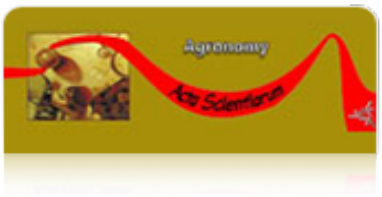


Acta Scientiarum. Agronomy
ISSN: 1679-9275
ISSN: 1807-8621
Editora da Universidade Estadual de Maringá - EDUEM

Madail, Rafael Hansen; Pio, Leila Aparecida Sales; Rezende, Renata Alves Lara Silva; Pasqual, Moacir; Silva, Sebastião de Oliveira e
Banana leaf anatomy characteristics related to ploidy levels
Acta Scientiarum. Agronomy, vol. 44, e55709, 2022
Editora da Universidade Estadual de Maringá - EDUEM

DOI: https://doi.org/10.4025/actasciagron.v44i1.55709

Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v44i1.55709





# Banana leaf anatomy characteristics related to ploidy levels

**Rafael Hansen Madail[1*], Leila Aparecida Sales Pio[1], Renata Alves Lara Silva Rezende[1], Moacir Pasqual[1] and Sebastião de Oliveira e Silva[2]**

[1]Departamento de Agricultura, Universidade Federal de Lavras, Campus Universitário, Aquenta Sol, s/n, 37200-000, Lavras, Minas Gerais, Brazil. [2]Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil. *Author for correspondence. E-mail: rafaelmadail@ifsul.edu.br

**ABSTRACT.** Many important crops have cultivars with different ploidy and provide a diverse choice of materials for breeding programs. Therefore, it is essential to accurately assess the ploidy of all breeding materials. Increased DNA content is known to have various effects on external and internal morphology, an effect known as the "nucleotypic effect". Thus, anatomical assessment can be used as a tool for determining ploidy in plants, making the chromosome counting technique or flow cytometry unnecessary. This study aimed to evaluate the leaf anatomical characteristics of banana cultivars and understand the relationship between these features and ploidy levels. Thirteen accessions were evaluated, including diploid, triploid, and tetraploid genotypes, and cultivars, resulting from in vitro propagation after 90 days of acclimatization. Five fully expanded young leaves were collected from each cultivar, fixed in FAA70 (formaldehyde–acetic acid–ethanol) and preserved in 70% alcohol. Transverse and paradermal sections of the abaxial and adaxial regions were taken, and variables such as size and stomatal density, leaf thickness in the midrib and fourth vascular bundle region, and thickness of the epidermis, hypodermis, and parenchyma were measured. Results for leaf thickness, stomatal size, and density proved to be appropriate parameters for characterizing banana ploidy levels.

**Keywords:** *Musa* sp.; leaf internal morphology; genetic diversity; plant microtechnique.

Received on September 8, 2020.
Accepted on February 4, 2021.

## Introduction

Plant anatomy has long been recognized for its importance in the field of taxonomy. The variations that occur within cultivars, species, genera, and families are reflected in the histology of plants (Ahmad et al., 2011).

More recently, quantitative anatomy has gained strength in the field of taxonomy and is especially useful for confirming the differences that simple anatomical descriptions are unable to distinguish. This technique has important applications, particularly in the characterization of crop cultivars that have different ploidy levels (Silva et al., 2019, Zeng, Liu, Du, & Kang, 2019). This field is of great interest for understanding the adaptive characteristics resulting from DNA content changes in plants. The widespread occurrence of polyploids suggests some adaptive advantage for these materials relative to their diploid progenitors (Hull-Sanders, Johnson, Owen, & Meyer, 2009), and it is extremely important that the ploidies of plant accessions are assessed early and accurately in breeding programs (Moon et al., 2013). In addition, plant anatomy knowledge is essential when asexual propagation is used to disseminate a species, which will thereby help in identifying the structural features necessary for successful propagation (Nassar, Graciano-Ribeiro, Fernandes, & Araújo, 2008).

It has long been observed that an increased DNA content leads to an increased cell volume, which is clearly observable in some plant structures, such as the size of the pollen grains and seeds, and the size and density of the stomata and chloroplasts (Beaulieu, Leitch, Patel, Pendharkar, & Knight, 2008).

Thus, ploidy has traditionally been designated by morphological and anatomical characteristics, and this process has provided good results. Phenotypic changes derived from variations in DNA content are known as the nucleotypic effect. Cavalier-Smith (2005) proposed that this phenomenon is balanced by cell growth, which maintains a constant ratio between the volume of the core, which is dedicated to transcription, and the volume of the cytoplasm, which is dedicated to protein synthesis.

Little information is available in the literature regarding the anatomy of the genus *Musa,* especially in terms of cultivar characterization and the effects of different ploidy levels (Vandehout, Ortiz, Vuylsteke,

Swennen, & Bai, 1995; Sumardi & Wulandari, 2010). Neither of these anatomical characteristics are included among those recommended by the International Plant Genetic Resources Institute (IPGRI, INIBAP, & CIRAD, 1996) as descriptors for banana cultivars. Therefore, this study aimed to contribute to the knowledge of banana leaf anatomy, characterization of its cultivars, and comprehension of histological modifications related to the ploidy level.

## Material and methods

The plants used in the experiment consisted of the apexes of 13 banana genotypes and cultivars with different ploidy levels from EMBRAPA Mandioca e Fruticultura Tropical active germplasm collection. These explants were cultured in vitro on MS medium and kept in a growth chamber with a light intensity of 36 µMol $m^{-2}$ $s^{-1}$ with a photoperiod of 16h and a temperature of 25 ± 2°C (Braga, Sá, & Mustafá, 2001). The materials were multiplied on MS medium for two generations, with each generation lasting for 30 days. The plants were subsequently transferred to a greenhouse and placed in 0.5 L pots in a substrate composed of 1 part soil: 1 part sand: 1 part manure and maintained under an intermittent mist system with 50% shading. Weekly watering was performed with liquid MS medium without sugar.

After 90 days of acclimatization, five of the youngest fully expanded leaves were collected from each banana accession. The materials were fixed in FAA70 (formaldehyde–acetic acid–ethanol) and then stored in 70% ethanol.

The samples collected were sectioned between the edge and midrib of the leaves in transverse and paradermal (abaxial and adaxial) directions, clarified in sodium hypochlorite (1% active chlorine) and triple washed in distilled water. The transverse sections were stained with safrabau solution (1% safranin and 0.1% astra blue in 7:3 ratio) and paradermal sections were stained with 0.1% safranin and then mounted on semipermanent slides with water and glycerin. The materials were observed under a Ken-a-vision microscope (Kansas City, MO, USA) and photographed. Measurements were performed using the Image Tool program (UTHSCSA Image Tool version 3.0). Sixteen transverse sections and twenty paradermal sections were evaluated for each banana accession.

The thickness of the leaf blade in the midrib and the fourth vascular bundle region, the thickness of the upper and lower epidermis, the upper and lower hypodermis, and the palisade and spongy parenchyma were measured in the transverse sections. The lengths, polar and equatorial diameters of the stomatal guard cells and the stomatal density (number of stomata per $mm^2$ of leaf surface) were measured in the paradermal sections. Quantitative data were subjected to an analysis of variance, and the mean values were compared using the Scott-Knott test at 5% probability.

## Results and discussion

Most publications on the relationship between anatomy and ploidy level in plant species are limited to the analyses of stomatal characteristics, with some exceptions exploring the inner structure of the leaf blade. It is important to use all the possible anatomical information not only to understand its relationship with ploidy levels, but also to characterize the anatomy of species and cultivars to assist in providing adequate choices for breeding programs.

Figure 1 shows images of the leaf blade: adaxial and abaxial epidermis of diploid (Malbut), triploid (Prata anã), and tetraploid (Princesa) cultivars to provide an anatomical overview. This image shows that the diploid accession has the thinnest leaf blade, the triploid accession is intermediate, and the tetraploid accession has the thickest leaf blade. The stomatal density is lower, and there are larger stomata on the adaxial surface of the tetraploid cultivar. The size of the adaxial stomata increased with increased ploidy.

Table 1 shows the results of blade thickness measured at the midrib and the fourth vascular bundle regions. Despite showing significant differences ($p < 0.0001$), the blade thickness in the central vein region was not a suitable parameter for distinguishing between the banana plant cultivars according to ploidy. The tetraploid cultivar FHIA 02 had the highest midrib blade thickness, with a value of 258.19 µm, and the value was higher than that observed for all the remaining cultivars. The rest of the tetraploid cultivars showed great variations in this parameter, with values between 218.09 µm for BRS Platinum and 165.18 µm for genotype 102.

These values overlapped with the values observed in triploid and even diploid cultivars, such as Malbut, in which the blade thickness at the midrib was 168.38 µm.

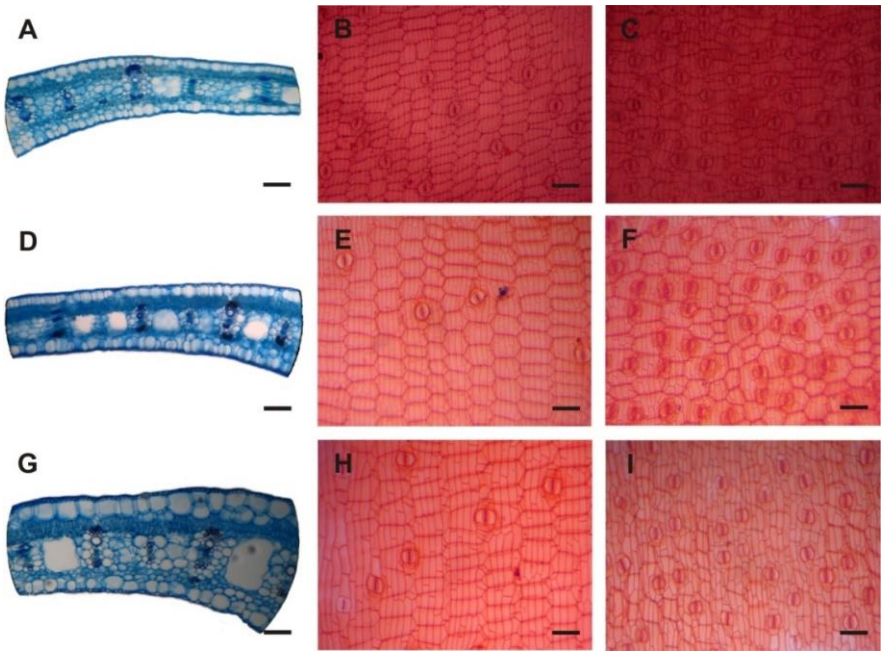


**Figure 1.** Photomicrographs of banana cultivars with different ploidy levels. A, B, and C – Diploid Malbut cultivar (leaf blade. adaxial. and abaxial surfaces). D, E, and F - Triploid Prata Anã cultivar (leaf blade. adaxial and abaxial surfaces). G, H, and I – Tetraploid Princesa cultivar (leaf blade adaxial and abaxial surfaces). Bars in A, D, and G were 150 µm and the other bars were 100 µm.

**Table 1.** Measurements of the leaf blade thicknesses (µm) in the midrib region and the fourth vascular bundle for banana accessions with different ploidy levels.

| Acessions | Genome | Midrib Region | Fourth Vascular Bundle |
|---|---|---|---|
| Malbut | AA | 168.38 D | 137.18 D |
| NBA | AA | 142.39 F | 142.20 D |
| Caipira | AAA | 155.44 E | 150.80 C |
| ThapMaeo | AAB | 210.66 B | 192.59 A |
| Prata Anã | AAB | 163.16 D | 170.45 C |
| Maçã | AAB | 170.71 C | 151.59 C |
| FHIA 02 | AAAB | 258.19 A | 197.88 A |
| Bucanero | AAAA | 192.50 C | 205.47 A |
| Princesa | AAAB | 202.37 C | 177.02 B |
| Garantida | AAAB | 198.00 C | 202.35 A |
| Tropical | AAAB | 176.44 D | 201.06 A |
| 102 | AAAB | 165.18 D | 155.89 C |
| BRS Platina | AAAB | 218.09 B | 178.96 B |
| C.V.(%) | | 4.90 | 6.48 |

Means followed by the same letter within the column belong to the same group according to the Scott-Knott test at 5% probability.

Sun et al. (2015), while working on polyploidization in pears, found that thicker leaves occurred in neopolyploids when compared to their diploid parents. However, the authors reported that these results varied depending on the evaluated polyploid.

Jellings and Leech (1984) evaluated different wheat cultivars and found that leaf thickness and tissue values did not always correlate for all plant materials. The authors proposed that the combination of nucleotypic and genetic effects creates an anatomical "identity" for each genotype; in other words, the imposition of genetic effects over genome size-related effects modulates the inner leaf structure.

Statistical analysis of blade measurements in the fourth vascular bundle region showed significant differences ($p < 0.0001$). The cultivars were separated into four different groups, of which the first two groups with the largest leaf thickness measurements were formed by tetraploid cultivars, an intermediate group was formed by triploid cultivars and the last group was formed by diploid cultivars that had lower leaf blade thickness values (Table 1).

Table 2 shows the results obtained for the thickness of abaxial (Ab. E.), and adaxial (Ad. E.) epidermis, thickness of abaxial (Ab. H.), and adaxial (Ad. H.) hypodermis, and thickness of palisade (P.P.) and spongy (S.P.) parenchyma.

**Table 2.** Measurements (µm) of leaf tissues from banana accessions with different ploidy levels[1].

| Accessions | Genome | Leaf tissues[2] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ab. E. | Ad. E. | Ab. H. | Ad. H. | P.P. | S.P. |
| Malbut | AA | 6.42 C | 7.10 C | 20.85 D | 21.05 C | 26.37 D | 21.44 B |
| NBA | AA | 6.94 C | 8.07 B | 19.22 D | 24.17 C | 28.99 D | 22.04 B |
| Caipira | AAA | 6.31 C | 6.69 C | 26.05 C | 26.03 B | 29.31 D | 20.90 B |
| ThapMaeo | AAB | 7.23 C | 8.60 B | 37.55 A | 23.79 C | 36.90 B | 28.04 A |
| Prata Anã | AAB | 7.33 C | 9.44 A | 24.71 C | 31.08 B | 45.18 A | 24.64 A |
| Maçã | AAB | 7.74 B | 8.13 B | 22.10 D | 23.39 C | 31.68 C | 21.06 B |
| FHIA 02 | AAAB | 7.75 B | 9.69 A | 23.17 D | 23.89 C | 43.57 A | 25.33 A |
| Bucanero | AAAA | 8.37 B | 9.97 A | 31.85 B | 27.05 B | 37.51 B | 22.39 B |
| Princesa | AAAB | 7.34 C | 9.89 A | 23.87 D | 27.36 B | 33.43 C | 17.57 B |
| Garantida | AAAB | 7.88 B | 10.61 A | 25.88 C | 42.01 A | 40.20 B | 23.21 B |
| Tropical | AAAB | 9.42 A | 10.79 A | 27.25 C | 28.57 B | 43.37 A | 26.94 A |
| 102 | AAAB | 7.07 C | 8.38 B | 27.98 C | 28.38 B | 33.62 C | 19.03 B |
| BRS Platina | AAAB | 6.58 C | 7.75 B | 23.88 D | 29.71 B | 40.40 B | 21.87 B |
| C.V.(%) | | 15.46 | 14.31 | 17.64 | 12.66 | 13.04 | 17.68 |

[1]Means followed by the same letter within the column belong to the same group according to the Scott-Knott test at 5% probability. [2] Ab. E. – Abaxial epidermis. Ad. E. – Adaxial epidermis. Ab. H. – Abaxial hypodermis. Ad. H. – Adaxial hypodermis. P.P. – Palisade parenchyma. S. P. – Spongy parenchyma

The thickness of the adaxial and abaxial epidermis correlated with the ploidy level for some of the cultivars. Regarding the thickness of the lower epidermis, the tetraploid cultivar Tropical stood out from the other cultivars for having the greatest value; however, the other tetraploid cultivars FHIA 02, Bucanero, and Garantida had greater epidermal thickness than the other diploid and triploid cultivars, apart from the triploid Maçã. For the rest of the cultivars, there were overlaps regarding ploidy levels. For example, the diploid genotype NBA had a thicker epidermis (6.94 µm) than the tetraploid cultivar BRS Platinum (6.58 µm). For the variable thickness of the adaxial epidermis, it was possible to characterize some cultivars according to ploidy: the tetraploid FHIA 02, Bucanero, Princesa, Tropical, and Garantida showed greater epidermal thickness. The triploids Maçã and Thap Maeo had intermediate values and diploid Malbut had a lower value. The other cultivars showed overlapping values, which cannot be used to infer the ploidy in relation to the epidermal thickness.

According to Ahmad et al. (2011), leaf epidermis is one of the most important anatomical characteristics for taxonomic studies, and its contribution clarifies questions about the systematics of many botanical families. Thus, the epidermis can also be an important tool for characterizing cultivars, especially in those with different ploidy levels, as observed by Baker, Yarkhunova, Vidal, Ewers, and Weinig (2017), Cao, Zhang, Gao, Wang, and Jia (2018), Van Laere et al. (2011), and Wang et al. (2017).

One hypothesis for explaining the fact that in this study, some cultivars showed no epidermal thickness values compatible with ploidy levels is that cultivation in tropical regions can subject plants to high irradiance levels. Leaf epidermis thickening is a well-known strategy used by plants to filter excessive environmental radiation, which can increase reactive oxygen species formation and lead to photodamage (Reyes-Diaz et al., 2016). Thus, one of the important adaptive characteristics of the species in tropical high-radiance regions is a thick epidermis. Sumardi and Wulandari (2010) also did not find a relationship between ploidy and epidermal thickness in their study of banana cultivars from Indonesia.

The thickness of the abaxial and adaxial hypodermis did not correlate with the ploidy level of the cultivars. Both the adaxial and abaxial hypodermis showed overlapping mean values for most cultivars at all ploidy levels. In relation to the thickness of the lower hypodermis, the diploid Malbut and NBA showed lower values for hypodermal thickness, which were suitable for determining the ploidy level. However, the triploid Maçã and tetraploids FHIA 02, Princesa, and BRS Platina also showed low values for the same variable. About the variable thickness of the adaxial hypodermis, it was possible to distinguish the tetraploid Garantida, with increased thickness (42.01 µm) from the triploids Prata Anã (31.08 µm) and Caipira (26.03 µm) and from the diploids Malbut (21.05 µm) and NBA (24.17 µm).

Hypodermis is a common feature in xerophytic species because of its capacity to store water in dry environments, although this is not the only function of the structure (Mascarenhas, Harley, & Scatena, 2020). It can be a mechanical support system to maintain the leaf architecture in windy places and, most importantly, it can act as an extra filter system to remove excess environmental irradiance, thereby protecting the photosystems from damage (Retamales & Scharschkin, 2015). Thus, it is expected that the hypodermis and epidermis will show similar results in its relationship with the ploidy level because it is a highly ecologically influenced trait.

The palisade parenchyma thickness was effective in differentiating diploids Malbut (26.37 µm) and NBA (28.99 µm) from the other cultivars. Tetraploid cultivars with the highest values were FHIA 02 (43.57 µm) and Tropical (43.37 µm), and the triploids Thap Maeo (36.90 µm) and Maçã (31.68 µm) had intermediate values. However, two triploid cultivars presented notable differences, with Prata Anã having a high value (45.18 µm), a pattern that was observed for both the parenchyma measurements, and Caipira (29.31 µm) showing no significant difference from diploid cultivars, a trend that was observed in other measured variables such as the thickness of the epidermis, hypodermis, and midrib region.

The thickness of the spongy parenchyma also showed no correlation with the ploidy levels of cultivars, and the tetraploids Tropical and FHIA 02, along with the triploids Thap Maeo and Prata Anã had the highest values.

The proportion of the chlorenchyma (palisade + spongy parenchyma) thickness relative to the blade thickness was 33.91% on average. The Princesa and Bucanero tetraploid cultivars showed lower values, with an average of 28.81 and 29.15%, respectively, and the triploid cultivar Prata Anã had the highest value, with an average of 40.96% of the blade thickness formed by the chlorenchyma.

One interesting feature that could be observed was the increase in the ratio of palisade parenchyma (PP) / spongy parenchyma (SP) related to the increase in ploidy. Diploid cultivars had an average PP/SP ratio of 1.27, while the average value was 1.75 for tetraploid cultivars. The investment in biomass is regulated by water and carbon economy needs driven by environmental features. Thus, plants from places where water and irradiance are not limiting factors can invest in carbon gain by increasing the PP/SP ratio.

Aspinwall et al. (2013) reported that genotypic variations that could be linked to climate were independent of the ploidy effect. Genome duplication creates an impact that leads to initial instabilities (Comai, 2005) that, according to Butterfield and Wood (2015) will be mitigated by the plants' need to "maintain homeostasis through plastic control of traits that affect physiology." Since most cultivars in this study are well established, it is possible that the initial effects of genome increase in the polyploids were "modulated" by environmental factors, leading to a lack of direct correspondence between some anatomical traits and ploidy level.

With respect to the stomata, banana leaves are characterized as amphy-hypostomatic, meaning that they have stomata on both sides but in a greater quantity on the abaxial surface.

Table 3 shows the stomatal characteristics of the banana cultivars. For the abaxial stomatal density variable, diploid Malbut had the highest density (119.57 stomata $mm^{-2}$), triploids Caipira (73.29 stomata $mm^{-2}$), and Prata Anã (73.43 stomata $mm^{-2}$) had intermediate values, and tetraploids Bucanero, Princesa, Tropical, and Garantida had the lowest values, ranging from 54.43 to 65.14 stomata $mm^{-2}$. A similar behavior was observed for the adaxial stomatal density. The diploid genotype Malbut had a higher value (31.57 stomata $mm^{-2}$), similar to that of the triploid Prata Anã (29.57 stomata $mm^{-2}$). Triploid Thap Maeo (24.00 stomata $mm^{-2}$) had an intermediate value and the tetraploids Garantida, Tropical, and 102 yielded lower values, ranging from 18.43 to 19.86 stomata $mm^{-2}$.

**Table 3.** Stomatal density (stomata $mm^{-2}$), polar and equatorial diameters (µm) in abaxial and adaxial surfaces from banana accessions with different ploidy levels[1].

| | | Stomatal characteristics[2] | | | | | |
|---|---|---|---|---|---|---|---|
| Accessions | Genome | Ab. D. | Ad. D. | Ab. P. | Ab. E. | Ad. P. | Ad. E. |
| Malbut | AA | 119.57 A | 31.57 A | 21.52 E | 12.36 D | 23.74 D | 11.23 E |
| NBA | AA | 82.43 B | 23.57 B | 22.37 E | 14.63 C | 23.74 D | 12.78 D |
| Caipira | AAA | 73.29 C | 15.86 D | 25.01 D | 15.07 C | 31.34 C | 15.04 C |
| ThapMaeo | AAB | 60.57 D | 24.00 B | 29.80 C | 15.64 C | 32.06 C | 17.63 B |
| Prata Anã | AAB | 73.43 C | 29.57 A | 30.63 C | 15.36 C | 32.08 C | 16.74 B |
| Maçã | AAB | 88.86 B | 15.57 D | 28.25 D | 16.11 C | 31.65 C | 16.05 B |
| FHIA 02 | AAAB | 73.43 C | 22.14 B | 32.38 B | 19.48 A | 37.36 A | 19.69 A |
| Bucanero | AAAA | 64.71 D | 22.29 B | 35.27 A | 17.89 B | 39.08 A | 18.81 A |
| Princesa | AAAB | 58.00 D | 15.86 D | 33.81 A | 17.67 B | 37.48 A | 17.60 B |
| Garantida | AAAB | 65.14 D | 19.43 C | 30.95 C | 18.44 B | 33.84 B | 17.23 B |
| Tropical | AAAB | 54.43 D | 19.86 C | 34.34 A | 19.50 A | 37.74 A | 18.74 A |
| 102 | AAAB | 69.43 C | 18.43 C | 30.79 C | 17.54 B | 34.95 B | 17.36 B |
| BRS Platina | AAAB | 75.86 C | 23.14 B | 32.67 B | 19.33 A | 37.10 A | 19.65 A |
| C.V.(%) | | 15.44 | 19.78 | 7.60 | 11.69 | 7.84 | 10.13 |

[1]Means followed by the same letter within the column belong to the same group according to the Scott-Knott test at 5% probability. [2]Ab. D. – Abaxial stomatal density, Ad. D. – Adaxial stomatal density, Ab. P. – Abaxial polar diameter Ad. P. – Adaxial polar diameter, Ab. E. – Abaxial equatorial diameter, Ad. E. – Adaxial equatorial diameter.

In relation to the size of the stomata, the variable polar diameter of the abaxial side was lower in diploids Malbut (21.52 µm) and NBA (22.37 µm) and the triploid values were greater than or equal to that of the tetraploids. The equatorial diameter of the abaxial surface was lower only for the diploid Malbut (12.36 µm). The NBA diploid and all the triploid cultivars had intermediate values, and the tetraploid cultivars showed higher values that could be divided into two groups: one group was formed by Bucanero, Princesa, Garantida, and 102, with values ranging from 17.54 to 18.44 µm, and another group was formed by FHIA 02, Tropical, and BRS Platina, with values ranging from 19.33 to 19.50 µm.

A similar behavior was observed in the adaxial stomata, where the diameter was lower in the diploids (Malbut and NBA with 23.74 µm), and the triploids had intermediate values ranging from 31.34 to 32.08 µm. The tetraploids had higher values that could be divided into two groups: one group formed by Garantida (33.84 µm) and 102 (34.95 µm) and the other group formed by the other cultivars, ranging from 37.10 to 39.08 µm. For the variable polar diameter, the diploids Malbut (11.23 µm) and NBA (12.78 µm) showed lower values, and the triploid Caipira had an intermediate value (15.04 µm). However, the other triploids along with tetraploids Princesa, Garantida, and 102 had similar values ranging from 16.05 to 17.63 µm. Finally, the tetraploids FHIA 02, Bucanero, Tropical, and BRS Platina showed higher values ranging from 18.74 to 19.69 µm.

As expected, the stomatal density was inversely proportional to the ploidy level. This relationship is explained by the fact that one of the nucleotypic effects associated with increased ploidy is increased stomatal size and therefore, reduced stomatal density. The stomata of the abaxial surface showed good results in relation to the recorded ploidy, revealing a tendency in which higher stomatal densities were found in cultivars with lower ploidy, with reference to the Malbut cultivar, and the lowest densities were observed in the tetraploid cultivars (Figure 1).

Hodgson et al. (2010) stated that the stomatal length and genome size are positively correlated and that it appears to be a general feature of eudicots and monocots. According to them, polyploidy in closely related lineages appears to cause genome size doubling and concomitant increase in guard-cell length.

According to Butterfield and Wood (2015), the increase in stomatal size due to genome duplication will be compensated by environmental pressure that leads to a reduction in stomatal number. This is fundamental to maintaining gas exchange at rates similar to those of diploid plants.

Simmonds (1948) and Vandehout et al. (1995) evaluated the relationship between ploidy and anatomical characteristics of banana accessions, and suggested that the stomatal measurements be performed on the adaxial side for easy measurement because this surface has fewer stomata. However, Vandehout et al. (1995) found no clear relationship between ploidy and stomatal density. In the present study, the results obtained for the adaxial surface were also not found to be a suitable parameter for ploidy evaluation. The small number of stomata on the adaxial surface of the evaluated samples did not yield good results; thus, it is recommended to evaluate the stomatal density on both the surfaces to achieve proper results.

Simmonds (1948) believed that stomatal evaluation is a valuable tool for the determination of ploidy in banana cultivars. The author suggests that stomatal evaluation should be performed before laborious chromosomal counting. After that, chromosomal evaluation should be performed within groups with different ploidy levels, preferably in materials that present dubious information. This approach would provide reliable results in less time, use fewer resources, and avoid excess workload.

## Conclusion

Some of the evaluated traits showed results different from those expected for its ploidy level. This can result from the effect of environmental factors on the genome, thereby "modulating" the ploidy effect to physiological adjustments. The blade thickness in the fourth vascular bundle region of the leaf and stomatal size and density are suitable parameters for characterizing banana cultivars according to their ploidy level.

## References

Ahmad, F., Khan, M. A., Ahmad, M., Arshad, M., Zafar, M., Khan, M., ... Rehman, Z. (2011). Foliar epidermal anatomy as an aid to the identification of grasses in tribe Aveneae (subfamily Pooideae. Poaceae) from salt range of Pakistan. *Journal of Medicinal Plants Research*, *5*(1), 81-87. DOI: https://doi.org/10.5897/JMPR.9000652

Aspinwall, M., Lowry, D., Taylor, S., Juenger, T., Hawkes, C., Johson, M., ... Fay, P. (2013). Genotipic variation in traits linke to climate and aboveground productivity in a widespread C4 grass: evidence for a functional trait syndrome. *New Phytologist*, *199*(4), 966-980. DOI: https://doi.org/10.1111/nph.12341

Baker, R., Yarkhunova, Y., Vidal, K., Ewers, B., & Weinig, C. (2017). Polyploidy and the relationship between leaf structure and function: implications for correlated evolution of anatomy, morphology and physiology in Brassica. *BMC Plant Biology*, *17*(3), 1-12. DOI: https://doi.org/10.1186/s12870-016-0957-3

Beaulieu, J. M., Leitch, I. J., Patel, S., Pendharkar, A., & Knight, C. A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist*, *179*(4), 975-986. DOI: https://doi.org/10.1111/j.1469-8137.2008.02528.x

Braga, M., Sá, M., & Mustafá, P. (2001). Avaliação de um protocolo para multiplicação in vitro de bananeira (*Musa* sp.) cv. Caipira (AAA). *Revista Brasileira de Fruticultura*, *23*(2), 215-219. DOI: https://doi.org/10.1590/S0100-29452001000200002

Butterfield, B., & Wood, T. (2015). Local climate and cultivation, but no ploidy, predict functional trait variation in *Bouteloua gracilis* (Poaceae). *Plant Ecology*, *216*(10), 1341-1349. DOI: https://doi.org/10.1007/s11258-015-0510-8

Cao, Q., Zhang, X., Gao, X., Wang, L., & Jia, G. (2018). Effects of ploidy level on the cellular, photochemical and photosynthetic characteristics in Lilium FO hybrids. *Plant Physiology and Biochemestry*, *133*, 50-56. DOI: https://doi.org/0.1016/j.plaphy.2018.10.027

Cavalier-Smith, T. (2005). Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. *Annals of Botany*, *95*(1), 147-175. DOI: https://doi.org/10.1093/aob/mci010

Comai, L. (2005) The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, *6*(11), 836-845. DOI: https://doi.org/10.1038/nrg1711

Silva, H. A, Scapim, C. A., Vivas, J. M. S., Amaral Júnior, A. T., Pinto, R.J. B., Rossi, R. M., ... Baleroni, A. G. (2019). Effect of ploidy level on guard cell length and use of stomata to discard diploids among putative haploids in maize. *Crop Science*, *60*(3), 1199-1209. DOI: https://doi.org/10.1002/csc2.20004

Hodgson, J., Sharafi, M., Jalili, A., Díaz, S., Montserrat-Martí, G., Palmer, B., ... Simmons, E. (2010). Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany*, *105*, 573-584. DOI: https://doi.org/10.1093/aob/mcq011

Hull-Sanders, Johnson, R. H., Owen, H. A., & Meyer, G. (2009). Effects of polyploidy on secondary chemistry, physiology, and performance of native and invasive genotypes of *Solidago gigantea* (Asteraceae). *American Journal of Botany*, *96*(4), 762-770. DOI: https://doi.org/10.3732/ajb.0800200

IPGRI, INIBAP, & CIRAD. (1996). *Descriptors for Banana* (*Musa* spp.). IPGRI; Rome, IT: INIBAP; Montpellier, FR: CIRAD.

Jellings, A., & Leech, R. (1984). Anatomical variation in first leaves of nine *Triticum* genotypes. and its relationship to photosynthetic capacity. *New Phytologist*, *96*(3), 371-384. DOI: https://doi.org/10.1111/j.1469-8137.1984.tb03573.x

Mascarenhas, A., Harley, A., & Scatena, V. (2020). Leaf and scape anatomy of *Leyothrix* Ruhland (Eriocaulaceae) from a taxonomic and ecological perspective. *Flora*, *262*, 151-158. DOI: https://doi.org/10.1016/j.flora.2019.151518

Moon, Y., Cha, Y., Choi, Y., Yoon, Y., Koo, B., Ahn, J. ..., Park, K. (2013). Diversity in ploidy levels and nuclear DNA amounts in Korean *Miscanthus* species. *Euphytica*, *193*(3), 317-326. DOI: https://doi.org/10.1007/s10681-013-0910-6

Nassar, N. M., Graciano-Ribeiro, D, Fernandes, S. D., & Araújo, P. C. (2008). Anatomical alterations due to polyploidy in cassava. *Manihot esculenta* Crantz. *Genetics and Molecular Research*, *7*(2), 276-283. DOI: https://doi.org/10.4238/vol7-2gmr399

Retamales, H., & Scharaschkin, T. (2015). Comparative anatomy and micromorphology of the Chilean Myrtaceae: Taxonomic and ecological implications. *Flora*, *217*, 138-154. DOI: https://doi.org/10.1016/j.flora.2015.10.005

Reyes-Díaz, M., Meriño-Gergichevich, C., Inostroza-Blancheteau, C., Latsague, M., Acevedo, P., & Alberdi, M. (2016). Anatomical, physiological, and biochemical traits involved in the UV-B radiation response in highbush blueberry. *Biologia Plantarum*, *60*(2), 355-366. DOI: https://doi.org/10.1007/s10535-015-0580-4

Simmonds, N. (1948) Genetical and cytological studies of *Musa*; stomatal size and plant vigor in relation to polyploidy. *Journal of Genetics*, *49*, 57-68. DOI: https://doi.org/10.1007/BF02986383

Sumardi, I., & Wulandari, M. (2010). Anatomy and morphology of five Indonesian banana cultivars (*Musa* spp.) of different ploidy levels. *Biodiversitas*, *11*(4), 167-175. DOI: https://doi.org/10.13057/biodiv/d110401

Sun, Q., Sun, H., Bell, R., Linguang, L., Guangfang, Z., Xin, L., & Wei, Z. (2015). Field performance of vegetative form traits of neopolyploids produced by in vitro colchicine treatment in *Pyrus communis*. *Scientia Horticulturae*, *193*, 182-187. DOI: https://doi.org/10.1016/j.scienta.2015.06.047

Van Laere, L., França, S., Vansteenkiste, H., Van Huylenbroek, J., Steppe, K., & Van Labeke, M. (2011). Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. *Acta Physiologiae Plantarum*, *33*(4), 1149-1156. DOI: https://doi.org/10.1007/s11738-010-0643-2

Vanderhout, H., Ortiz, R., Vuylsteke, D., Swennen, R., & Bai, K. V. (1995). Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica*, *83*(2), 117-122. DOI: https://doi.org/10.1007/BF01678038

Wang, Z., Fan, G., Dong, Y., Zhai, X., Deng, M., Zhao, Z., ... Cao, Y. (2017). Implications of polyploidy events on the phenotype, microstructure, and proteome of *Paulownia australis*. *PLoS One*, *12*(3), 1-21. DOI: https://doi.org/10.1371/journal.pone.0172633

Zeng, Q., Liu, Z., Du, K., & Kang, X. (2019). Orizalin-induced chromosome doubling in triploid *Populus* and its effect on plant morphology and anatomy. *Plant Cell, Tissue and Organ Culture*, *138*(3), 571-581. DOI: https://doi.org/10.1007/s11240-019-01654-y