ISSN: 1984-5529 Científica Jaboticabal v.45, n.3, p.231–239, 2017

http://dx.doi.org/10.15361/1984-5529.2017v45n3p231-239

Leaf morpho-anatomy of *Jatropha curcas* in vitro: Response to light conditions and temperature

Morfoanatomia foliar de *Jatropha curcas* in vitro: Resposta às condições de luz e temperatura

Claudinéia Ferreira NUNES[1*]; Dalilhia Nazaré dos SANTOS[2]; Thaís Cainã Teixeira VALENTE[3]; Moacir PASQUAL[2]; Joyce Dória Soares RODRIGUES[2]; Karine Simões FERREIRA[4]; Evaristo Mauro de CASTRO[3]

[1] Corresponding author. Professora Doutora - Instituto de Ciências Agrárias (ICA), Universidade Federal de Minas Gerais (UFMG), Avenida Universitária, 1000 – Bairro Universitário, CEP: 39.404 – 547, Montes Claros, MG, Brazil. Email: Claudineia.f.nunes@gmail.com

[2] Doutora; Professores Doutores - Departamento de Agricultura, Laboratório de Cultura de Tecidos – Universidade Federal de Lavras (UFLA),Lavras, MG, Brazil. Email: dalilhians@gmail.com; mpasqual@dag.ufla.br; joycerodrigues01@yahoo.com.br

[3] Mestre; Professor Doutor - Departamento de Biologia – Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil. Email: thaistv7@hotmail.com; emcastro@ufla.br

[4] Mestre - Universidade Federal de São João Del-Rei (UFSJ), Sete Lagoas, MG, Brazil. Email: ksferreira@yahoo.com.br

Recebido em: 08-10-2015; Aceito em: 13-04-2017

Abstract

Jatropha curcas L. plant is adapted to diverse climatic conditions with high requirement of heat stroke and drought resistant, having a special position among the attractive crops to biofuel program. This species has been studied from the perspectives of plant breeding, biotechnology and development of production systems. However, there are few reports of research regarding its anatomical characteristics. Thus, the objective of this study was to evaluate the effects of different conditions of light and temperature on the germination *in vitro* of *J. curcas* embryo and describe the leaf structure of seedlings through anatomical and micromorphological parameters. Embryos were excised and cultured in test tubes containing 15 ml of MS medium and grown in different temperature (25 °C and 30 °C) and light conditions (white, red, far red, and absence of light). The cultures were kept in BOD germinators chambers, with a 16 h photoperiod. Phytotechnical, anatomical and micromorphological (via electron scanning microscopy) characteristics were measured. The species *J. curcas* has a higher germination speed index when grown at 30 ºC. There is an increased of number of normal seedlings in white light and this point characterizes the species as neutral photoblastic. *J. curcas* presents uniseriate epidermis, palisade unistratified parenchyma, and spongy parenchyma composed of irregular cells. It is an amphistomatic species, with more stomata on the abaxial surface.

Additional keywords: euphorbiaceae; growing environment; leaf anatomy; physic nut.

Resumo

Jatropha curcas L. é uma planta adaptada às mais diversas condições climáticas, com alta exigência de insolação e resistente à seca, tendo uma posição especial entre as culturas atrativas ao programa de biocombustíveis. Dentre as pesquisas em pleno desenvolvimento para essa espécie estão as abordagens de melhoramento genético, biotecnologia e desenvolvimento de sistemas de produção, mas há poucos relatos de pesquisa em relação as suas características anatômicas. Assim, o objetivo deste estudo foi avaliar os efeitos de diferentes condições de luz e temperatura na germinação *in vitro* de embriões de *Jatropha curcas* e descrever a estrutura foliar das plântulas por meio de parâmetros anatômicos e micromorfológicos. Embriões foram excisados e inoculados em tubos de ensaio contendo 15 mL de meio de cultura MS, sendo cultivados em diferentes temperaturas (25°C e 30°C) e condições de luz (branca, vermelha, vermelha extrema e ausência de luz). As culturas foram mantidas em germinadores tipo BOD, com fotoperíodo de 16 h. Foram realizadas avaliações fitotécnicas, anatômicas e micromorfológicas (via microscopia eletrônica de varredura). A espécie *Jatropha curcas* apresenta maior índice de velocidade de germinação quando cultivada a 30 °C e maior número de plântulas normais na luz branca, sendo caracterizada como fotoblástica neutra. A *Jatroha curcas* apresenta epiderme unisseriada, parênquima paliçádico uniestratificado e parênquima lacunoso constituído por células irregulares. É uma espécie anfiestomática, com maior presença de estômatos na face abaxial.

Palavras-chave adicionais: ambiente de cultivo; anatomia foliar; euphorbiaceae; pinhão-manso.

Introduction

Due to gradual depletion of world petroleum reserves and the impact on environmental pollution, there is an urgent need for suitable alternative fuel sources. Vegetable oil is a promising alternative because it is renewable and environment-friendly (Verma & Verma, 2014). Special interest has been shown in the cultivation of the plant species, for example, *Jatropha curcas* Linnaeus. This specie is commonly known as *pinhão manso*, it is a plant of the family Euphorbiaceae that has substantial phenotypic plasticity, being considered a source of biodiesel with high biomass yield.

Because of its importance as an energy source, the species has been given special attention by various fields of science, with the objective of improving its genetic potential and developing appropriate conditions for its commercial propagation. To achieve this objective, simple and efficient in vitro techniques have been established, contributing to the development of plants adapted to the field conditions. However, the success of in vitro propagation depends on factors related to plant growth and development. For the genus Jatropha, the physiological aspects involved in the germination of the species is an important step in the in vitro cultivation process because it strongly influences the number of plants obtained.

Plant development and growth are directly related to the culture conditions and are dependent on various factors considered important in germination physiology. For example, culture conditions can be adjusted to provide ideal conditions of light and temperature, which determine the amount and speed of germination (Menezes et al., 2004).

For some species light is a factor of importance in seedling germination and survival. The responsible for photomorphogenic processes, controlling the germination, is a pigment denominated phytochrome, main photoreceptor of the plants. The light signals captured by phytochrome may or may not initiate seed germination, and their mode of action depends on the type of incident radiation (Araújo Neto et al., 2003).

The effects of light conditions on the quality of seedlings of *J. curcas* were observed by Matos et al. (2011) and Pascuali et al. (2012), who used appropriate physiological indices and a luminous environment to evaluate the plant plasticity produced by variations in the available light.

Another important factor in the germination process is temperature, which affects the overall germination and the germination rate. It tends to influence water absorption speed and determining biochemical reactions on the germination (Carvalho & Nakagawa, 2012). Studies demonstrated the influence of different temperatures on germination potential and seedling vigor in *J. curcas* (Martins et al., 2008; Vanzolini et al., 2010; Mota et al., 2012; Pascuali et al., 2012).

It is important to emphasize that to meet the demand in future, a large quantity of quality planting material will be need. Tissue culture has allowed mass propagation of superior genotypes, thus enabling the commercialization of healthy and uniform plant material (Kaviani, 2015). In this context, the *in vitro* culture of embryos is extremely important to obtain sources of explants, essential for mass multiplication. In addition, the *in vitro* culture of embryos can be used as an important tool for physiological, morphological and anatomical manipulation. Studies using *in vitro* culture of embryos have produced significant results regarding increased rates of germination, uniformity of plants and conversion of viable seedlings for species such as *Jatropha podagrica* Hook (Jesus et al., 2003) and *Jatropha curcas* (Nunes et al., 2008).

The species *J. curcas* has been studied from the perspectives of mass production system development, plant breeding and biotechnology (Santos et al., 2012; Santos et al., 2013; Nunes et al., 2013), but there are few reports discussing its anatomy. Pioneering studies were performed for the genus *Jatropha*, but descriptive studies do not present a specific approach for *Jatropha* species (Dehgan & Webster, 1979; Olowokudejo, 1993). Also studies on the anatomical characterization of oil secretory cells in the fruits and seeds of *J. curcas* are found in literature (Librea & Tolentino, 2012). Current studies reinforce the proposal of using anatomical diagnosis to help adjusting culture conditions aiming to improve survival rate and quality plants grown *in vitro* (Rodrigues et al., 2014).

Study on the morpho-anatomy characterization of leaf of *J. curcas* in response to ligth and temperature conditions are a pioneering attempt to contribute to the limited literature, suggesting significant contribution to maximize the use of the species. Therefore, the work was done with the objective of studying micromorphological and anatomical parameters to characterize the leaf structure of *Jatropha curcas* seedlings subjected to culture of embryos in different conditions of light and temperature.

Material and methods

The work was conducted using as plant material, seeds of the matrix plant variety Oracília collected from the germplasm bank of *J. curcas* located in the town of Janaúba, Minas Gerais, Brazil. The *Jatropha curcas* seeds were established *in vitro* following the methodology developed by Nunes et al. (2008). For this the embryos were placed in individual test tubes measuring 25 x 150 mm, containing 15 mL of MS (Murashige & Skoog, 1962) culture medium.

To evaluate embryos development, we used germination chambers of type Biological Organism Development (BOD), with a daily photoperiod of 16 h. The treatments combined two temperatures (25 °C and 30 ºC) and four light conditions (white, red, far red light and dark) (Figure 1A). For white light, tube racks were coated with two layers of transparent cellophane and exposed to the light produced by four fluorescent

lamps (20 W) fixed on the door of the incubator chamber. The procedure for simulating red and red distant light was obtained as follows: The red light was obtained by passing the white light spectrum through two sheets of red cellophane wrapped around each tube rack. For the far red light, the grids were covered with two sheets each of blue and red cellophane (Menezes et al., 2004). The absence of light was obtained using laminated paper and fabric (TNT) surrounding the tube racks.

Phytotechnical observations were made over 10 days under safety light that is green light. The germination speed index (GSI) (Maguire, 1962) was evaluated as follows:

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \ldots + \frac{Gn}{Nn} \quad (1)$$

Where: G1, G2, Gn = number of seedlings germinated in the first, second, and, eventually, the last count; N1, N2, Nn = number of days from the first, second, and last count.

Percentage of germination and seedling length (normal vs. abnormal) were also evaluated at 10 days after inoculation. Embryos were considered germinated after growth of the embryonic axis and cotyledonary leaf expansion. The seedling was considered normal if it showed development of plumula and root in the same primary structure, cotyledon expansion, and occasionally the development of lateral roots (Figure 1B). Seedlings with hyperhydric aspect, stunted growth of the primary root and no expansion of the cotyledons were considered abnormal (Figure 1C).

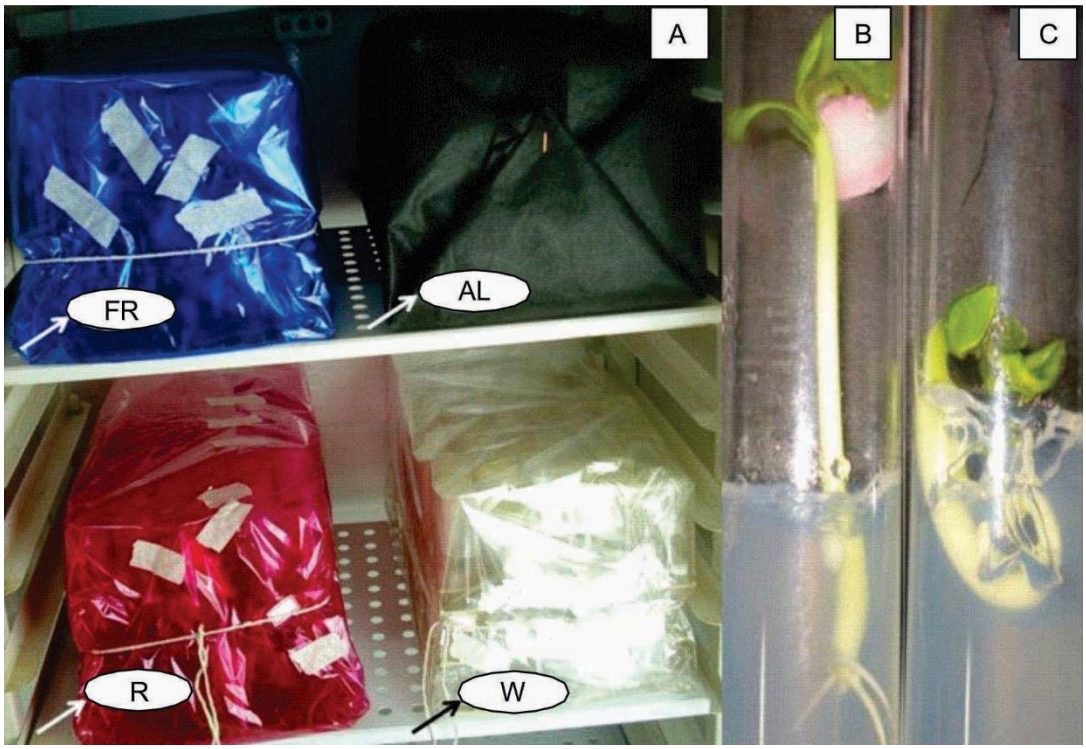

Figure 1 -.(A) Tube racks in the B.O.D. incubator chamber with temperature set at 25 °C and a photoperiod of 16 h/8 h, covered with cellophane to make light conditions white, red, and far red or covered with fabric TNT (Nonwoven fabric) to exclude light. (B) *In vitro* development of normal seedling. (C) Abnormal seedling in a test tube containing 15 mL of MS growing medium (Murashige & Skoog, 1962). (FR) = Far red, (AL) = Absence of light, (R) = Red, (W) = White.

For anatomical and micromorphological features, in each treatment were collected six leaves of seedlings cultured for 30 days *in vitro*. The leaves were fixed in FAA (formaldehyde, glacial acetic acid, and 70% ethanol at ratio of 0.5:0.5:9.0) for 72 hours and were preserved in 70% ethanol. Transverse sections of the middle third of the leaves were prepared according to the protocol from Kraus & Arduim (1997). The sections were observed and photographed under a light microscope (Olympus BX 60) coupled to a digital camera (Canon A630). The parameters evaluated were thickness of adaxial and abaxial epidermis, palisade and spongy parenchyma (characteristics of leaf blade).

For micromorphological characteristics and definition of the epidermis and stomates, portions of the middle region of the sheet were fixed as described by Karnovsky (1965) and then prepared as described by Robards (1978). Samples were then coated with gold (20 nm) and analyzed by scanning electron microscopy (SEM) LEO-EVO, following pre-determined protocol by Alves (2004).

The experimental design was completely randomized in a 2 x 4 factorial arrangement (two temperatures and four light conditions), with 6 replicates of 12 embryos each. Data were analyzed with the software Sisvar (Ferreira, 2011), with means compared by Scott- Knott test at 5% probability.

Results and discussions

The germinated embryos of *Jatropha curcas* from the second to seventh day were stimulated by both temperatures (25 °C and 30 °C), but more efficient germination occurred at 30 °C, corresponding to a GSI of 5.63 (Figure 2A). Similarly, germination of cryopreserved seeds of *J. curcas* occurred beginning on the third day of inoculation *in vitro* (Silva et al., 2011).

Similar behavior was observed for germination percentage regardless of the temperature at which embryos were exposed registered a rate of over 90% (Table 1). Embryos showed no differences between light conditions and were able to germinate under all light stimuli evaluated. They may not exhibit photosensitivity; germination was triggered in conditions of little or no light. The use the form of the phytochrome to classify the reaction of the seed to light is recommended in the literature (Takaki, 2001). It can thus be concluded that the *J. curcas* phytochrome has sufficient activity to induce germination in the absence of a light source.

Several authors have used the term photoblastism to group seeds into different categories based on light stimuli. Because germination can be stimulated or inhibited by light, plants can be classified as positively or negatively photoblastic (Atroch et al., 2001), with other species whose seeds remain undifferent to the presence of light for germination. Thus, *J. curcas* may be considered neutral photoblastic, triggering germination of embryos regardless of the presence or absence of light stimulation.

Although there is no significant difference between temperatures tested in the present study, *J. curcas* requires high temperature for germination. This supports the hypothesis that temperature regulation can be used to influence germination capacity and germination rate. Similar responses were observed in *J. curcas* seeds grown in sand or paper, with temperatures of 20-30 °C (Martins et al., 2008). In other experiments, also using paper substrate, the germination of seeds of *J. curcas* at both 25 ºC and 20-30 °C was satisfactory (Vanzolini et al., 2010).

After germination, the seedlings grown *in vitro* require an average of five days to show fibrous root, hypocotyl and cotyledons, thus generating normal seedlings. Morphologically abnormal seedlings considered epicotyls had atrophied, retaining achlorophyllous cotyledons and seedlings that developed only the hypocotyl (Figure 1C). Silva et al., 2011 evaluated the leaf morphoanatomy of *J. curcas* seedlings from cryopreserved germplasm and observed the formation of callus on the adaxial side of the cotyledons and in the hypocotyl base, corresponding to an abnormal growth and development.

Seedlings were positively influenced by light, showing increased formation of normal seedlings under white light and absence of light in comparison to red and far red light, in which abnormal seedlings were observed (Table 1). The changes in light levels to which a species is adapted may condition different physiological responses in their biochemical, anatomical and growth (Atroch et al., 2001).

Table 1. Germination speed index (GSI), germination percentage (%G), normal seedling (%NS) and abnormal seedling (% ANS) of *Jatropha curcas* L. grown *in vitro* under different light conditions *.

Light condition	GSI	%G	%NS	%ANS
Absence	5.09 a	98.61 a	74.53 a	4.98 b
White	5.08 a	97.91 a	78.10 a	4.67 b
Red	5.08 a	96.52 a	62.81 b	5.98 a
Far red	5.19 a	96.52 a	64.65 b	5.82 a
CV (%)	5.86	4.52	16.68	19.52

* Averages followed by the same letters in each column do not differs by the Scott-Knott test at 5% probability.

Cultivation conditions are factors that deserve attention. The time required for germination depends on the tested species and the environmental conditions in which it is grown, and those conditions may produce a greater or lesser degree of germination and seedling development than is considered normal. For example, *J. curcas* embryos grown in MS medium supplemented with highest concentration of sucrose produced an 83.68% germination rate, along with the absence of abnormal seedlings at the end of the evaluation (Nunes et al., 2008).

The incidence of normal seedlings was also improved by the higher temperature (30 °C, Figure 2B). The appropriate condition for germination of seeds and/or embryos is that which produces the highest possible number of normal seedlings in the shortest period of time. This condition was observed with white light at 30 ºC. For length of normal seedlings, the association between the factors of light and temperature showed a significant response (Figure 2C).

Greater length was obtained when seedlings were grown in the dark at 30 ºC, and the shortest length was observed in the treatment with far red light at 25 ºC. When a plant is exposed to light, upon emergence from the soil or growing conditions *in vitro*, it is influenced by its environment. One type of change that can be induced by the environment is a decrease in the elongation rate of the stem, such that stems are shorter in the presence of light than in the dark. The elongation of the seedlings in the condition of no light could be considered as an adaptive strategy in the search for light.

When assessing the stem length of seedlings grown in vitro as a function of light, it should be consid-

ered that the sensitivity of the seedlings to the light regime could be influenced by other factors, such as the endogenous concentration of plant hormones. For Scalon et al. (2008), the endogenous hormonal level directly influences the initial growth of the seedlings, without ruling out the influence of environmental factors. Giberelin is a good example of an endogenous hormone, which is involved in stem elongation, internodes length, leaf area and dry matter accumulation (Stefanini et al., 2002).

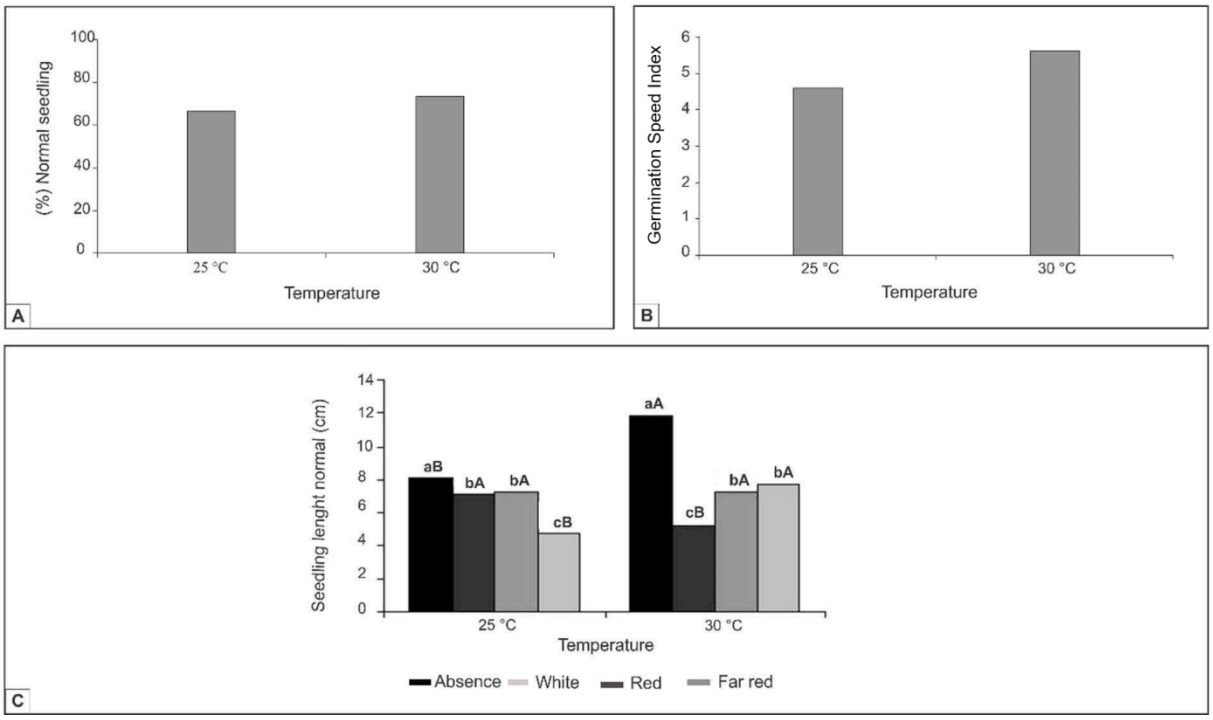

Figure 2 - Normal seedling (A), Germination speed index (B) and Seedling length normal (C) of *Jatropha curcas* grown *in vitro* under different light conditions and temperatures*.

* Averages followed by equal letters, lower case in the line and upper case in column, do not differ at 5% probability by the Scott-Knott test.

Observations regarding the anatomical structures pointed out that seedlings grown under 25 °C exhibited the highest thicknesses in all tissues of the leaf blade under all light regimes. Whereas, the environment condition of 30 °C provided smaller thicknesses (Table 2). The epidermis of *J. curcas* leaves is uniseriate, comprising small cells of irregular sizes (Figure 3B). The adaxial epidermis was thicker than the abaxial side, where the red light at 25 °C provided a superior effect on the thickness of the epidermis. In contrast, the absence of light at 30 °C reflected in lower responses for the same characteristic. With the obtained results, it could be inferred that the luminous condition and the temperature promote anatomical changes in seedlings of *J. curcas*, being able to contribute to that seedlings with thicker leaf epidermis are more easily adapted to the condition of acclimatization.

Similar features were observed for *J. curcas* plants subjected to different salt concentrations in *ex vitro* conditions (Melo et al., 2011). Another work showed the adaxial epidermis being thicker than that of the abaxial side (Ferreira et al., 2003). These features should be consequence of the high relative humidity and external sucrose offered of the *in vitro* conditions. The triggering for stomata development involves a 'default' fate of some cells of the meristemoid cells (Tricker et al., 2012), which is blocked on the adaxial surface of the leaves of plants of *J. curcas* cultivated *in vitro*.

Light and temperature influence plant anatomy and physiology, altering the qualities of plants. In the *in vitro* conditions, increased temperature caused a reduction of leaf blade thickness. Unlike the findings of Dickison (2000), the leaf structure may be greatly influenced by the temperature and level light during cultivation, and the increase of either variable can thicken the tissues that form the leaf blade. According to other authors (Alquini et al., 2006), the leaf tissue and especially the epidermis, are most susceptible to structural changes because it is in direct contact with the environment.

Regarding the parenchyma (Figure 3A), the palisade parenchyma is unistratified, and the thickest layer of cells is observed in leaf tissue grown under red light at 25 °C or in the absence of light at 30 °C (Table 2). In histological analysis, Silva et al. (2011) observed leaves from *J. curcas* seedlings derived from cryopreserved germplasm, focusing on the palisade parenchyma on the adaxial epidermis and spongy parenchyma facing the abaxial epidermis.

For these evaluations, the spongy parenchyma consists of approximately seven layers of irregular cells,

resulting in large spaces (Figure 3A) that are thickened after exposure to white light at 25 °C (Table 2). These observations corroborate results of other research (Melo et al., 2011), who noted a similar number of layers of spongy parenchyma cells in irregular plant leaves of *J. curcas* grown under salt stress. Such variations are associated with responses to the environment because parenchyma tend to have multiple layers in the presence of abundant light, allowing the absorption and dispersion of light without changing the vitality of the leaf (Castro et al., 2009), thus improving the adaptability of plants to different environments. The anatomical analysis also allowed the visualization of a beam formed by a number of xylem cells and another series of phloem cells along the midrib of the leaf (Figure 3C).

Table 2 - Adaxial epidermis (ADE), abaxial epidermis (ABE), palisade parenchyma (PP), spongy parenchyma (SP) of leaf tissue of *Jatropha curcas* L. under different conditions of light and temperature *.

Light conditions	Temperature (ºC)	
	25	30
	ADE (µm)	
Absence	17.30 bA	9.91 cB
White	17.20 bA	13.51 bB
Red	20.59 aA	16.89 aB
Far red	14.64 cA	15.32 aA
	ABE (µm)	
Absence	16.74 bA	10.09 bB
White	18.90 aA	12.54 aB
Red	19.60 aA	12.30 aB
Far red	15.19 bA	12.62 aB
	PP (µm)	
Absence	34.31 cA	26.09 bB
White	52.03 aA	28.42 bB
Red	53.71 aA	36.05 aB
Far red	47.12 bA	28.49 bB
	SP (µm)	
Absence	147.60 aA	92.65 aB
White	167.05 aA	111.78 aB
Red	159.30 aA	113.54 aB
Far red	159.62 aA	103.42 aB

* Average followed by different letters, lower case in vertical and upper case in horizontal, within each evaluated variable, differ at 5% probability by the Scott-Knott test.

Anatomical sections of the leaves obtained with scanning electron microscopy (SEM) showed that, at different temperatures and light conditions, the stomata are arranged at random, becoming superficial and/or prominent and becoming reniform, narrow or wide in shape. Leaves exhibit some wax in the form of flakes or particles, along with a slightly wavy epidermis (Figure 3D). The cells on the adaxial and abaxial surfaces are polygonal in shape and vary in size (Figure 3E). The stomata of the leaves grown at 25 °C (Figure 3F) were apparently close to the surface, regardless of lighting conditions, and the most prominent stomata were found in the 30 °C conditions (Figure 3G).

In general, we observed the presence of stomata on both sides of the leaves, with more stomata on the lower epidermis, characterizing the plant as amphistomatic (Figure 3, H and I). Amphistomatic leaves with greater presence of stomata on the abaxial surface was also observed in *E. heterophylla* (Ferreira et al., 2003). The occurrence of stomata on both faces of the leaves like observed to *J. curcas*, too was observed to *J. gossypifolia, J. chevalieri, J. kamerunica, J. neriifolia* and *J. atacorensis* (Olowokudejo, 1993). This confirms that the predominant type of stomata in the genus *Jatropha* is undoubtedly the paracytic, which is considered the most common in the family Euphorbiaceae (Raju & Rao, 1977). Under field conditions, when comparing the parameters that were presented above, Figueiredo et al. (2015) show that leaves of species *J. curcas* are amphistomatic, with differences in the stomatal density between the adaxial and abaxial surfaces. Additionally, Rodrigues et al. (2014) observed that in vitro conditions promoted stomata absence on adaxial epidermis, and higher stomatal index.

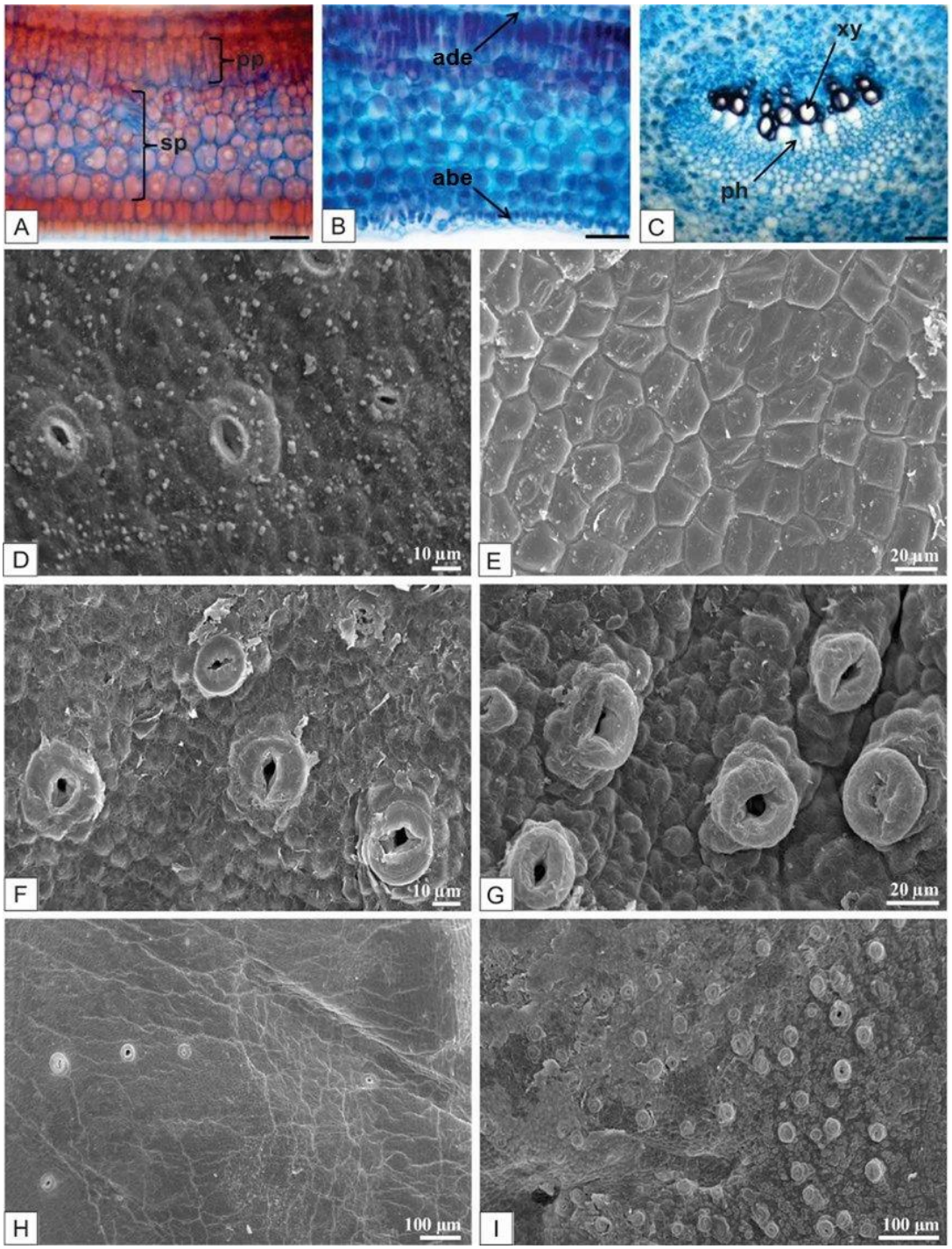

Figure 3 - Photomicrographs of transverse sections of leaves of *Jatropha curcas* incubated in a growth chamber (B.O.D.) at constant temperature of 30 °C/16 hour photoperiod under far red light (A) or red light (B). Bar = 28.5 µm; (pp), palisade parenchyma; (sp) spongy parenchyma; (ade) adaxial epidermis; (abe) abaxial epidermis. Cross-section showing the xylem and phloem (C). Bar = 20.0 µm; ph = phloem; xy = xylem. Electron micrographs of leaves of *Jatropha curcas* under different conditions of light and temperature, with 16-hour photoperiod. Epidermis with the presence of wax in the form of flakes or particles (D). Adaxial cells with polygonal morphology (E). Surface stomata (25 °C) (F). Prominent stomata (30 °C) (G). Stomata on the epidermis of the adaxial face (H). Stomata in the epidermis of the abaxial face (I).

In the *in vivo* conditions, the behavior of the plants and the morphological and anatomical characteristics exhibited by the leaves can be explained by the fact that this is a species adapted to arid and semi-arid conditions, further enhancing their tendency to have better and faster germination at higher temperatures. These results corroborate with observations of authors who describe this species as demanding in insolation, with strong resistance to drought (Teixeira, 2005; Andréo-Souza et al., 2010). They also confirm

results who indicated that this species adapts to a variety of environments and environmental conditions (Saturnino et al., 2005).

Conclusion

This study showed that the species *Jatropha curcas* exhibits a higher germination speed index in temperature 30 ºC and an increased number of normal seedlings in white light, and it is characterized as neutral photoblastic. This species presents seedlings with uniseriate epidermis, palisade unistratified parenchyma, and a spongy parenchyma composed of irregular cells. It is an amphistomatic species with more stomata on the abaxial surface.

Acknowledgements

The authors gratefully acknowledge the financial support of the Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Alquini Y, Bona C, Boeger MRT, Costa GG, Barros CF (2006) Epiderme. In: Appezzato-da-Glória B, Carmello-Guerreiro SM (2006) Anatomia Vegetal. 1 ed. Viçosa: Ed.UFV, p. 87-108.

Alves E (2004) Introdução à microscopia eletrônica. 1. ed. Lavras: UFLA/FAEPE, 88 p.

Andréo-Souza Y, Pereira AL, Silva FFS, Ribeiro-Reis RC, Evangelista MRV, Castro RD, Dantas BF (2010) Efeito da salinidade na germinação de sementes e no crescimento inicial de mudas de pinhão-manso. Revista Brasileira de Sementes 32(2): 83-92.

Araújo Neto JC, Aguiar IB, Ferreira VM (2003) Efeito da temperatura e da luz na germinação de sementes de *Acacia polyphylla* DC. Revista Brasileira de Botânica 26(2):.249-256.

Atroch EMAC, Soares AM, Alvarenga AA, Castro EM (2001) Crescimento, teor de clorofilas, distribuição de biomassa e características anatômicas de plantas jovens de *Bauhinia forticata* Link submetidas a diferentes condições de sombreamento. Ciência & Agrotecnologia 25(4): 853-862.

Carvalho NM, Nakagawa J (2012) Sementes: ciência, tecnologia e produção. 5. ed. Jaboticabal: FUNEP, 590p.

Castro EM, Pereira FJ, Paiva R (2009) Histologia vegetal: estrutura e função de órgãos vegetativos. 1. ed. Lavras: Editora UFLA, 234p.

Dehgan B, Webster GL (1979) Morphology and infrageneric relationships of the genus *Jatropha* (Euphorbiaceae). University of California Publications in Botany, California 74:1-73.

Dickison WC (2000) Integrative plant anatomy. 1. ed. San Diego: Academic Press, 533p

Ferreira DF (2011) SISVAR: A computer statistical analysis system. Ciência & Agrotecnologia 35(6):1039--1042.

Ferreira EA, Procópio SO, Silva EAM, Silva AA, Rufino RJN (2003) Estudos anatômicos de folhas de espécies de plantas daninhas de grande ocorrência no Brasil. IV - *Amaranthus deflexus, Amaranthus spinosus, Alternanthera tenella* e *Euphorbia heterophyl.* Planta Daninha 21(2): 263-271.

Figueiredo KV, Oliveira MT, Arruda ECP, Silva BCF, Santos MG (2015) Changes in leaf epicuticular wax, gas exchange and biochemistry metabolism between *Jatropha mollissima* and *Jatropha curcas* under semi-arid conditions. Acta Physiologiae Plantarum 37:108.

Jesus MAS, Pasqual M, Dutra LF, Chagas EA (2003) Cultivo *in vitro* de embriões zigóticos de Jatropha. Revista Ceres 50(288):183-189.

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in eletron microscopy. Journal of Cell Biology 27:137-138.

Kaviani B (2015) Some useful information about micropropagation. Journal of Ornamental Plants 5(1): :29-40.

Kraus JE, Arduim M (1997) Manual básico de métodos em morfologia vegetal. 1. ed. Seropédica: UFRRJ, 198 p.

Librea ML, Tolentino VS (2012) Anatomical characterization of oil cells and oil cavities in *Jatropha curcas* L. using light and electron microscopy. Biology Education for Social and Sustainable Development, p.145–152.

Maguire JD (1962) Speed of germination - aid in selection and evaluation for seedling emergence and vigor. Crop Science 2(2):176-177.

Martins CC, Machado CG, Cavasini R (2008) Temperatura e substrato para o teste de germinação de sementes de pinhão-manso. Ciência & Agrotecnologia 32(3): 863-868.

Matos FS, Gamboa I, Ribeiro RP, Mayer ML, Neves TG, Leonardo BRL, Souza AC (2011) Influência da intensidade luminosa no desenvolvimento de mudas de *Jatropha curcas* L. Revista Agrarian 4(14):265-272.

Melo GM, Cunha PC, Pereira JAF, Willadino L, Ulisses C (2011) Alterações anatômicas em folhas e raízes de *Jatropha curcas* L. cultivadas sob estresse salino. Revista Ciência Agronômica 42(3): 670-674.

Menezes NL, Franzin SM, Roversi T, Nunes EP (2004) Germinação de sementes de *Salvia splendens* Sellow em diferentes temperaturas e qualidades de luz. Revista Brasileira Sementes 26(1):32-37.

Mota LHS, Heinz R, Garbiate, MV, Scalon SPQ, Cremon T, Neto ALV (2012) Efeito da temperatura e tratamentos pré-germinativos na germinação e vigor de plântulas de *Jatropha curcas* L. Revista Biotemas 25(3): 29-37.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473- 479.

Nunes CF, Pasqual M, Santos DN, Custódio TN, Araujo AGD (2008) Diferentes suplementos no cultivo *in vitro* de embriões de pinhão-manso. Pesquisa Agropecuária Brasileira 43(1): 9-14.

Nunes CF, Santos DN, Pasqual M, Valente TCT, Oliveira ACL, Alves E, Setotaw TA (2013) Morphogenesis and regeneration of adventitious shoots in *Jatropha curcas* L.. Australian Journal of Crop Science 7(10): 1511-1519.

Olowokudejo JD (1993) Comparative epidermal morphology of West African species of *Jatropha* L. (Euphorbiaceae). Botanical Journal of the Linnean Society 111(2):139-154.

Pascuali LC, Silva FS, Porto AG, Filho AS, Meneghello GE (2012) Germinação de sementes de pinhão manso em diferentes temperaturas, luz e substratos. Semina: Ciencias Agrárias 33(4):1435-1440.

Raju VS, Rao PN (1977) Variation in the structure and development of foliar stomata in the Euphorbiaceae. Botanical Journal of the Linnean Society 75(1):69-97.

Rodrigues SP, Picoli EAT, Oliveira DC, Carneiro RGS, Isaias RMS (2014) The effects of in vitro culture on the leaf anatomy of *Jatropha curcas* L. (Euphorbiaceae). *Bioscience Journal* 30(6):1933-1941.

Robards AW (1978) An introduction to techniques for scanning electron microscopy of plant cells. In: Hall JL. Electron microscopy and cytochemistry of plant cells. 1 ed. New York: Elsevier, p. 343-444.

Santos DN, Nunes CF, Soares JDR, Valente TCT, Alves E, Labory CRG, Pasqual M (2013) Cytological characterization of *Jatropha curcas* callus in diferente periods of cultivation. Crop Breeding and Applied Biotechnology 13(4):228-233.

Santos DN, Nunes CF, Val ADB, Cancado GMA, Pasqual M (2012) Biotecnologia Aplicada ao Pinhão-manso. In: Cançado GMA, Nogueira LL (2012) Biotecnologia Aplicada à Agropecuária. 1 ed. Caldas: EPAMIG, p. 507-536.

Saturnino HM, Pacheco DD, Kakida J, Tominaga N, Gonçalves NP (2005) Cultura do pinhão-manso (*Jatropha curcas* L.). Informe Agropecuário 26(229):44-78.

Scalon SPQ, Kodama FM, Scalon filho H, Mussury RM (2008) Crescimento inicial de mudas de sangra-d'água (Croton urucurana Baill.) sob sombreamento e aplicação de giberelina. Revista Brasileira de Plantas Medicinais 10(3):61-66.

Silva RC, Camillo J, Luis ZG, Scherwinski-Pereira JE (2011) Potencial germinativo e morfoanatomia foliar de plântulas de pinhão-manso originadas de germoplasma criopreservado. Pesquisa Agropecuária Brasileira 46(8):836-844.

Stefanini MB, Rodrigues SD, Ming LC (2002) Ação de fitorreguladores no crescimento da erva-cidreira brasileira. Horticultura Brasileira 20(1):18-23.

Takaki M (2001) New proposal of classification of seeds based on forms of phytochrome instead of photoblastism. Revista Brasileira de Fisiologia Vegetal 13(1):103-107.

Teixeira CT (2005) Potencialidades de oleaginosas para produção de biodiesel. In: Produção de oleaginosas para biodiesel. Informe Agropecuário 26(229):18-27.

Tricker PJ, Gibbings JG, López CMR, Hadley P, Wilkinson MJ (2012) Low relative humidity triggers RNA-directed de novo DNA methylation and suppression of genes controlling stomatal development. Journal of Experimental Botany 63(10):3799-3813.

Vanzolini S, Meorin EBK, Silva RA, Nakagawa J (2010) Qualidade sanitária e germinação de sementes de pinhão-manso. Revista Brasileira de Sementes 32(4):009-014.

Verma KC, Verma SK (2014) Biophysicochemical Evaluation of Wild Hilly Biotypes of *Jatropha curcas* for Biodiesel Production and Micropropagation Study of Elite Plant Parts. Applied Biochemistry and Biotechnology 175(1):549-559.

View publication stats