Australian Journal of Crop Science AJCS

AJCS 12(12):1837-1843 (2018) ISSN:1835-2707
doi: 10.21475/ajcs.18.12.12.p1104

Effect of lighting spectrum and naphthaleneacetic acid (NAA) on *in vitro* development of cactus pear [*Opuntia ficus-indica* (L.) *Mill*]

Selma Silva Rocha*[1], Luciana Cardoso Nogueira Londe[2], Jessica Guerra Calaes[3], Júlio Cesar Gomes Pereira[4], Marcela Caroline Batista da Mota[5], Wander Silva Viana[6], Renata Aparecida Neres Faria[7], Núbia Nunes Xavier[8]

[1]Universidade Estadual de Montes Claros, Janaúba- MG, Brazil
[2]Empresa de Pesquisa Agropecuária de Minas Gerais, Unidade Regional Epamig Norte de Minas, Nova Porteirinha- MG, Brazil
[3]Universidade Federal de Minas Gerais, Montes Claros- MG, Brazil
[4]Engenheiro Agrônomo, Universidade Estadual de Montes Claros, Janaúba- MG, Brazil
[5]Universidade Federal de Lavras, Lavras-MG, Brazil
[6]Universidade Estadual de Montes Claros, Janaúba- MG, Brazil
[7] Engenheira Agrônoma, Universidade Estadual de Montes Claros, Janaúba- MG, Brazil
[8]Universidade Estadual de Montes Claros, Janaúba- MG, Brazil

***Corresponding author: selmauniagro@gmail.com**

Abstract

Modification of *in vitro* culture environment can improve the development of plants, obtaining higher morphological and physiological quality. The objective of this work was to analyze the influence of different luminous spectrums as well as different doses of auxin NAA on *in vitro* development of cactus pear (*Opuntia ficus-indica* (L.) Mill). The experiment was conducted in a completely randomized design in factorial scheme 4 x 3 (Luminous spectrums x NAA doses), corresponding to four luminous spectrums: red, green, blue and white, and three doses of NAA 1, 2 and 3 mg. L^{-1}. A total of 12 treatments with 6 replications were applied including 1 plant per repetition. Cactus pear plants were introduced on MS culture medium solidified with 7% agar, and pH adjusted to 5.8. We observed higher development of plants (growth and rooting) under luminous spectrums, 30 and 60 days after plant establishment (red and green). At concentrations of 1 and 2 mg L^{-1} of NAA we observed better height of the plants, 30 and 60 days after establishment. The luminous spectrums red and green influenced the *in vitro* development of cactus pear plants. The best doses to promote better plant growth are 1 and 2 mg. L^{-1} of NAA.

Keywords: Cactus pear cultivar Giant, Light, Multiplication.

Introduction

The Brazilian semiarid occupies an area of 969,589.4 km^2 and is marked by a very unstable climate (Pereira Jr, 2007). It is characterized by extreme stages of drought within rainy periods that are the limitation for the production systems. According to Oliveira et al. (2010), in this area it is necessary to evaluate the cactus species because they better adapted to the climatic conditions, being the cactus pear (*Opuntia ficus-indica (L.)* Mill) an alternative to be used during periods of drought.

Most plants have the leaves as a photosynthetic apparatus. Cactus pear uses the cladodes to perform photosynthesis, because their leaves are lost during its development, which makes it have great tolerance to the adverse conditions, besides the Chrysolactic acid metabolism (Farias et al., 2000). It is also known as the CAM mechanism (Crassulacean acid metabolism).

According to Peixoto et al. (2008), the cactus pear presents slow growth, and the availability of seedlings occurs two years after planting, resulting in difficulty in the acquisition of vegetative propagules. Thus, the technique of *in vitro* cultivation presents an alternative to multiply this species in a short time.

The cactus pear is grown on *in vitro* system. They multiply very easily, especially with the addition of growth regulators such as naphthaleneacetic acid (NAA), which stimulates cell expansion and is mainly used to induce rooting of plant segments (Pasqual, 2004a).

A plant to have a good development needs another item like luminosity. The primary source of brightness in growth is the cold white light. However, the use of other spectral bands such as red, blue, green and yellow or a combination of them result in increases or reduction on the *in vitro* development of species (Gu et al., 2012; Chen et al., 2004). Several studies have shown the efficiency of different wavelengths on *in vitro* cultures, through meshes, or colored cellophane.

It is important to know the effects of different light filters on *in vitro* cultures, since the efficiency in light quality exerts morphogenetic changes in plants. The use of light filters at in *vitro* growth rooms may be a promising technique. The variation in light quality with the use of colored screens may be an alternative, reducing the use of growth regulators. The quality and luminous intensity determines the efficiency of the photosynthesis, consequently obtaining plants with greater productivity.

Thus, the objective of the work was to determine the influence of light filters, as well as different concentrations of NAA on *in vitro* development of cactus pear and also to determine type of wavelengths more efficient in a multiplication of this culture.

Results

Spectrum analysis at 30 days

There was no significant interaction ($p > 0.01$) among the factors lights and doses to none of the analyzed characteristics 30 days after establishment of the plants. For plant height (HGT), there was significant difference ($p < 0.01$) to the factors isolated doses and lights.

After 30 days of establishment only the red and green spectrum promoted better growth of plants. There is no significant difference for the characteristics number and length of roots in none of the submitted spectrum (Table 1).

Doses analysis at 30 days

Application of doses of 1 and 2 mg L^{-1} promoted growth of aerial part of plants (HGT). However, the highest dose (3 mg L^{-1}) reduced the growth, but to the number and length of roots these doses showed no significant difference (Table 2).

Spectrum analysis at 60 days

60 days after establishment of the culture, there was no significant interaction ($p > 0.01$) among the light and dose treatments on many of the analyzed features. However, dose and light treatments caused significant difference ($p < 0.01$) on height of plants (HGT), and length of roots (LR).

After 60 days of establishment, better elongation of aerial part of plants was observed, when subjected to the green light spectrum. We observed that under white light spectrum, there was a smaller plant growth. About rooting the luminous spectrum, red and green promoted root growth (Table 3).

Doses analysis at 60 days

With the smaller doses of NAA (1 and 2 mg L^{-1}), there was a higher growth of aerial part of the plants. However, lower growth was observed under the dose of 3 mg L^{-1}. The same fact was observed after 30 days of establishment. There is no significant difference for the features, number and length of roots (Table 4).

In this way, the luminous spectrums, red and green were more efficient to develop pear cactus plants, 30 and 60 days after establishment. The doses of 1 and 2 mg L^{-1} only promoted the growth of aerial part of plants, both 30 and 60 days of *in vitro* establishment.

Discussion

The plants have achieved good growth, when subjected to bright green and Red spectrums. Pereira (2007) studied different light qualities in *in vitro* cultivation of *Coffea arabica*, in which it obtained greater shoot length using green and red light, similar to our results. Araújo et al. (2009a), working with *Cattleya loddigesii* cultivated *in vitro*, obtained higher growth in height of these, under cultivation in red cellophane paper.

Some studies have concluded that red radiation promotes an increase in shoot length of seedlings (Appelgren, 1991; Marks and Simpson, 1999a). However, this characteristic is dependent on the plant species (Antonopolou et al., 2004; Hunter and Burritt, 2004). The red, green and blue pigments, as reported in the literature, are fundamental for photosynthesis and in growth rooms. The efficient quality of light reflects on more productive seedlings, according to Lee et al. (1985). The light intensity has a pronounced influence on photosynthesis *in vitro*.

In this sense, the red light would be an alternative to improve the photosynthetic efficiency of the cactus pear, through the increase of the cladodes, according to Oliveira et al., 2007. The cactus pear presents low cladodes area index, compared to legume species, resulting in a lower rate of biomass accumulation. As observed by Saebo et al. (1995), red light is important for the development of the photosynthetic apparatus of plants and may increase the accumulation of starch in many species by inhibiting the translocation of photoassimilates out of the leaves, increasing the concentration of carbohydrates.

The green spectrum is indicated in several studies, as little efficient in the photosynthetic process and consequently in the development of plants, due to being little absorbed. However, according to Hall and Rao (1980), even if this spectrum is not significantly absorbed by chlorophyll, it is important for photosynthesis. According to Klein (1992), the importance of the green spectrum is due to its insistence on being absorbed and reflection from chloroplast to chloroplast. After several reflections, the absorption is performed efficiently in the photosynthetic process.

In the present work, the green light spectrum was efficient in the stretching aerial part of the cactus pear, and may have occurred successive reflections of this light pigment, enough to promote such elongation.

According to Economou and Read (1987), the luminous intensity, besides influencing the growth and the proliferation of the shoots, can directly affect the formation of roots, while in excess can reduce the formation of the same ones. There was no significant difference between the rooting and the spectra. This may be due to the high light intensity in the growth room, where its development is inhibited.

The smaller doses of NAA (1 and 2 mg L^{-1}) promoted the *in vitro* growth of plants. The auxins are more used to stimulate the rhizogenesis in plants, and for the growth of aerial part, there are few reports in the literature analyzing its effect. In this work, smaller concentrations of NAA

Table 1. The Average height of the plant (HGT), average number of roots (NR) and average length of roots (LR) of cactus pear (*Opuntia ficus-indica* (L.) *Mill)* at 30 days of *in vitro* establishment.

LIGHT	Variables		
	Height (cm)	**Number of roots**	**Root length (cm)**
Red	16.26 a	10.61 a	1.35 a
Green	15.86 a	8.50 a	1.51 a
Blue	7.39 b	9.44 a	1.27 a
White	6.51 b	9.89 a	1.27 a
CV (%)	58.98	56,07	35.04

Means followed by distinct letters in the column differ from each other, by the Tukey test at 5% probability. CV = coefficient of variation.

Fig 1. Red light exposed on the plants *in vitro*.

Table 2. The average height of the plant (HGT), average number of roots (NR) and average length of roots (LR) of cactus pear (*Opuntia ficus-indica* (L.) *Mill)* at 30 days of *in vitro* establishment.

DOSE (mg.L^{-1})	Variables		
	Height (cm)	**Number of roots**	**Root length (cm)**
1	14.46 a	9.88 a	1.21a
2	13.09 a	11.04 a	1.45a
3	6.97 b	7.92 a	1.38 a
CV (%)	58.98	56.07	35.04

Means followed by distinct letters in the column differ from each other, by the Tukey test at 5% probability. CV = coefficient of variation.

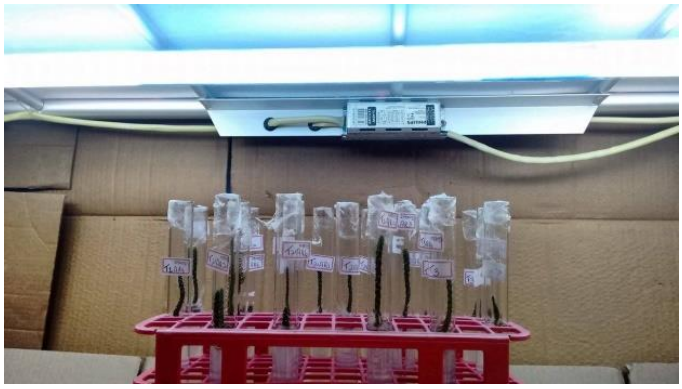

Fig 2. Blue light on the plants.

Table 3. The average height of the plant, (HGT) average number of roots (NR) and average length of roots (LR) of cactus pear (*Opuntia ficus-indica* (L.) *Mill)* at 60 days of *in vitro* establishment.

LIGHT	Variables		
	Height (cm)	Number of roots	Root length (cm)
Red	34.44 ab	12.33 a	1.98 a
Green	30.30 a	10.22 a	1.69 a
Blue	20.62 bc	11.89 a	1.38 b
White	16.85 c	11.67 a	1.51 b
CV (%)	53.41	56.67	32.17

Means followed by distinct letters in the column differ from each other, by the Tukey test at 5% probability.
CV = coefficient of variation

Fig 3. Green light on the plants.

Table 4. The average height of the plant (HGT), average number of roots (NR) and average length of roots (LR) of cactus pear (*Opuntia ficus-indica* (L.) *Mill)* at 60 days of *in vitro* establishment.

DOSE ($mg.L^{-1}$)	Variables		
	Height (cm)	Number of roots	Root length (cm)
1	33.59 a	12.12 a	1.91b
2	31.28 a	13.04 a	1.40 b
3	11.78 b	9.42 a	1.62 b
CV (%)	53.41	56.67	32.17

Means followed by distinct letters in the column differ from each other, by the Tukey test at 5% probability. CV = coefficient of variation

Fig 4. White light on the plants.

promoted considerable stretching of shoot in cactus pear plants. The lowest height in the plants is explained because the auxins act more in the elongation of roots. It would need a medium containing a cytokinin to promote the stretch of aerial part.

We observed that the Auxin only served in the growth of aerial part of plants *in vitro.* According to George (1996), auxins promote increased cell growth and stretching, root and callus formation, mainly associated with a cytokinin. We suggest that if there was a cytokinin associated with auxin, the results for rhizogenesis could have been different, since this regulator would potentiate the action of auxin NAA, obtaining more satisfactory results. After 30 days of *in vitro* culture, the behavior of plants subject to bright green and red spectrum revealed the importance of these spectra for the development of cactus pear.

To understand how light is reflected from colored cellophane paper, Souza (2008) explained that white light from the sun or a lamp falls on the colored cellophane part being absorbed by the pigment, while some parts will be reflected from the paper and plant part. The reflected wavelength will be precisely what will give the sensation of the color of cellophane. That is cellophane identified as red reflecting. Therefore, since cellophane has some degree of

transparency it will allow that same wavelength is crossed from its surface by changing the zeta-ratio of the environment on the opposite side of the light incidence.
The knowledge of the light incident in growth rooms interfering with *in vitro* cultures is of paramount importance, since this is a factor, among others, that will define the quality of the plant under greenhouse conditions and later in the field.
The red and green light was well utilized by the cytochromes responsible for photosynthesis, promoting a good development in cactus pear, but this could only be proven with more advanced analyzes, such as spectrophotometry and chromatography.
Erig and Schuch (2004), observed better results for leaf number and multiplication rate of *Rubus idaeus* under green cellophane. In this study, we observed that these plants cultivated under a red mesh showed higher levels of total dry biomass (Melo and Alvarenga et al., 2009). For this reason, better studies on the use of colored screens in forage palm crop, especially on red screen are important, as this light can modify the area of radiation absorption and this crop can acquire greater accumulation in biomass. The red screens transfer more light from the spectrum to the distant red and red waves and diffuse the light passing through the mesh, being efficient in the development of the plant (Li 2006).
Although the blue spectrum did not show significant results comparing with red and green light, this spectrum is also efficient in the development of plants. The light blue is important in processes of pigment synthesis, enzymes, development of chloroplastideos, stomatal opening and closing and several other photomorphogenic processes (Taiz and Zeiger, 2004).
The different luminous spectrums did not influence the number of roots. This was also observed by Araújo (2009b), in *Cattleya loddigesii* L. cultivated *in vitro.* For root length, the red and green lights were responsive. According to George (1996), red light stimulates rooting in many species. This fact was not observed at 30 days of *in vitro* cultivation, showing that the time of permanence of the plants to the lights influenced the *in vitro* rhizogenesis of cactus pear.
Although in most growing rooms cold white light is used as the main source of luminosity *in vitro* cultures, this spectrum did not show responsive results in any of the characteristics analyzed for the cactus pear.
The lowest doses of NAA (1 and 2 mg L^{-1}), promoted the growth of *in vitro* plants. According to Pasqual (2004b), auxins stimulate cell expansion and are mainly used to induce rooting of plant segments. In this study, different doses of NAA did not influence on *in vitro* rhizogenesis. Pasqual and Hoshika (1992) stated that the rooting of *Mammillaria bocasana* (*Cactaceae*) may occur independently regardless of NAA concentration. There for it can be suggested that endogenous concentration of this hormone in plants is sufficient. Also, excess of this hormone causes no inhibition and there is no influence after exogenous application of NAA on *in vitro* rhizogenesis.
Marks and Simpson (1999b) concluded that with variation in spectral quality, it is possible to manipulate the *in vitro* growth of several species in an alternative way to the addition of growth regulators to the culture medium. From this study, it can be confirmed that it is possible to obtain more developed cactus pear seedlings under a light-colored spectrum than only under cold white light in growth rooms.

Materials and Methods

Disinfection and production of in vitro plants

The experiment was developed in the laboratory of Biotechnology of Agricultural Research Company of Minas Gerais – EPAMIG, North Field trial EPAMIG Gorutuba, Nova Porteirinha, MG, from October to December 2015.
The cladodes were previously selected from cactus pear of cultivar Giant (*Opuntia ficus-indica Mill*) (uniforms cladodes with no deformation and attack by pests and diseases were choosen). They were removed from the experimental field of the EPAMIG as source of explant for the subculture.
After selection, the cladodes were sent to the laboratory to pass the fumigation process of immersion in alcohol 70% for 5 minutes. After that cladodes were soaked for 25 minutes in sodium hypochlorite (2.0%) and later the triple washing with distilled and autoclaved water.
In laminar flow hood, the cladodes were excised by removing the areolas (explant source), and then they were placed in MS (Murashige and Skoog, 1962) culture medium, supplemented with 30 g L^{-1} of sucrose, 0.1 g. L^{-1} of inositol and 7 g. L^{-1} agar. The explants were cultivated for 90 days until the third subculture. During this period of initial establishment, no phytoregulators was used for the development of explant. The explants were incubated in a growth room with controlled temperature (25 ± 2° C) and under photoperiod of 16 hours of light (30 W/m^2).

Statistical analysis

The experiment was conducted in a completely randomized design in factorial scheme 4 x 3 (Luminous spectrums x NAA doses). The luminous spectrums were red, green, blue and white light, and the NAA doses were 1, 2 and 3 mg. L^{-1}. A total of 12 treatments with 6 replications, including 1 plant per repetition was used.

In vitro cultivation in different luminous spectrums

At the end of the third subculture, the necessary number of plants was formed and used for the experiment. The plants excised from the basal segments in 0.5 cm length and introduced into the culture medium MS. They Supplemented with NAA in the concentrations of NAA (1, 2 and 3 mg. L^{-1}), solidified with agar 7%, 0.01mg. L^{-1} with mio inositol and pH adjusted to 5.8. All media were initially sterilized and packed in baby jar with 20 ml of medium (50 ml total capacity), being sealed and autoclaved for 20 minutes at 120 °C.
After multiplication under the laminar flow hood, the plants were brought to the growth room and subjected to different light spectrums: spectrum white (conventional growth room), colorful spectrum: blue, red and green. To obtain these spectrums two sheets of cellophane paper to coat the white lamps were used. Each luminous spectrum showed the following wavelength: blue, (425-490 nm) red (640-700 nm), green (490-550 nm) and white (350-750 nm).

Evaluation of the experiment

After 30 and 60 days, the plants were evaluated using a digital caliper and measures: height, number of roots and length of roots of plants were held in centimeters.

Statistical analyses

The data were subjected to analysis of variance and averages compared by Tukey test at 5% of significance.

Conclusion

Application of colored filters of red and green influenced the *in vitro* development of cactus pear cultivar Giant. Application of NAA at concentrations of 1 and 2 mgL^{-1} promoted aerial shoot elongation in cactus pear plants during 30 and 60 days in the growth room. This suggests that the use of colored filters, under the conditions of this work, is a viable alternative to phyto-hormones. The coloured filters could promote the same result in the development of the plant like the hormone. We suggest that they are capable of being replaced with hormones, reducing expenses with of reagents.

Acknowledgements

To the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), for research funding and grants awarded.

References

ANTONOPOLOU C, DIMASSI K, THERIOS I, CHATZISSAVVIDIS C (2004) The influence of radiation quality on the *in vitro* rooting and nutrient concentrations of peach rootstock. Biologia Plantarum, Thessaloniki, 48 (4): 549-553.

ARAÚJO AG, PASQUAL M, RODRIGUES FA, RODRIGUES JD, DE CASTRO EM, SANTOS AM (2009a) Crescimento *in vitro* de *Cattleya loddigesii* Lindl. em diferentes espectros luminosos associados com ácido giberélico. Revista Ceres Viçosa. 56 (5):542-546.

ARAÚJO AG, PASQUAL M, RODRIGUES FA, RODRIGUES JD, DE CASTRO EM, SANTOS AM (2009b) Crescimento *in vitro* de *Cattleya loddigesii* Lindl. em diferentes espectros luminosos associados com ácido giberélico. Revista Ceres Viçosa. 56 (5):542-546.

APPELGREN M (1991) Effects of light quality on stem elongation of Pelargonium in vitro. Scientia Horticulturae. 45(3-4): 345-351.

CHEN JT, CHANG WC (2004) Induction of Repetitive Embryogenesis from Seed- derived Protocorms of *Phalaenopsis amabilis* var. Formosa Shimadzu. *In Vitro* Cellar e Develpmental Biology– Plant. 40(3): 290-293.

ECONOMOU AS, READ PE (1987) Light treatments to improve efficiency of in vitro propagartion systems. Hortscience, Alexandria. 22 (5): 751-754.

FARIAS I, LIRA MA, SANTOS DC et al (2000) Manejo de colheita e espaçamento da palma forrageira, em consorcio com sorgo granífero, no Agreste de Pernambuco**.** Pesquisa Agropecuária Brasileira. 35 (2): 341-347.

GU A et al. (2012) Regeneration of Anthurium andraeanum from leaf explants and evaluation of microcutting rooting and growth under different light qualities. Hortscience. 47 (1) :88-92.

GEORGE EF (1996) Plant propagation by tissue culture, the technology. 2nd.Edington: Exegetics Limited, (pt 1): 15-74.

HALL DO, RAO KK (1980) Coleção Temas de Biologia: Fotossíntese. Editora Pedagógica e Universitária Ltda, São Paulo. 89.

Hunter DC, Burritt DJ (2004) Light quality influences adventitious shoot production from cotyledon explants of lettuce (*Lactuca sativa L.*). *In Vitro* Cellular and Developmental Biology- Plant. (40): 215-220.

KLEIN RICHARD M (1992) Effects of green light on biological systems. Biological Reviews. 67 (2): 199-284.

LEE N, WEZSTEIN Y, SOMMER HE (1985) Effects of quantum flux density on photosynthesis and chloroplast ultraestructure in tissue-culture plantlets and seedlings of Liquidambar styraciflua L. towards improved acclimatization and field survival. Plant Physiology. (78): 637-641.

LI JC (2006) Uso de mallas en invernaderos. Horticultura Internacional n. extra, 86-91.

MARKS TR, SIMPSON SE (1999a) Effect of irradiance on shoot development *in vitro*. Plant Growth Reg. 28 (2): 133-142.

MARKS TR, SIMPSON SE (1999b) Effect of irradiance on shoot development *in vitro*. Plant Growth Reg. 28 (2): 133-142.

MELO AAM, ALVARENGA AA (2009) Sombreamento de plantas de *Catharanthus roseus (L.)* G. Don 'Pacifica White'por malhas coloridas: desenvolvimento vegetativo. Ciência e Agrotecnologia. 33 (2):514-520.

MURASHIGE T, SKOOG F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 15 (3): 473-497.

OLIVEIRA JPF, BARRETO MLJ, LIMA JÚNIOR DM, AGUIAR EM, SILVA TO (2010) Algarobeira (*Prosopis juliflora*): uma alternativa para alimentação de ovinos no nordeste brasileiro. Revista Verde de Agroecologia e Desenvolvimento Sustentável. 5 (2): 01-04.

OLIVEIRA RA et al. (2007) Área foliar em três cultivares de cana-de-açúcar e sua correlação com a produção de biomassa. Pesquisa Agropecuária Tropical. 37 (2): 71-76.

PASQUAL M (2004a).Propagação de plantas ornamentais. UFLA-FAEPE, Lavras-Brazil. 106.

PASQUAL M (2004b) Propagação de plantas ornamentais. UFLA-FAEPE, Lavras-Brazil, 106.

PASQUAL M, HOSHIKA (1992) E. Efeitos do ácido naftaleno acético e 6benzilaminopurina sobre a proliferação in vitro de cactos Gynmnocalicium buldiamur L. e *Mammillaria bocasana* L. Pesquisa Agropecuária Brasileira. 24: 589-593.

PEIXOTO MJA et al. (2008) Desenvolvimento de *Opuntia ficus-indica (L.) Mill*., em diferentes substratos, após micropropagação *in vitro*. Acta Scientiarum Animal Sciences. 28 (1):17-20.

PEREIRA IS et al. (2007) Diferentes qualidades de luz no cultivo *in vitro* de *Coffea arabica L*, São Paulo-Brazil, 3.

Pereira Júnior, José de Sena **(**2007). "Nova delimitação do semi-árido brasileiro, Brasília Câmara dos deputados, Brazil.

SÆBØ A et al. (1995) Light quality affects photosynthesis and leaf anatomy of birch plantlets *in vitro*. Plant Cell, Tissue and Organ Culture. 41 (20): 177-185.

SOUZA DMS (2008) Influência da qualidade da Luz na germinação de sementes de espécies arbóreas nativas.Trabalho de Conclusão de Curso (Graduação em Engenharia Florestal) – Universidade Federal Rural do Rio de Janeiro, Brazil.

TAIZ L, ZEIGER E (2004). Fisiologia vegetal. 3. ed. Porto Alegre, Brazil: Artmed, 719 p.