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Leaf anatomy of genotypes of banana plant grown under coloured shade nets

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This study aimed to evaluate the effect of spectral light quality on different anatomical features of banana tree plantlets grown under coloured shade nets. Banana plants of five genotypes (Maçã, Thap Maeo, Caipira, BRS Platina and Princesa), obtained from micropropagation, were grown under white, blue, red and black nets, with shade of 50%, in a completely randomized design. After 90 days of acclimatization under nets, the leaves were collected and analyzed anatomically following basic protocol of plant microtechnique. Cultivation under white net provided greater thickness of epidermis cells, hypodermis on the adaxial face and palisade parenchyma; and greater stomatal density on the adaxial face; both the red and white nets, however, increased stomatal density on the abaxial face. The use of white net, during the acclimatization phase, is recommended for cultivation of banana plantlets obtained of micropropagation.

Key words: *Musa* sp., acclimatization, spectral quality, anatomical plasticity.

INTRODUCTION

According to FAO (2012), the banana production worldwide was around 101.9 million tons, and Brazil was responsible for 6.8 million tons, being ranked 5th in the world rankings of the major producing countries. Currently, much of the production of banana plantlets is derived from micropropagation which is a method that promotes the formation of large number of plants in a short time and free of pathogens (Pereira et al., 2005).

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Abbreviations: AdE, Epidermis on the adaxial face; **AbE,** epidermis on the abaxial face; **AdH,** hypodermis of the adaxial face; **AbH,** hypodermis of the abaxial face; **PP,** palisade parenchyma; **SP,** spongy parenchyma; **MR,** midrib; **AdD,** stomatal density of the adaxial face; AbD, stomatal density of the abaxial face; **PD/ED Ad,** polar diameter/equatorial diameter ratio of stomata on the adaxial; **PD/ED Ab,** abaxial faces.

Before being taken to the field, the micropropagated plantlets need a period of acclimatization in the nursery to adapt to the natural environment (Nomura et al., 2012). At this stage, the plants need to be passed to the new climatic conditions gradually; thereby avoiding any type of injury that may occur. The acclimatization phase is extremely important to complete the process of plantlets formation, so it is often considered a limiting factor to the micropropagation process, making it necessary to know the ideal *ex vitro* environment to better adaptation of these plants (Costa et al., 2008). Several researches were reported about the factors that can hold up the nursery phase, such as those related to type of substrate (Nomura et al., 2008) and shading (Scaranari et al., 2009). In this regard, considerable attention has been given to studies that deals with the ability of plant adaptation to light conditions of environment, whether intensity or quality.

It is known that solar radiation is an essential environmental factor for direct or indirect regulation of plant growth and development (Morini and Muleo, 2003). Among the characteristics of radiation, the spectral quality can have significant effects on the morpho-physiological processes of the plant (Schuerger et al., 1997), which can result in changes in plant behavior and the development of different adaptive straregies.

In the literature, we can find numerous reports demonstrating that changes in light quality leads to adaptive changes in plants, such as the rearrangement of the anatomical structure of the leaf to maintain its functionality (Araújo et al, 2009;. Santiago et al, 2001; Hunsche et al., 2010; Solomakhin and Blanke, 2010). This organ has a high degree of anatomical plasticity that contributes to improving the adaptability of plants in different environments (Silva and Nogueira, 2012). The use of coloured shade nets is a way to modify the natural radiation providing an increase in the amount of diffuse light, by combining physical protection with differential refraction of the solar radiation (Henrique et al, 2011; Oren-Shamir et al, 2001).

Thus, studies in environments with different light qualities may assist in obtaining more information about the anatomical changes of the plant, so that the plantlets can be better acclimatized. In this context, this study was performed to evaluate the effect of the spectral quality of light, through the use of coloured nets, on characteristics of leaf anatomy of plantlets of different genotypes of banana plants.

MATERIAL AND METHODS

The experiment was conducted under field conditions from September to December 2010, at Department of Agriculture of Federal University of Lavras. The city of Lavras is located at 21°14'S and 45°00'W GRW with an altitude of 918 m, south of Minas Gerais. According to the Köppen classification, the regional climate is Cwa, but it shows characteristics of Cwb with two distinct seasons defined as follows: a cold and dry season from April to September; and a hot and humid season from October to March (Brazil, 1992).

The plant material used in the experiment was micropropagated banana plantlets of the following cultivar groups: AAB (Maçã and Thap Maeo), AAAB (BRS Platina and Princesa) and AAA (Caipira). The material was donated by Embrapa Cassava and Fruit Culture (Embrapa Mandioca e Fruticultura) and the plants were at their second subculture stage. The plantlets were grown in 24-well polyethylene trays containing a mixture of red latosol (oxisol) and bovine manure (1:1), fertilized according to the recommended method (Ribeiro et al., 1999), and irrigated daily for 90 days.

When the plantlets had approximately 15 cm-high, they were placed inside of structures individually covered with blue and red ChromatiNet-shade nets and white and black shade nets, with 50% of shading. According to the manufacturer, the blue net reduces waves in the range of red and far-red and adds blue waves, and the red net reduces blue, green and yellow waves and adds waves in the range of red and far-red. The white and black nets are not photoconverters; the white net does not interfere on the transmitted light spectrum, and the black net does not change the spectrum, just reduces the irradiance. The average intensity of radiation (measured with USB-850 spectroradiometer Red Tie) inside the nets are shown in Figure 1. The maximum temperature, minimum temperature and photoperiod were varied during the experiment according to the climatic conditions of the city of Lavras.

Initially, plants were also arranged in the condition in full sun, but those plants did not survive in this environment, and then they were discarded from the analysis of the experiment. Thus, we used a 5×4 factorial scheme (five genotypes and four shade nets colors); constituting 20 treatments in a completely randomized design with 5 replicates, each replicate consisted of 4 plants.

The sample collection for anatomical evaluation was performed after 90 days of the treatment regimen. Fully expanded younger leaves were removed from plants of each genotype, fixed in F.A.A.$_{70\%}$ and then stored in 70% ethanol. Paradermal sections of the abaxial and adaxial faces of the leaves were performed by hand using stainless steel blades, while the cross-sections were performed on a microtome table (model LPC). All sections were washed in 1% sodium hypochlorite; paradermal sections were stained with 1.0% safranin, and cross-sections were stained with safrablau solution (1.0% safranin and 0.1% of astra blue at a 7:3 ratio) and then mounted on semi-permanent slides with 50% glycerol (Johansen, 1940). The samples were viewed on an Olympus CX41 microscope coupled to a digital camera (Belcam DIV-3000) and photographed (20 fields for each treatment). Measurements were performed using ImageTool 3.0 software. The following characteristics were assessed: stomatal density, polar diameter/equatorial diameter ratio and thickness of epidermis, hypodermis, palisade parenchyma, spongy parenchyma and midrib. Data were subjected to analysis of variance using SISVAR software (Ferreira, 2011), and the means were compared by the Scott-Knott test at 5% of probability.

RESULTS AND DISCUSSION

Banana tree leaf has a unistratified epidermis on the adaxial and abaxial faces, being classified as amphi-hypostomatic. The mesophyll is characterized as dorso-ventral, showing palisade parenchyma oriented toward the epidermis of the adaxial face just beneath the hypodermis of the adaxial face, and spongy parenchyma is oriented toward hypodermis of abaxial face (Sumardi and Wulandari, 2010). The coloured shade nets promoted

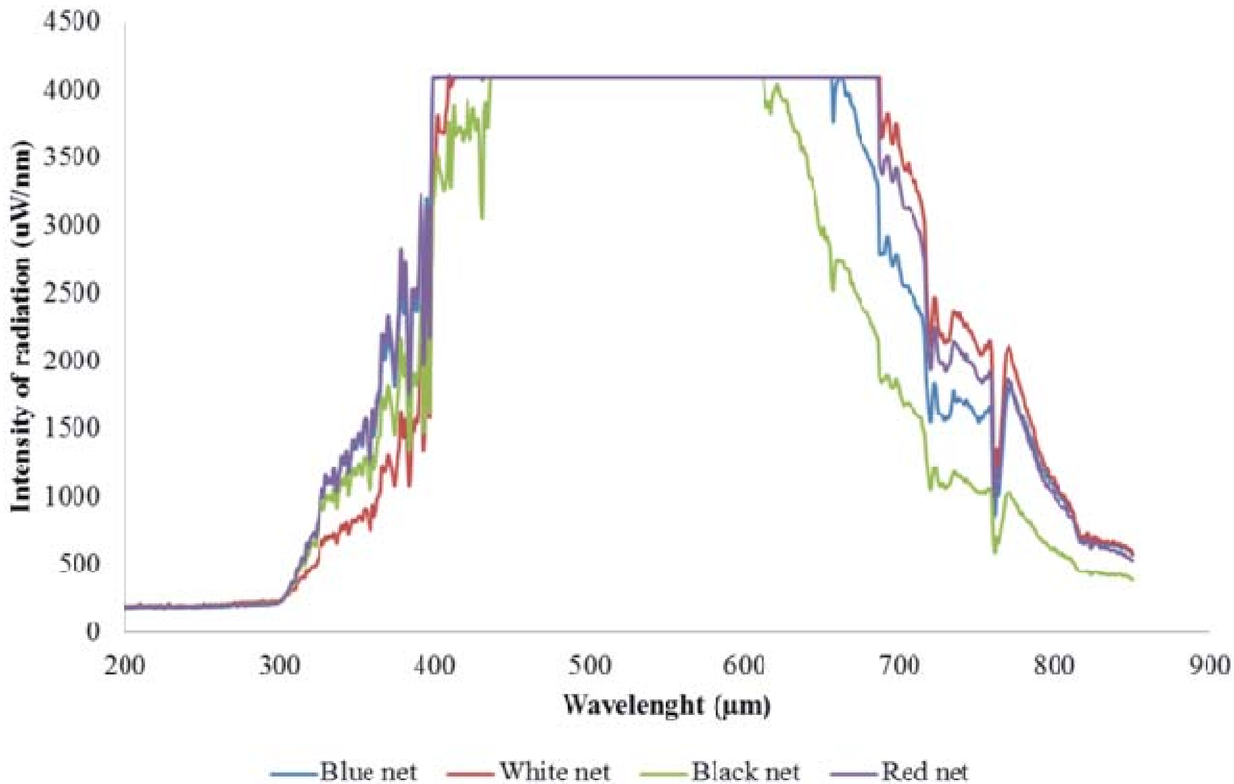

Figure 1. Intensities of radiation of different coloured shade nets according to wavelenght.

significant diferences in epidermis, hypodermis, palisade parenchyma, spongy parenchyma and midrib, as well as in stomatal features (Table 1).

Plants grown under white net showed greater size of epidermal cells on the adaxial face, except for the Caipira genotype which had greater thickness of epidermal cells when grown under red net (Table 2). When considering the epidermis of the abaxial face, the Maçã and Thap Maeo genotypes exhibited greater thickness under white net, while BRS Platina and Princesa genotypes were more prominent under blue net. This behavior was expected, since the same ploidy genotypes tend to exhibit similar behavior.

According to Sumardi and Wulandari (2010), differences in ploidy affect some phenotypic characteristics, with alterations that can often be assessed through observation of plant anatomy. In this study, under all nets, the size of the epidermal cells on the adaxial face was greater than on the cells of the abaxial face. According to Taiz and Zeiger (2004), the epidermis is typically transparent to visible light, and the individual cells are often convex. Convex epidermal cells can act as lenses and focus light so that the amount reaching some of the chloroplasts can be many times greater than the amount of ambient light. In this study, the epidermal cells also have convex shape; however, depending on net used, these cells are larger in size, which increases the concavity of the cell. Hence less light will reach the chloroplasts and the photosynthetic apparatus will be better protected against damage by excess light, being a form of anatomical plasticity of the epidermis. Costa et al. (2010) found no significant difference for thickness of the epidermis on the adaxial face to species *Ocimum selloi*, however, the authors observed greater values for the epidermis on the adaxial face under blue net compared to the red net. The same was observed for the abaxial face.

Also in relation to anatomical aspects of the epidermis, we evaluated the stomatal density and the polar diameter/equatorial diameter ratio of the stomata (Table 3), which are considered as features of high plasticity when plants are cultivated in environments with differences in spectral quality. On the adaxial face, the highest stomatal densities were found under white net, whereas on the abaxial face, greater densities were obtained under white net for Maçã and BRS Platina genotypes, and under red net for Thap Maeo, Caipira and Princesa genotypes.

Similar results were found by Martins et al. (2009) who reported that the cultivation of clove basil (*Ocimum gratissimum* L.) under red net promoted greater stomatal densities on the abaxial face, while adaxially, the cultivation without photoconverter nets was responsible for the greater density. Studies show that greater stomatal density can be found when leaves are exposed

Table 1. Summary of the analysis of variance for studied anatomical features.

SV	DF	Mean square										
		AdE	AbE	AdH	AbH	PP	SP	MR	AdD	AbD	PD/ED Ad	PD/ED Ab
Genotype (G)	4	80.396*	105.212*	4494.771*	2063.043*	2020.738*	1421.262*	254408.835*	118.961*	4706.060*	0.338ns	0.393*
Net (N)	3	15.531^{ns}	8.158ns	3070.403*	386.626*	1336.666*	930.510*	559803.348*	516.776*	19410.676*	0.915*	3.869*
G x N	12	32.332*	24.373*	636.077*	298.901*	207.519*	268.298*	101441.688*	44.346*	4916.080*	0.865*	0.687*
Error	380	10.164	7.253	74.616	68.797	48.546	46.423	4303.021	8.629	86.307	0.153	0.134
CV(%)		19.57	18.38	13.90	16.46	10.57	15.11	9.11	30.76	16.81	17.34	17.46
Average		16.29	14.65	62.12	50.40	65.94	45.11	720.29	9.55	55.28	2.25	2.09

* = significant (Scott-Knott test, 5% of probability); ns = non-significant. SV = Source of variation; DF = Degrees of freedom; CV = coefficient of variation; AdE, Epidermis on the adaxial face; AbE epidermis on the abaxial face; AdH, hypodermis of the adaxial face; AbH, hypodermis of the abaxial face; PP, palisade parenchyma; SP, spongy parenchyma; MR, midrib; AdD, stomatal density of the adaxial face; AbD, stomatal density of the abaxial face; PD/ED Ad, polar diameter/equatorial diameter ratio of stomata on the adaxial; PD/ED, Ab abaxial faces.

Table 2. Biometric measurements of leaves of five banana genotypes grown under different qualities of radiation.

Genotype	Shade net							
	Adaxial epidermis (µm)				Adaxial hypodermis (µm)			
	B	W	Bk	R	B	W	Bk	R
Maçã	16.32^{aB}	18.79^{aA}	16.90^{aB}	14.57^{cC}	58.65^{bB}	71.98^{aA}	53.67^{bC}	60.82^{bB}
Thap Maeo	15.76^{aA}	13.92^{bA}	14.42^{aA}	14.51^{cA}	53.01^{cB}	61.00^{bA}	55.75^{bB}	51.94^{cB}
Caipira	16.70^{aB}	17.27^{aB}	16.30^{aB}	19.08^{aA}	63.23^{bB}	75.77^{aA}	54.91^{bC}	53.60^{cC}
BRS Platina	17.26^{aA}	15.80^{bA}	16.69^{aA}	16.70^{bA}	83.68^{aA}	70.75^{aC}	67.59^{aC}	76.58^{aB}
Princesa	16.43^{aB}	17.97^{aA}	15.39^{aB}	15.05^{cB}	59.32^{bB}	66.15^{bA}	49.47^{bC}	54.66^{cC}
CV (%)	19.57				13.90			
	Abaxial epidermis (µm)				Abaxial hypodermis (µm)			
	B	W	Bk	R	B	W	Bk	R
Maçã	14.32^{bB}	16.65^{aA}	15.02^{aB}	13.83^{aB}	58.41^{aA}	48.99^{bB}	54.82^{aA}	56.24^{aA}
Thap Maeo	12.65^{cA}	12.26^{cA}	13.18^{bA}	13.34^{aA}	43.18^{cA}	42.23^{cA}	44.30^{bA}	46.33^{bA}
Caipira	14.54^{bB}	16.73^{aA}	15.93^{aA}	14.45^{aB}	50.79^{bB}	57.36^{aA}	52.98^{aA}	46.39^{bB}
BRS Platina	17.12^{aA}	14.70^{bB}	15.74^{aB}	15.48^{aB}	62.80^{aA}	50.83^{bB}	53.93^{aB}	53.84^{aB}
Princesa	15.79^{aA}	14.00^{bB}	13.06^{bB}	14.30^{aB}	50.80^{bA}	43.48^{cB}	43.48^{bB}	46.92^{bA}
CV (%)	18.38				16.46			

Means followed by the same lowercase letter in the column and uppercase letter in the row for each variable do not differ by the Scott-Knott test at 5% probability. B, Blue; W, white; Bk, black; R, red.

Table 3. Stomatal features of leaves of five banana genotypes grown under different qualities of radiation.

Genotype	Shade net							
	Adaxial density (stomata/mm^2)				Adaxial PD/ED			
	B	W	Bk	R	B	W	Bk	R
Maçã	10^{aA}	12^{cA}	8^{cB}	8^{aB}	1.83^{dC}	2.52^{aA}	2.06^{bB}	2.17^{aB}
Thap Maeo	9^{aC}	15^{aA}	11^{aB}	10^{aB}	2.28^{bA}	2.17^{bA}	2.36^{aA}	2.23^{aA}
Caipira	7^{bB}	10^{cA}	6^{dB}	9^{aA}	2.08^{cB}	2.49^{aA}	2.29^{aB}	2.19^{aB}
BRS Platina	7^{bC}	15^{aA}	10^{bB}	9^{aB}	2.63^{aA}	2.51^{aA}	1.98^{bB}	2.17^{aB}
Princesa	6^{bC}	13^{bA}	9^{bB}	8^{aB}	2.25^{bA}	2.28^{bA}	2.20^{aA}	2.40^{aA}
CV (%)	30.76				17.34			
	Abaxial density (stomata/mm^2)				Abaxial PD/ED			
	B	W	Bk	R	B	W	Bk	R
Maçã	45^{bB}	58^{aA}	47^{aB}	44^{dB}	1.95^{bB}	2.31^{aA}	1.90^{aB}	2.27^{bA}
Thap Maeo	51^{aB}	49^{bB}	47^{aB}	109^{aA}	1.93^{bC}	2.22^{aB}	2.00^{aC}	2.70^{aA}
Caipira	35^{cD}	60^{aB}	53^{aC}	83^{cA}	1.89^{bA}	2.19^{aB}	1.91^{aA}	2.17^{bB}
BRS Platina	43^{bB}	49^{bA}	49^{aA}	43^{dB}	2.17^{aA}	2.31^{aA}	1.93^{aB}	1.94^{cB}
Princesa	46^{bC}	53^{bB}	44^{aC}	97^{bA}	1.77^{bB}	2.03^{aB}	1.88^{aB}	2.50^{aA}
CV (%)	16.81				17.46			

Means followed by the same lowercase letter in the column and uppercase letter in the row for each variable do not differ by the Scott-Knott test at 5% probability. B, Blue; W, white; Bk, black; R, red.

to high irradiance, giving indications of better control of stomatal conductance, which will reduce water loss through transpiration (Rossatto et al., 2009). Variations in stomatal density on both face of the leaves show the anatomical plasticity of banana genotypes, depending on the growth environment.

Generally, the increase in stomatal density is correlated with a greater stomatal conductance, thus minimizing harmful effects on photosynthesis under different growing conditions (Lima Júnior et al., 2006), and to increased CO_2 uptake, which enables higher photosynthetic rates (Niinemets and Tenhunen, 1997).

The cultivation of plants under white net favored increase of the polar diameter/equatorial diameter ratio (PD/ED) of stomata on the adaxial face of epidermis, while on the abaxial face, different behaviors were observed depending on the genotype (Table 3). The PD/ED ratio of stomata is a marker of the functionality of this epidermal attachment. According to Khan et al. (2002), PD/ED ratio is related to the shape of the guard cells, which constitutes a key particularity for the functioning of stomata, with elliptical shapes (higher PD/ED ratio) being characteristic of functional stomata and rounded shapes (lower PD/ED ratio) characteristic of stomata with abnormal functioning. Variations in functionality highlight the anatomical plasticity of banana leaves of various genotypes in the face of different cultivation environments. Changes in the size and frequency of stomata show the ability that the plants have to rearrange these structures of the epidermis in response to environmental changes, so that there is greater activity of stomata in gas exchange and transpiration, appropriately (Rossatto et al., 2009).

When considering the thickness of the hypodermis, we found different behaviors among the genotypes under the different coloured nets (Table 2). The white net was responsible for the greatest values of thickness of hypodermis on the adaxial face, except for the BRS Platina genotype which experienced its best results hypodermis under blue net. The hypodermis is important in protecting the palisade parenchyma from excess solar radiation, especially during periods of peak

Table 4. Parenchymas thickness of leaves of five banana genotypes grown under different qualities of radiation.

Genotype	Shade net							
	Palisade parenchyma (μm)				Spongy parenchyma (μm)			
	B	W	Bk	R	B	W	Bk	R
Maçã	56.10^{bB}	64.17^{cA}	58.30^{bB}	59.99^{cB}	40.92^{cA}	40.72^{cA}	43.01^{cA}	36.40^{cB}
Thap Maeo	63.49^{aB}	73.60^{bA}	60.95^{bB}	65.61^{bB}	38.90^{cB}	37.96^{cB}	44.60^{cA}	45.19^{aA}
Caipira	57.37^{bC}	64.19^{cB}	69.01^{aA}	60.36^{cC}	41.94^{cB}	43.79^{bB}	59.30^{aA}	42.04^{bB}
BRS Platina	67.63^{aB}	78.29^{aA}	71.93^{aB}	71.79^{aB}	50.66^{aA}	48.92^{aA}	53.71^{bA}	49.79^{aA}
Princesa	63.54^{aB}	72.40^{bA}	66.80^{aB}	73.47^{aA}	45.34^{bA}	43.78^{bA}	47.51^{cA}	47.69^{aA}
CV (%)	10.57				15.11			

Means followed by the same lowercase letter in the column and uppercase letter in the row for each variable do not differ by the Scott-Knott test at 5% probability. B, Blue; W, white; Bk, black; R, red.

incidence of light on leaves (Boeger et al., 2007; Verdaguer et al.,2012). We presume that the white net shade provides a cooler and more humid environment because it reflects all wavelengths of light. Moreover, according to manufacturer, this net works as the full-sun condition, only serving to protect against insects. When considering the thickness of the abaxial hypodermis, the genotypes of the same ploidy showed similar behavior, in order that Maçã and Thap Maeo genotypes exhibited no differences between the nets, BRS Platina and Princesa genotypes had the thickest hypodermis under blue net, and Caipira genotype showed better results under white and black nets. According to Castro et al. (2009), the hypodermis shows no relevant photosynthetic activity, however, it can accumulate water, which is then available in photosynthetic processes.

In the present study, the thickness of the palisade parenchyma was always greater than the spongy parenchyma's (Table 4). All genotypes, with the exception of the Caipira genotype, showed thicker palisade parenchyma when grown under white shade net. When considering the thickness of the spongy parenchyma, we also found that the responses varied for all genotypes depending on the net used. The BRS Platina and Princesa genotypes leaves exhibited no significant differences in the parenchyma thickness. The Caipira genotype leaves showed greater development of spongy parenchyma when grown under black net. The Maçã genotype exhibited similar behavior under blue, white and black net, and the Thap Maeo genotype leaves had thicker spongy parenchyma under black and red nets. Spongy parenchyma has a large amount of intercellular spaces, which are crucial for gas storage. A considerable amount of gases can enter the mesophyll and be retained in the intercellular spaces; CO_2, in particular, can then be fixed by the palisade parenchyma (Castro et al., 2009). Therefore, the increase in spongy parenchyma contributes to an increase in the available area for CO_2 absorption (with subsequent assimilation); this increased area is often larger than that of the external surface of the leaf.

According to Appezzato-da-Glória and Carmello-Guerreiro (2006), the palisade parenchyma and the spongy parenchyma are considered as assimilators parenchymas which have photosynthetic activity. This fact is due to the presence of chloroplasts which convert light energy into chemical energy, and store it in the carbohydrates form. Thus, further development of these tissues can result in a higher photosynthetic efficiency, which can contribute to the rapid growth of the plant during the acclimatization phase. It is expected that thicker palisade parenchyma result in greater photosynthetic rates (Bolhar-Vordenkampf and Draxler, 1993), which are essential to the growth and development of plants. Usually, the palisade parenchyma is more developed than the spongy parenchyma, which optimizes photosynthesis and decreases the intercellular spaces of the spongy parenchyma, thus reducing the availability of water vapor subject to transpiration (Castro et al., 2009). Costa et al. (2010) found that the thickness of palisade parenchyma of leaves of *Ocimum selloi* (pepper basil) did not change when plants were grown under blue and red shade nets; however, the spongy parenchyma was thicker under these conditions. In the present study, the spongy parenchyma showed different responses depending on the genotype analyzed. Dimassi-Theriou and Bosabalidis (1997) found that the thickness of the palisade parenchyma increased when kiwi plants were subjected to higher solar radiation. In the present study, for most genotypes, the palisade parenchyma was thicker under the white net. White shade nets do not affect the spectrum of irradiated light; in other words, they produce a change in the quantity but not the quality of radiation. In cooler environments, thickening of the palisade parenchyma is favored, so as to enable increased photosynthetic activity (Boeger et al., 2007; Haberlandt, 1990).

In this study, the black net was responsible for providing the greatest thicknesses of midrib for all genotypes, with the exception of Thap Maeo genotype

Table 5. Midrib thickness of leaves of five banana genotypes grown under different qualities of radiation.

Genotype	Midrib (µm)			
	B	W	Bk	R
Maçã	638.08^{bC}	794.35^{bB}	875.06^{aA}	796.13^{bB}
Thap Maeo	710.93^{aB}	577.37^{dC}	705.46^{bB}	767.03^{bA}
Caipira	499.19^{dD}	643.95^{cB}	751.48^{bA}	588.40^{dC}
BRS Platina	626.40^{bC}	864.72^{aA}	889.14^{aA}	699.23^{cB}
Princesa	569.42^{cC}	684.86^{cB}	866.28^{aA}	858.30^{aA}
CV (%)	9.11			

Means followed by the same lowercase letter in the column and uppercase letter in the row for each variable do not differ by the Scott-Knott test at 5% probability. B, Blue; W, white; Bk, black; R, red.

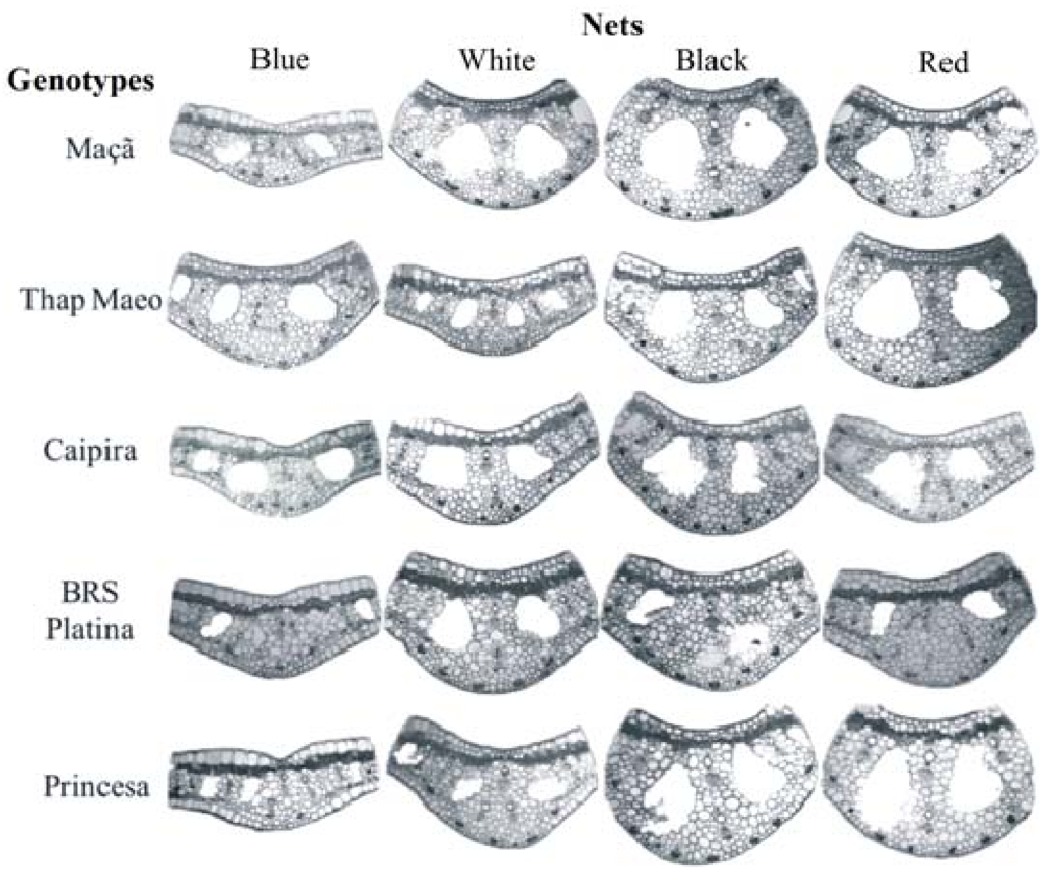

Figure 2. Photomicrographs of banana leaves showing the plasticity of midrib of five genotypes grown under environments with different light qualities. Bar = 200 µm.

that exhibited the greatest values for that feature under red net (Table 5).

Thicker midribs indicate adaptation to stressful conditions, such as situations of low irradiance. Figure 2 shows clearly the plasticity of the midrib of the banana leaves under different radiation qualities.

Conclusion

Banana leaves exhibit anatomical plasticity in response to changes in the spectrum of radiation. This plasticity can vary depending on the cultivated genotype; plants belonging to the same genomic group do not respond uniformly to changes in light quality. Cultivation under white net provided greater thickness of epidermal cells, hypodermis on the adaxial face, and palisade parenchyma and greater stomatal density on the adaxial face; both red and white nets, however, promoted an increase of stomatal density on the abaxial face. Thus, it may be recommended to use white net during the acclimatization phase for cultivation of micropropagated banana plantlets due to the development of anatomical features more favorable to the plant growth.

Conflict of Interests

The author(s) have not declared any conflict of interests

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