaxial. This data variation may be due to miss-timing of stomata opening during the leaf samples were taken.

3.3.3. Stomata size at closed profile

On the contrary of the stomata opens profile, morphometry stomata at closed profile showed that generally stomata size of bananas on abaxial were smaller than adaxial (width = 90.50%, length = 82.45%, size area = 68.01%). The pattern among and within genomic groups of stomata size (width, length, size area) at closed profile were more varied. However, consideration that the triploid bananas have larger stomata size than the diploid also applied to stomata at closed profile. Klutuk Ijo (BBw), Klutuk Wulung (BBw), were tend to have small stomata size, whilst Raja Lingi (AAB) and Sobo Londo (ABB) were tend to have large stomata size (Table 3).

Furthermore, if compared to the opens profile, the stomata width of closed profile on adaxial were shrunken 54.19%, but the stomata length were expanded 131.33%, and the stomata size area were subsided 73.44%. Meanwhile, the stomata width and length on abaxial were shrunken i.e. 47.82% and 82.18% respectively, and  the stomata size area were subsided more drastically 38.03%. Hence, the abaxial stomata in banana leaves were considered more sensitive to opening stimulation. Stomata anatomical features define the maximum theoretical conductance and also influence the speed of response [13].

The sizes of the guard cells of stomata and other leaf cells are correlated with genome size among angiosperms [12], including bananas. Upon this study, the wild bananas of M. balbisiana i.e. Klutuk Ijo and Klutuk Wulung were consistently to have smaller stomata size both at opens and closed profiles (width, length, size area) than M. acuminata i.e. Cici and Monyet, and all hybrids with at least one A genomes. More in details, the haploid B-genome size of banana is on average only 90% of the size of the A-genome [30]. That is why, bananas with A genomes were tend to have larger stomata size. Nonetheless, stomata size is negatively correlated with stomata number (per field-of-view) and/or stomata density. Interestingly, among bananas examined Sobo Londo (ABB) has the largest stomata size significantly on both adaxial and abaxial surfaces. It was presummably associated with its evolution and adaptation to the changing environments and climates.
| Local name (Genomic group) | Number of stomata (per field-of-view) | Width stomata – opens (µm) | Stomata area - opens (µm) | Width stomata - closed (µm) | Stomata area - closed (µm) |
|---------------------------|--------------------------------------|-----------------------------|---------------------------|-----------------------------|---------------------------|
| **Adaxial surface**       |                                      |                             |                           |                             |                           |
| Cici (AAw)                | 9.50 ± 0.71<sup>abc</sup>            | 18.75 ± 1.77<sup>abc</sup> | 9.38 ± 2.65<sup>ab</sup> | 173.44 ± 33.15<sup>abc</sup> | 25.00 ± 0.00<sup>bcd</sup> |
| Monyet (AAw)              | 10.50 ± 3.54<sup>e</sup>             | 19.38 ± 2.65<sup>abc</sup> | 6.88 ± 0.88<sup>ab</sup> | 134.38 ± 35.36<sup>abc</sup> | 20.00 ± 3.54<sup>ab</sup> |
| Emas (AAcv)               | 7.50 ± 0.71<sup>abcde</sup>          | 23.75 ± 0.00<sup>bc</sup>  | 10.00 ± 0.00<sup>ab</sup> | 237.50 ± 0.00<sup>bc</sup>  | 27.50 ± 3.54<sup>abcde</sup> |
| Jaran (AAcv)              | 8.20 ± 2.12<sup>bde</sup>            | 15.68 ± 0.88<sup>ab</sup>  | 6.88 ± 0.88<sup>de</sup> | 107.82 ± 19.89<sup>ab</sup> | 23.75 ± 1.77<sup>bcd</sup> |
| Billa (AAA)               | 9.00 ± 1.44<sup>de</sup>             | 21.88 ± 0.88<sup>bc</sup>  | 10.00 ± 0.00<sup>bc</sup> | 218.75 ± 8.84<sup>bc</sup>  | 28.75 ± 1.77<sup>bc</sup>  |
| Kreas (AAA)               | 10.00 ± 0.00<sup>bc</sup>            | 14.38 ± 0.88<sup>a</sup>   | 7.50 ± 0.00<sup>a</sup>  | 71.88 ± 4.42<sup>a</sup>    | 25.00 ± 0.00<sup>bcd</sup> |
| R. Lingi (AAw)            | 3.00 ± 2.83<sup>abc</sup>            | 25.00 ± 0.00<sup>a</sup>   | 7.50 ± 0.00<sup>a</sup>  | 187.50 ± 0.00<sup>bc</sup>  | 30.63 ± 2.65<sup>de</sup>  |
| R. Sajen (AAw)            | 5.00 ± 1.41<sup>bde</sup>            | 20.00 ± 3.54<sup>bc</sup>  | 7.50 ± 3.54<sup>ab</sup> | 156.25 ± 97.23<sup>abc</sup> | 33.75 ± 1.77<sup>c</sup>  |
| Sobo Londo (ABB)          | 2.00 ± 1.41<sup>b</sup>              | 24.38 ± 0.88<sup>a</sup>   | 11.25 ± 1.77<sup>c</sup> | 275.00 ± 53.03<sup>c</sup>  | 30.63 ± 2.65<sup>de</sup>  |
| Ebung (ABB)               | 3.50 ± 2.12<sup>bcd</sup>            | 22.50 ± 0.00<sup>c</sup>   | 8.13 ± 0.88<sup>c</sup>  | 182.84 ± 19.98<sup>bcd</sup> | 23.75 ± 1.77<sup>bcd</sup> |
| K. Wulong (BBw)           | 1.00 ± 0.00<sup>a</sup>              | 13.75 ± 1.77<sup>a</sup>   | 6.88 ± 0.88<sup>b</sup>  | 95.32 ± 24.30<sup>ab</sup>  | 20.63 ± 0.88<sup>bc</sup>  |
| K. Ijo (BBw)              | 1.00 ± 0.00<sup>a</sup>              | 13.75 ± 1.77<sup>a</sup>   | 7.50 ± 0.00<sup>a</sup>  | 103.13 ± 13.26<sup>ab</sup> | 16.88 ± 0.88<sup>a</sup>  |
| **Abaxial surface**       |                                      |                             |                           |                             |                           |
| Cici (AAw)                | 32.50 ± 0.71<sup>a</sup>             | 26.25 ± 1.77<sup>b</sup>   | 6.25 ± 1.77<sup>a</sup>  | 162.50 ± 35.36<sup>abc</sup> | 21.25 ± 1.77<sup>b</sup>  |
| Monyet (AAw)              | 29.00 ± 12.73<sup>a</sup>            | 24.38 ± 0.88<sup>abc</sup> | 12.50 ± 0.00<sup>b</sup> | 304.69 ± 11.05<sup>d</sup>  | 15.00 ± 3.54<sup>ab</sup>  |
| Emas (AAcv)               | 24.50 ± 2.12<sup>a</sup>             | 28.13 ± 2.65<sup>b</sup>   | 5.63 ± 0.88<sup>a</sup>  | 159.38 ± 39.77<sup>abc</sup> | 18.75 ± 1.77<sup>bc</sup>  |
| Jaran (AAcv)              | 24.50 ± 6.36<sup>a</sup>             | 23.75 ± 1.77<sup>bcd</sup>| 7.50 ± 0.00<sup>bc</sup> | 178.13 ± 13.26<sup>bcd</sup>| 21.88 ± 0.88<sup>bcd</sup> |
| Billa (AAA)               | 25.00 ± 7.07<sup>a</sup>             | 22.50 ± 0.00<sup>bc</sup>  | 12.50 ± 0.00<sup>b</sup> | 281.25 ± 0.00<sup>cd</sup>  | 22.50 ± 0.00<sup>bcd</sup> |
| Kreas (AAA)               | 24.00 ± 2.83<sup>a</sup>             | 27.50 ± 3.54<sup>b</sup>   | 10.00 ± 3.54<sup>bc</sup> | 268.75 ± 61.87<sup>bcd</sup> | 18.75 ± 1.77<sup>bcd</sup> |
| R. Lingi (AAw)            | 22.00 ± 7.07<sup>a</sup>             | 28.13 ± 4.42<sup>b</sup>   | 5.00 ± 0.00<sup>a</sup>  | 140.63 ± 22.10<sup>ab</sup> | 23.75 ± 5.30<sup>cd</sup>  |
| R. Sajen (AAw)            | 21.50 ± 2.12<sup>a</sup>             | 31.25 ± 1.77<sup>bc</sup>  | 7.50 ± 0.00<sup>bc</sup> | 234.38 ± 13.26<sup>bcd</sup> | 30.00 ± 0.00<sup>cd</sup>  |
| Sobo Londo (ABB)          | 25.00 ± 1.41<sup>a</sup>             | 32.50 ± 0.00<sup>a</sup>   | 13.75 ± 1.77<sup>c</sup> | 446.88 ± 57.45<sup>a</sup>  | 33.13 ± 0.88<sup>abc</sup> |
| Ebung (ABB)               | 24.00 ± 1.41<sup>a</sup>             | 26.25 ± 1.77<sup>b</sup>   | 5.00 ± 0.00<sup>a</sup>  | 131.25 ± 8.84<sup>a</sup>   | 20.00 ± 0.00<sup>bc</sup>  |
| K. Wulong (BBw)           | 42.00 ± 2.83<sup>a</sup>             | 20.00 ± 3.54<sup>a</sup>   | 6.25 ± 1.77<sup>a</sup>  | 128.13 ± 57.45<sup>a</sup>  | 13.13 ± 0.88<sup>abc</sup> |
| K. Ijo (BBw)              | 35.50 ± 6.36<sup>a</sup>             | 15.63 ± 0.88<sup>a</sup>   | 7.50 ± 0.00<sup>bc</sup> | 117.19 ± 6.63<sup>a</sup>   | 22.50 ± 0.00<sup>b</sup>   |

Note: values followed by different rates in the same column are not significantly different in Tukey Test with α = 5%.
3.4. Clustering pattern and similarity-distance based on stomata profiles of bananas

Clustering and similarity-distance analysis subjected to twelve bananas at six different genomic groups resulted in a dendogram which clearly separated into two main groups following its ploidy level and genomic groups, with stomata profile similarity 33.32% to 80.71% (distance 0.19 to 0.67) (Figure 3). Group 1 was consisted of all diploid bananas and one triploid banana (nested). Further, it was separated according to its genomic group into three sister sub-groups i.e. BBw, AAcv, and AAw plus AAA (nested). The triploid banana Kreas (AAA) was considered as an outlier nested to sub-group 3, probably due to variations.

Whilst, Group II was comprised of triploid bananas (AAA, AAB, ABB) which separated into two sub-groups (Figure 3). However, the genomic groups separation were unclear. Sub-group 1 was consisted of Bila (AAA) and Sobo Londo (ABB) with 72.50% similarity. Ebung (ABB) was close related to AAB group bananas (Raja Lingi and Raja Sajen) in sub-group 2 with similarity of 73.70% and 76.17%, respectively. This clustering pattern was confirmed that stomata profiles can be used as a complementary character to differentiate among ploidy level and genomic groups of bananas.

![Figure 3. Clustering pattern of twelve bananas at six different genomic groups based on stomata profiles both qualitative and quantitative traits](image)

4. Conclusion

Stomata profiles both qualitative and quantitative traits among ploidy level and genomic groups of bananas (AAw, AAcv, AAA, AAB, ABB, BBw) can be clearly differentiated, although there were some variation within. Therefore, stomata profiles can be used as a complementary taxonomic characters in bananas. Ploidy level in bananas is inversely correlated to the number of stomata, but positively correlated to stomata length and size. Further, the genome size is influenced to the stomata size, thus A genome bananas tend to have larger stomata size than B genome bananas. Research on stomata profiles of bananas using the whole mount method of paradermal layer suggested to be
conducted for a clearer results. Further researches related to the evolution and adaptation of stomata in bananas including leaf anatomy to environments and climate changes also interesting to be studied.

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