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STOMATAL CHARACTERIZATION IN LEAVES OF BANANA CULTIVARS OF THE TYPE PRATA AND MAÇÃ

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Abstract: In banana trees, traits such as stomatal density, stomatal index, polar and equatorial diameter act as morphological markers to understand relations between genetic, environmental and pathological factors. The present study was analyzed as stomatal characteristics in leaves of six banana cultivars of the type Prata and Maçã. The banana cultivars selected were: Prata-Anã, BRS Platina and Pacovan Ken and of the type Maçã: BRS Tropical, Maçã and BRS Princesa. The states and epidermal cells were counted, measuring the polar diameters (PD) and the equatorial diameter (ED). The difference in number of epidermal cells (NEC), number of stomata (NS) and stomatal density (SD) in the leaves of six banana cultivars was verified. A Prata Anã cultivar showed a higher content of leaf material abaxial and higher than the other cultivars under analysis. Regarding the application of the stomata (PD / ED), such as the cultivars BRS Platina and Pacovan Ken, of the Prata type, and the BRS Princesa, of the Maçã type, are more flexible stomata. The different cultivars of bananas of the type Prata and Maçã are among the anatomical and morphological characteristics of the stomata.

Keywords: *Musa* spp, morphogenetic characters, leaf anatomy.

1 INTRODUCTION

The banana tree (*Musa* spp.) is among the most important fruit trees, for the economic, food and social aspects in Brazil and the world, being the food base of several populations in need around the world (DONATO et al., 2021). It is an important source of income in many countries because it is cultivated in tropical and subtropical climates. According to the Food and Agriculture Organization of the United Nations (2022), world production reached 119.9 million tonnes of bananas by 2020, which are predominantly produced in Asia, America and Africa. In Brazil, fruit is part of the diet of a large part of the population, mainly small producers and low-income populations, being the country one of the main banana producers and consumers. Only in 2020, Brazil produced 6,637,308 tons, in 455,004 hectares of harvested area (IBGE, 2022).

Considering the importance of culture, studies on anatomical structures together with morphological and physiological aspects are extremely important to understand the behavior of culture in different locations. Several authors have worked with anatomical structures, among the studies that highlight those that aim at the description of cultivars, behavior regarding pests and diseases, tolerance to water deficiency, propagation processes, micropropagation, in vitro and ex vitro cultivation, acclimatization of seedlings and seed propagation, to obtain new varieties by means of genetic improvement, increasing their agronomic performance and thus converting a greater potential of banana production with privileged anatomical structures. (COSTA et al., 2009; MADAIL et al., 2011; ROSA, 2016; FREITAS et al., 2017).

Relevant parameters to leaf anatomy were mainly related to stomata, which are small openings that can occur in any part of the plants, being more common and abundant in the leaves, and are more related to the gas exchanges, important for the photosynthetic process, being the stomata made up of guard cells that act as mediators of gas and water vapor exchange between plants and the atmosphere (TAIZ, ZEIGER, 2017).

Stomata can be anatomical indicators of the different responses of the plant to environmental stimuli, as well as their structures in relation to the size, shape, in the same plant. In the case of the water deficit signal, for example, the exit of guard cells occurs, thus the osmotic potential of the cells increases and, thus, the water shuts off during closure in parts of the stomata (CASTRO et al., 2009). In the same way, the intensity of the volume of water by the plants can also be observed by the variation in the size of the stomata and the distribution of the stomata in the leaves (OLIVEIRA, E. C.; MIGLIORANZA, 2014; CAMARGO; MARENCO, 2011).

The stomatal structure can also be related to the entry of microorganisms in plants, where a higher stomatal density for example, besides interfering in the photosynthetic capacity, can contribute to a greater chance of contamination, due to the possibility of the pathogen penetrating the plant through the stomata. (SILVA et al., 2005).

For Araújo et al. (2014), some anatomical foliar characteristics such as the size, location, shape of the stomata and lenticels; quantity and quality of cuticle wax covering the epidermis; and the presence of thick cell walls can prevent infections and allow the identification of the inherent characteristics of susceptible and tolerant genotypes in certain banana diseases, such as Sigatoka, caused by the infection of the fungus *Mycosphaerella* sp.

In banana trees, morphoanatomic traits such as stomatal density, stomatal index, polar and equatorial diameter act as morphological markers to characterize cultivars such as Prata Anã, (ARAÚJO et al., 2014), Maçã, BRS Platina and BRS Princesa (SILVA et al., 2014).

The objective of the present study was to analyze the stomatal characteristics of leaves of six banana cultivars of the Prata and Maçã type, in order to assist and strengthen brazilian banana farming and provide subsidies to understand the relation with morphology, environmental and pathological factors.

2 METHODS

The experiment was carried out at the Experimental Farm of the Universidade Federal do Espírito Santo, Centro Universitário Norte do Espírito Santo (CEUNES), São Mateus campus, at 18º40'32 "S, 39º51'39"W of latitude and longitude, and at 37.7 m of altitude. The region has an average annual rainfall of 1,200 mm, the climate according to the Köppen classification, is dry sub-humid, with average temperatures ranging from 25 ° C to 30 ° C in summer and 19 ° C to 21 ° C in winter, (PEZZOPANE, et al., 2010).

The selected banana cultivars of the Prata type were: Prata-Anã, BRS Platina and Pacovan Ken and the Maçã type: BRS Tropical, Maçã and BRS Princesa. The vegetative material was derived from micropropagation. The anatomical studies were conducted using the middle third of the third leaf expanded from the apex, leaf is used worldwide for foliar tissue analysis, collected from four plants for each replicate (DONATO et al., 2021).

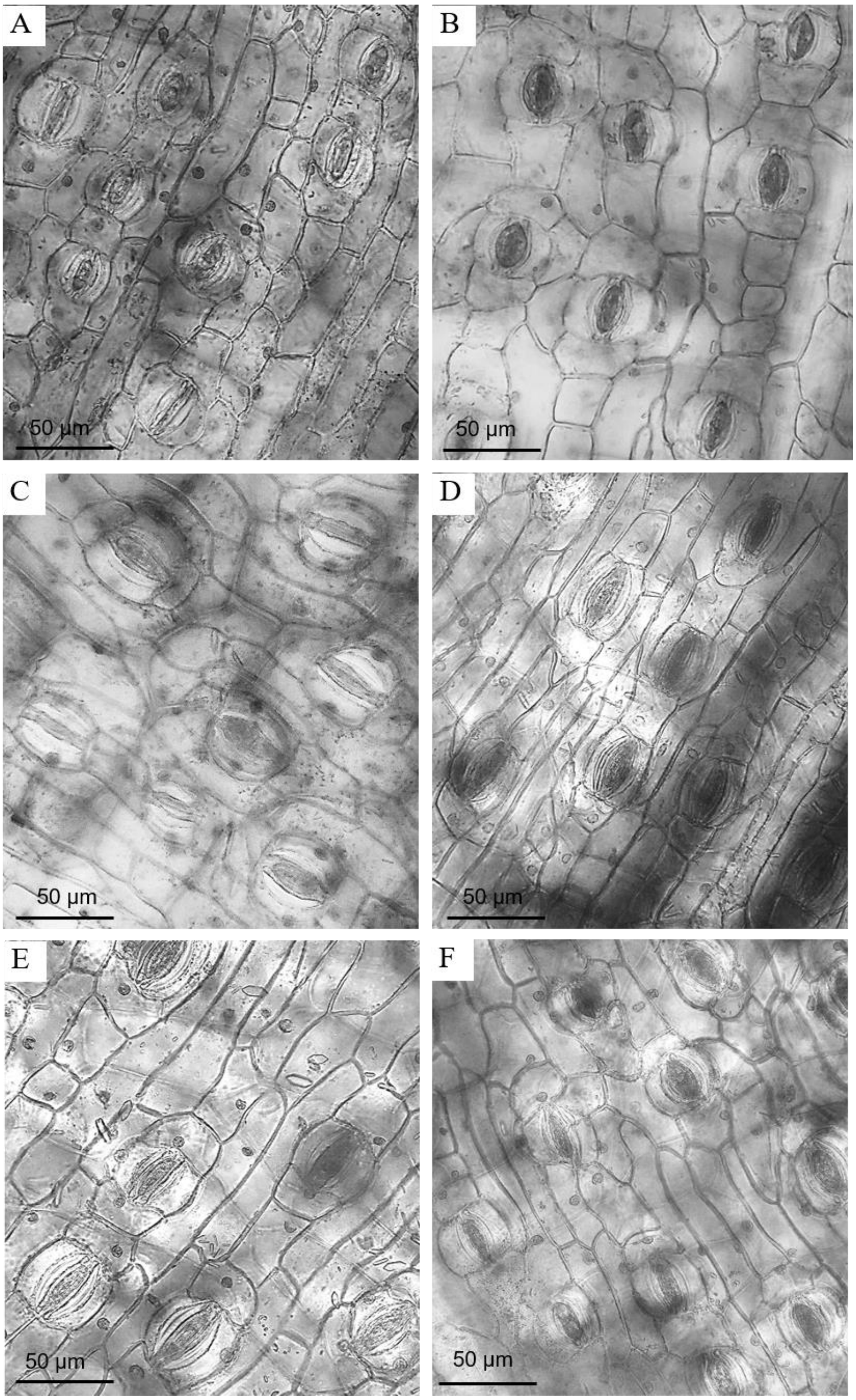
Paradermical cuts of the abaxial face of banana leaves, obtained by free hand were placed in 70% ethylic solution and later mounted on slides with 70% alcohol. The material was photographed and the counting of the epidermal cells and stomata using the software ANATIQUANTI (AGUIAR et al., 2007) was carried out using a Motic BA 210 photomicroscope and Motican 3.0MP camera. Measurements of polar diameters (PD) and equatorial diameters (ED) using the Image Pro Plus software were also performed. The functionality of the stomata was also calculated through the PD/ED ratio

The experimental design was completely randomized with 4 replicates, 6 treatments (cultivars) and at least 10 observations/measurements for the paradermal cuts. With the data obtained, analyzes of variance (F-test) were performed using the statistical software Sisvar (FERREIRA, 2008) and the means gruped by the Scott-Knott test at 5% probability.

3 RESULTS AND DISCUSSION

The banana leaves show tetracytic type stomata, characterized by four subsidiary cells, two parallel to guard cells and the remaining polar pair (Figure 1). The same type of stomata was observed by COSTA et al. (2009) in micropropagated banana plantations (AAAB).

Figure 1 - Frontal view of the abaxial leaf surface of banana tree. A - Prata anã. B - Tropical. C - BRS Platina. D - Pacovan ken. E - BRS Princesa. F - Maçã.



Source: Authors.

As to the distribution, the stomata are present on both faces of the epidermis, but are more numerous in the abaxial face. In relation to the localization of the stomata in the leaves, the banana trees are classified as hypoestomatic due to the stomata appearing in greater quantity in the epidermis of the abaxial face (CASTRO, 2009). Also, according to Soto BALLESTRERO (1992), although the largest number of stomata on the adaxial surface is frequent, clones such as Gran Enamo have a large number of stomata on both surfaces. In addition, the author reports that in addition to differences between cultivars, there may be a difference between the same clones.

It was possible to verify difference in the number of epidermal cells (NEC); number of stomata (NS) and stomatal density (SD) in the leaves of six banana cultivars (Table 1). Studies that relate foliar anatomy and plant production have verified that cultivars of the same species, as well as species of the same genus, may have different stomatal densities (SILVA et al., 2005).

Table 1 - Number of epidermal cells (NEC); number of stomata (NS); stomatal index (SI) and stomatal density (SD) of the abaxial epidermis of leaves of six banana cultivars.

Cultivar	Rated traits			
	NEC	**NS**	**SI (%)**	**DS (mm²)**
Prata Anã	118.75 a	15.05 a	11.26 a	137.91 a
BRS Platina	102.92 b	10.82 b	9.51 a	99.19 b
Pacovan Ken	102.92 b	10.82 b	9.51 a	99.19 b
BRS Tropical	90.97 c	9.22 b	9.14 a	84.53 b
BRS Princesa	88.50 c	10.05 b	10.19 a	92.09 b
Maçã	88.20 c	10.87 b	10.92 a	99.65 b
CV (%)	**4.42**	**13.52**	**12.50**	**13.77**

Averaged is followed by distinct letters within each evaluated variable differ from one another by the Scott-Knott test at 5% probability.

Source: Authors.

In relation to the number of epidermal cells (NEC), cultivars of the Prata type presented higher NEC than those of the Maçã type. The cultivar Prata Anã showed the largest NEC in relation to the others, followed by the cultivars BRS Platina and Pacovan Ken. The cultivar Prata Anã also presented higher number of stomata (NS) in the abaxial leaf epidermis and, consequently, higher stomatal density (SD) than the other cultivars analyzed.

There was no SD statistical difference between the other cultivars, in which they presented much lower values. There was no statistical difference in relation to the stomatal index (SI) among the cultivars evaluated. These characteristics may also be associated to higher rates of stomatal, transpiratory and photosynthetic conductance of the plants of the Prata Anã cultivar, as observed by CARVALHO et al. (2001) in Conilon coffee plants.

RIBEIRO et al. (2012), studying the foliar anatomy of cassava as a function of the potential for the different environmental forms, which had an intensity in the transpiration, and concomitantly, a greater ease in cassava cultivation than the greater stomatal density (SD), which is increased amount of radiation and lower water availability (SOUZA et al., 2007).When a number of stomata per unit area is larger, it is considered a strategy of water conservation of leaves that develop in soils with less capacity of water retention and under high luminosity (LLERAS, 1977; LARCHER, 2000). Lower leaf stomatal densities are found in response to the acclimation period of seedlings and increased plant age, possibly due to a higher growth rate of epidermal cells and other foliar tissues (COSTA et al., 2009).

Already on an evolutionary scale, according to HAWORTH et al. (2018), among the stomatal factors involved in genetic diversity to increase the carbon and water use efficiencies of C3 plants, increases in stomatal density are related to the stomatal rapid reaction capacity to environmental pressures.

Sigatoka leaf spot is one of the main diseases of the banana crop that affects the leaves, leading to a decrease in the photosynthetically active area of the plants and, consequently, decreasing their ability to fix carbon, which is directly linked to the productive yield of the crop. The use of cultivars resistant to this disease is one of the practices adopted for management, however, more than 90% of all crops established in Brazil are planted with cultivars susceptible to this disease (DONATO et al., 2021). In this way, the early identification, through techniques such as the quantification of stomata, of possible cultivars with resistance to sigatoka leaf spot can be an interesting alternative for plant genetic improvement programs.

It is worth noting that the largest of the varieties presented by the cultivar Prata Anã is due to the fact that it is the only one among the studies on susceptibility to Yellow Sigatoka. A similar result was observed by COLLADO et al. (2004), in which the authors observed a lower number of stomata, both in the abaxial and adaxial parts, in clones resistant to Black Sigatoka.

It is worth mentioning the low values of coefficients of variation (CV). According to the classification of PIMENTEL GOMES (2009), the NEC characteristic is low where the value found was 4.42%, and average for the characteristics NS, SI, SD, where the coefficients found were 13.52, 12.50 and 13.77%, respectively. All these values confirm a high experimental precision from the foliar analyzes performed and low variation among the evaluated plants within of each the cultivar. The characteristics related to the functionality of the stomata and stomatal area of the six banana cultivars of the Prata and Maçã type can be observed in Table 2. In relation to the polar diameter (PD), the banana trees of the Maçã type presented bigger diameters than those of the Prata type, being BRS Princesa the one with the highest PD and the Prata Anã with the lowest PD. Again, the cultivars of the Maçã type presented higher values of equatorial diameter (ED), and BRS Tropical presented the highest ED value, similar to BRS Princesa and Maçã cultivars. Lower dimensions of ED were found in cultivars BRS Platina, Pacovan Ken, and Prata Anã, both of the Prata type.

Table 2 - Polar diameter (PD); Equatorial Diameter (ED); Functionality (PD/ED) and area of the abaxial epidermis of leaves of six banana cultivars.

Cultivar	Rated traits			
	PD	**ED**	**PD/ED**	**Area**
Prata Anã	27.32 b	14.17 b	1.98 b	393.67 b
BRS Platina	29.30 b	12.16 b	2.42 a	357.79 b
Pacovan Ken	29.65 b	13.43 b	2.26 a	404.03 b
BRS Tropical	31.48 a	15.90 a	2.01 b	504.23 a
BRS Princesa	33.61 a	14.78 a	2.32 a	496.89 a
Maçã	32.32 a	15.82 a	2.07 b	517.20 a
CV (%)	**9.51**	**16.90**	**14.27**	**24.19**

Average is followed by distinct letters within each evaluated trait differ from one another by the Scott-Knott test at 5% probability.

Source: Authors.

Regarding the functionality of the stomata (PD / ED), BRS Platina and Pacovan Ken, of the Prata type, and the BRS Princesa, of the Maçã type, presented stomata more functional, due to the greater relation, differing statistically from the others cultivars under study. The lowest PD / ED values were found in the cultivars Prata Anã, BRS Tropical and Maçã. According to COSTA et al. (2009), the elliptic shape (higher PD/SD) is characteristic of functional stomata, while the round shape is associated with a lower PD/SD ratio, in which stomata do not have normal functionality. However, according to the authors, the PD/SD ratio may present a specific result according to each species and culture condition.

The largest stomatal areas were found in the cultivars of the Maçã type, being the largest area of the cultivar Maçã, followed by BRS Tropical and BRS Princesa, differing from the other cultivars. The smallest stomatal areas were found in cultivars BRS Platina, Prata Anã and Pacovan Ken, Prata type. Oliveira and Miglioranza (2014), studying density and stomatal distribution in cassava (*Manihot esculenta* Crantz) observed that it is possible to affirm that the reduction in the size of stomata is an important event in the regulation of gas exchange, since leaves with smaller stomata present greater efficiency in the use of water because they present a smaller size of the stomatal pores, thus conditioning, less water loss due to transpiration (BOEGER; WISNIEWSKI, 2003).

When related to anatomical characteristics and fungal infections, Araújo et al. (2014), there was a trend of increase in stomatal size, increase in PD / SD ratio, and significant increase in stomatal density. The authors verified the characteristics mentioned in six cultivars (Japira, BRS Platina, Calipso, Preciosa, Prata Anã, Grand Naine), with the exception of cultivar BRS Platina, which has been increasing the stomatal density. In this way, the authors have suggested that the cultivar is promising for commercial plantings in regions where the pressure of the body of Yellow Sigatoka is high.

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4 CONCLUSIONS

The different cultivars of bananas of the Prata and Maçã type are presented as anatomical and morphological characteristics of the stomata, with a higher density for the greater stomatal density of 'Prata Anã.

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