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How does UV-B radiation affect the initial growth of common bean (*Phaseolus vulgaris* L.)? Physiological and structural aspects

Como a radiação UV-B afeta o crescimento inicial do feijoeiro-comum (*Phaseolus vulgaris* L.)? Aspectos fisiológicos e estruturais

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ABCTRACT

Increased UV-B radiation, a topic of concern due to climate change, can affect plant development through structural and physiological changes. Common bean (*Phaseolus vulgaris* L.) is a legume of great economic relevance, being one of the basic components of the human population's diet. Seeds of *P. vulgaris* var. Carioca were sown and grown in a growth chamber, and at 12 days of age were exposed for 15 min daily to UV-B radiation for a period of 14 days. Growth, leaf anatomy, gas exchange and pigments were evaluated. It was observed that UV-B radiation negatively affected the initial growth of bean plants, which had a reduction in height, number of leaves, fresh and dry leaf masses, leaf area, as well as reduction of stem and root dry masses. There was a reduction in the epidermis thickness, increase in the thickness of the palisade parenchyma and the leaf blade. It was verified yet reduction of the photosynthetic and photoprotectors pigments, reduction of the photosynthetic rate and alterations on the absorbent compounds of the UV-B radiation. The results obtained showed that the increase in UV-B radiation was harmful to the initial development of this crop.

Palavras-chave: Agriculture, Anatomy, Beans, Pigments.

RESUMO

O aumento da radiação UV-B, tema preocupante em virtude das mudanças climáticas, pode afetar o desenvolvimento das plantas, por meio de mudanças estruturais e fisiológicas. O feijoeiro-comum (*Phaseolus vulgaris* L.) é uma leguminosa de grande relevância econômica, sendo um dos componentes básicos da alimentação humana. Sementes de *P. vulgaris* var. Carioca foram germinadas e cultivadas em uma câmara de crescimento, sendo aos 12 dias de idade expostas a 15 min diários de radiação UV-B, por um período de 14 dias. Foram avaliados crescimento, anatomia foliar, trocas gasosas e pigmentos. Observou-se que a radiação UV-B afetou negativamente o crescimento inicial do feijoeiro, o qual teve redução em altura, número de folhas, massa fresca, massa seca e área foliares, bem como, redução da massa seca de caule e raiz. Houve redução na espessura da epiderme e aumento nas espessuras do parênquima paliçádico e do limbo. Verificou-se, ainda, redução dos teores de pigmentos fotossintéticos e fotoprotetores, redução da taxa fotossintética e alterações nos compostos absorventes da radiação UV-B. Os resultados obtidos mostraram que o aumento da radiação UV-B foi prejudicial para o desenvolvimento inicial desta cultura.

Key words: Agricultura, Anatomia, Feijão, Pigmentos.

1 INTRODUCTION

Radiation is essential since it is a primary source of energy for the replenishment of the organic matter consumed in the food chain and for the energy balance in the planet. For plants it is not only an energy source, but also a stimulus for its development, and in excess, it becomes a stress factor. Above all, solar radiation controls many development processes, acting as an indication for

germination, directed growth and development of the external form of the plant (Larcher, 2000; Jordan, 2017).

Although UV-B light is relatively the smallest component of sunlight, which corresponds to less than 0.5% of the total light energy reaching the Earth's surface (Lidon et al., 2012), it has the highest energy in the daylight spectrum and therefore, has a substantial impact on the biosphere (Kataria et al., 2014).

Plants have the ability to identify the radiation by different sensors and are able to transform into biochemical information. It is known that radiation can affect various physiological processes. Large amounts of photosynthetically active radiation and increased absorption of UV radiation produce a stressful situation. The energy of the photons of UV radiation is so high that, upon reaching the molecules of the cells, it withdraws electrons from its structure, leaving them ionized, irreversibly compromising its structure and function (Kerbauy, 2008; Jordan, 2017).

However, depending on the wavelength, amount of photons, and time of exposure, UV radiation can cause much greater damage, such as mutations, lesions or even plant death (Holá et al., 2015). The strong radiation introduces an amount of energy in the leaf greater than its capacity of use in photosynthesis, overloading the processes, being able to destroy the photosynthetic pigments and the structures of thylakoids (Rai and Agrawal, 2017).

The UV-B-induced photomorphological changes in the leaves include leaf size reduction and foliar winding, chlorosis and tissue necrosis, increased leaf thickness, degradation of photosynthetic pigments and synthesis of phenolic compounds, which are considered UV-B filters, such as anthocyanins and flavonoids (Ravindran et al., 2010).

One of the most important effects of UV radiation is the accumulation of flavonoids, anthocyanins or other UV absorbing compounds in the leaf epidermis (Tohidi-Moghadam et al., 2012). Flavonoid compounds, as secondary metabolites, play an important role in protecting plants that have been damaged by UV-B. Some plants are more tolerant to UV-B than others because they produce a variety of secondary metabolites that effectively absorb UV-B and prevent it from penetrating leaf mesophyll cells (Ravindran et al., 2010).

The common bean (*Phaseolus vulgaris* L.) is an annual herbaceous plant of the Fabaceae family. It has a short life cycle, with an average duration of 90 days depending on the cultivation method and environmental conditions (Almeida et al., 1971). It is the most important food legume for direct consumption in the world (Foyer et al. 2016) and is considered one of the main sources of protein (Luna-Vital et al., 2015). *P. vulgaris* is produced in a range of crop systems and environments in regions as diverse as Asia, the Americas and Africa (Foyer et al. 2016). Some environmental factors can limit bean productivity, such as temperature, solar radiation and photoperiodism, and

among them, one of the most important affecting the development of bean plant is radiation (Didonet and Silva, 2004).

The present work aimed to evaluate the effects of UV-B radiation on morphology, leaf anatomy and physiology of bean (*Phaseolus vulgaris* L.) in their initial phase of growth.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

Seeds of Brazilian beans, *Phaseolus vulgaris* L., var. Carioca were sown in 290 mL tubes containing a mixture of vegetable soil and organic compound in a proportion of 1:1. The obtained plants were kept in a growth chamber (Shellab Model LI15, Oregon, USA) under a 12 hours photoperiod, photosynthetic active radiation of 300 µmol $^{m-2\ s-1}$ provided with four 32 W white light lamps (GE - F32T8/SP41/ECO), constant temperature of 27 ºC and relative humidity of 60 %. After 12 days of sowing, 10 plants were equally divided into two conditions: the control, which remained in the initial growth conditions, and the UV-B treatment. UV-B treatment consisted of 15 minutes daily supplementation of radiation using two special lamps (Sankyo Denki G15T8E, Kanagawa, Japan) suspended 30 cm above the plants. Plants received a daily dose of 0.22 KJ $^{m-2}$ of UV-B radiation (3 KJ $^{m-2}$ at the end of 14 days) and the radiation UV was 5.5 µmol $^{m-2\ s-1}$ (quantified with the UV Spectrum Technologies meter, Illinois, USA).

2.2 GROWTH ANALYSIS

In both control and UV-B treatment, the height of the aerial part (cm), stem diameter (mm), number of leaves, total leaf area, total fresh leaf mass, fresh stem mass, fresh root mass, and dry mass of leaf, stem and root were measured. Juvenile plants height was measured using a ruler, diameter measurements were made with a pachymeter and leaf number was counted manually. The mass measurements were made in a precision balance and the dry mass was obtained by weighing the leaves after drying in an oven at 60 °C until constant weight. The leaf area was measured using an Area Meter LI-COR 3100 (Lincoln, USA).

2.3 GAS EXCHANGE

Gas exchange measurements were performed on the fully expanded leaves located on the second stem node, after two hours of the light period begins, using an infrared gas analyzer (LI-6400XT, LI-COR, Lincoln, NE, USA) coupled with red/blue light source (LI-6400-02B LED). The system was kept constant under irradiance of 300 µmol photons $^{m-2\ s-1}$ (defined by the light curve), 400

μmol CO_2 mol^{-1} air and temperature of 27 °C. Net CO_2 assimilation rate (A) and stomatal conductance (gs) were measured.

2.4 PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC PIGMENTS CONTENT

The content of chlorophylls and carotenoids was quantified from the ethanolic extract read in a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific, Waltham, USA) at wavelengths 470, 648 and 664 nm. The pigment concentrations were determined according to Lichtenthaler and Buschmann (2001): Chlorophyll *a* = (13.36 x A_{664}) – (5.19 x A_{648}); Chlorophyll *b* = (27.43 x A_{648}) – (8.12 x A_{664}); Total Chlorophyll = Chlorophyll *a* + Chlorophyll *b*; Carotenoids = [(1000 x A_{470}) – (2.13 x chlorophyll *a*) – (97.64 x chlorophyll *b*)] / 209. Where: A_{470} = absorbance at 470 nm; A_{664} = absorbance at 664 nm; A_{648} = absorbance at 648 nm. Results were expressed in mg per gram of fresh mass (mg g^{-1} MF).

Flavonoids were measured using the colorimetric method using aluminum chloride based on Park et al. (1998), with modifications. The reaction mixture consisted of 500 μL of ethanolic extract, 100 μL of aluminum chloride 10% in 80% ethanol, 100 μL of potassium acetate 1 M in water and 4.3 mL of distilled water. The substances were mixed and the mixture remained at a temperature of 25ºC for 30 min, being subsequently determined its absorption at 428 nm in a spectrophotometer (Thermo Scientific®, Genesys 10S). Quercetin was used for the calibration curve (100, 80, 60, 40, 30, 20, 10 and 0 μg mL^{-1} of quercetin) and the results were expressed in mg equivalent of quercetin per gram of fresh mass.

2.5 LEAF ANATOMY

Leaf anatomy analyses were performed on samples of the middle third of fully expanded leaves located in the second node from the stem apex. The leaf samples were stored in 70% ethanol and later cross sections were made with a tabletop microtome. The quantitative anatomical analysis was performed by measuring the thickness of the leaf blade, the adaxial and abaxial surfaces of the epidermis, and the palisade and spongy parenchyma.

The stomatal density (number of stomata per mm^2) was determined on the abaxial surface of the leaves by the epidermal printing on glass slides technique, using instant glue. The calculation of the stomatal density was based on the number of stomata present in a known leaf area.

Quantitative analyses were performed using Image J1 software (US National Institutes of Health, Bethesda, MD, USA). Photomicrographs were obtained with a digital camera coupled to an optical microscope (Nikon Eclipse 50i, Nikon Tec. Corporation, Tokyo, Japan) and to a computer with image capture software (Nikon NIS-Elements, Nikon Tec. Corporation, Tokyo, Japan).

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2.6 STATISTICAL ANALYSIS

All data were submitted to statistical analysis using InfoStat software (Version 2011, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). The t-test was used to compare means considering $p \leq 0.05$.

3 RESULTS

3.1 GROWTH ANALYSIS

UV-B radiation negatively affected the development of bean plants (Table 1), causing damage on their growth by reducing plant height, leaf number, total leaf area, fresh and dry leaf masses and stem and root dry masses. In addition, leaf changes such as necrosis, distortion (Figure 1) and abscission of newly emerged leaves were observed.

3.2 GASEOUS EXCHANGES AND UV-B ABSORBING COMPOUNDS

Gas exchange was significantly altered with UV-B radiation (Table 1). The net CO_2 assimilation (photosynthesis) showed lower values for plants treated with UV-B; however, the stomatal conductance did not show significant changes.

UV-B absorbing compounds values differed in a specific point. An increase in the absorbance of UV-B treatment can be observed in the wavelength range for UV-B radiation, considered between 280 and 320 nm (Figure 2).

3.3 PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC PIGMENTS CONTENT

The chloroplastid pigments analysis from plants exposed to UV-B radiation revealed a decrease only in the concentrations of chlorophyll *a* and carotenoids (Table 1).

3.4 LEAF ANATOMY

UV-B radiation caused some structural changes in plants. It was observed a decrease in the thickness of the adaxial and abaxial surfaces of epidermis, as well as an increase in the thickness of the palisade parenchyma and leaf blade. The stomatal density of the leaves submitted to UV-B radiation was higher than that found for the control (Table 1).

4 DISCUSSION

The UV-B radiation has significantly affected the physiology, biochemistry and anatomy of *P. vulgaris* plants. The results obtained in this study, such as reduction in total leaf area and fresh and dry masses, number of leaves and height of plants corroborate those found by Boeger and Poulson

(2006) in *Arabidopsis thaliana* leaves. Results from Liakoura et al. (2003) study also showed a reduction of specific leaf area in leaves of sunny plants (*Arbutus andrachne* L., *Arbutus unedo* L., *Quercus coccifera* L. and *Quercus ilex* L.) compared to shadow plants in relation to UV-B and UV-A rays.

The reduction of dry and fresh leaf mass can be explained as a reaction to stress caused by UV-B radiation in plant development and metabolism (Boeger and Poulson, 2006). In addition, since UV-B radiation can adversely affect cell division, this effect possibly influences the leaf area reduction (Nogues et al., 1998; Raghuvanshi and Sharma, 2016). Concomitantly, the smaller expansion in epidermal cells (observed by the smaller epidermis thickness in the adaxial and abaxial surfaces) can lead to a reduction in leaf area. Hectors et al. (2010) found a correlation between leaf area and cell areas of epidermis; in *Arabidopsis* subjected to UV radiation was possible identify that reduction in leaf area was due to smaller cell area without reduction in cell number.

The reduction in leaf area associated with the lower concentration of photosynthetic pigments seems to be the cause of the decrease in growth and the reduction of stem length and root dry mass verified under UV-B radiation treatment in *P. vulgaris*. These factors can lead to lower absorption of sunlight, as well as affect photosynthetic activity, leading to a decrease in photosynthesis, indirectly affecting plant growth (Raghuvanshi and Sharma, 2016).

The higher absorbance of plants treated with UV-B radiation indicates an investment by plants in photoprotection. Compounds capable of absorbing this radiation, such as flavonoids and sinapic esters, protect because their strong absorption between 280 and 340 nm. Also, flavonoids scavenge free-radical, offering additional protection to cells (Jansen et al. 1998, Riquelme et al., 2007). Despite the photoprotective mechanism, a reduction of chlorophyll *a* and carotenoids content was observed, a possible consequence of the high amount of energy released by UV-B radiation that reaches the leaves causing overload of photosynthetic processes and low CO_2 assimilation yield. The reduction of chlorophyll *a* and carotenoids, also observed by Raghuvanshi and Sharma (2016) in bean plants, probably is related with the decrease in photosynthesis of plants treated with UV-B, since no changes were observed in stomatal conductance.

The higher stomatal density obtained under UV-B radiation treatment can be associated to the reduction of leaf area. Boeger and Wisniewski (2003) obtained similar results and related to water retention mechanisms in order to reduce transpiration, which is reinforced in this study by the lower values of stomatal conductance.

Thickness and leaf area, in stressful circumstances, tend to be inversely proportional and compensatory between them (Smith et al., 1997; Boeger and Poulson, 2006). In this study, thick leaves were observed under UV-B radiation, which can be advantageous. Thicker leaves with reduced

areas provide a longer path for radiation, reducing the possible damage caused by excessive solar radiation (Lambers et al., 2008). It is also possible to verify that the greater leaf thickness in *P. vulgaris* was a result of the palisade parenchyma thickening.

The results obtained in this study show that the exposure of young plants of *P. vulgaris* var. Carioca to UV-B radiation over a period of 14 days has a detrimental effect on growth of this species. However, observed changes in the UV-B radiation absorption spectra, thickness of palisade parenchyma, stomatal density and stomatal conductance indicate a defense response, enabling the survival of these plants.

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Table 1 - Effects of UV-B radiation on growth, gaseous exchanges, pigment contents and leaf anatomy of *P. vulgaris* plants.

Variables evaluated	Control	UV-B
Growth		
Height (cm)	25.08 ± 1.42*	18.5 ± 1.82
Diameter (mm)	4.24 ± 0.11	4.43 ± 0.23
Leaf number	3.60 ± 0.24*	2.60 ± 0.24
Total Leaf Area (cm^2)	181.76 ± 15.14*	87.64 ± 4.28
Fresh Leaf Mass (g)	4.19 ± 0.08*	2.30 ± 0.22
Fresh Stem Mass (g)	2.42 ± 0.09	2.21 ± 0.11
Fresh Root Mass (g)	4.78 ± 0.55	3.74 ± 0.64

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Dry Stem Mass (g)	0.24 ± 0.01*	0.16 ± 0.02
Dry Leaf Mass (g)	0.51 ± 0.03*	0.25 ± 0.03
Dry Root Mass (g)	0.33 ± 0.04*	0.23 ± 0.03
Root/Shoot Ratio (g g^{-1})	1.29 ± 0.10	1.08 ± 0.09
Gaseous exchanges		
A (µmol CO_2 m^{-2} s^{-1})	2.98 ± 0.15*	0.52 ± 0.24
g_s (mol H_2O m^{-2} s^{-1})	0.03 ± 0.008*	0.01 ± 0.002
Pigment contents		
Chlorophyll *a* (mg g^{-1} MF)	2.07 ± 0.24*	1.05 ± 0.09
Chlorophyll *b* (mg g^{-1} MF)	0.58 ± 0.09	0.49 ± 0.05
Total Chlorophyll (mg g^{-1} MF)	2.32 ± 0.45	1.72 ± 0.26
Carotenoids (mg g^{-1} MF)	0.54 ± 0.06*	0.28 ± 0.03
Chlorophyll *a/b*	0.97 ± 0.07	0.90 ± 0.06
Total Chlotophyll/carotenoides	1.76 ± 0.04	1.96 ± 0.15
Flavonoids (mg quercetin g^{-1} MF)	33.4 ± 3.33	28.10 ± 2.96
Leaf anatomy		
Adaxial surface epidermis (µm)	25.90 ± 0.18*	22.83 ± 0.80
Abaxial surface epidermis (µm)	24.42 ± 0.01*	20.17 ± 0.24
Palisade parenchyma (µm)	63.59 ± 6.74*	90.58 ± 4.14
Spongy parenchyma (µm)	105.42 ± 4.98	107.18 ± 6.23
Leaf blade (µm)	205.30 ± 2.94*	240.18 ± 5.62
Stomatal density (n° mm^{-2})	109.78±7.10*	178.85±18.67

Asterisks indicate a significant difference between control and UV-B treatment ($P \leq 0.05$, t-test). Values are shown as means ± standard error (n = 5).

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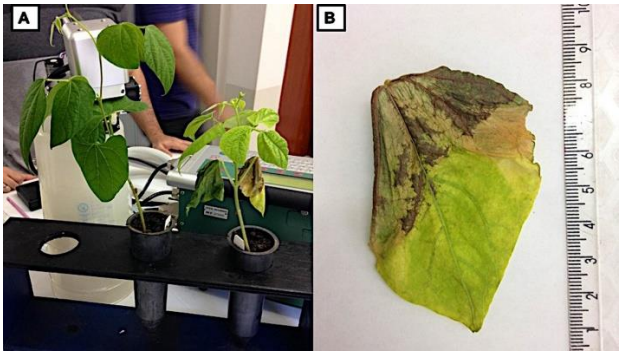

Figure 1 - General appearance of young plants of *P. vulgaris*. A - Control (left) and UV-B treatment (right); B - Leaf with damage caused by UV-B radiation.

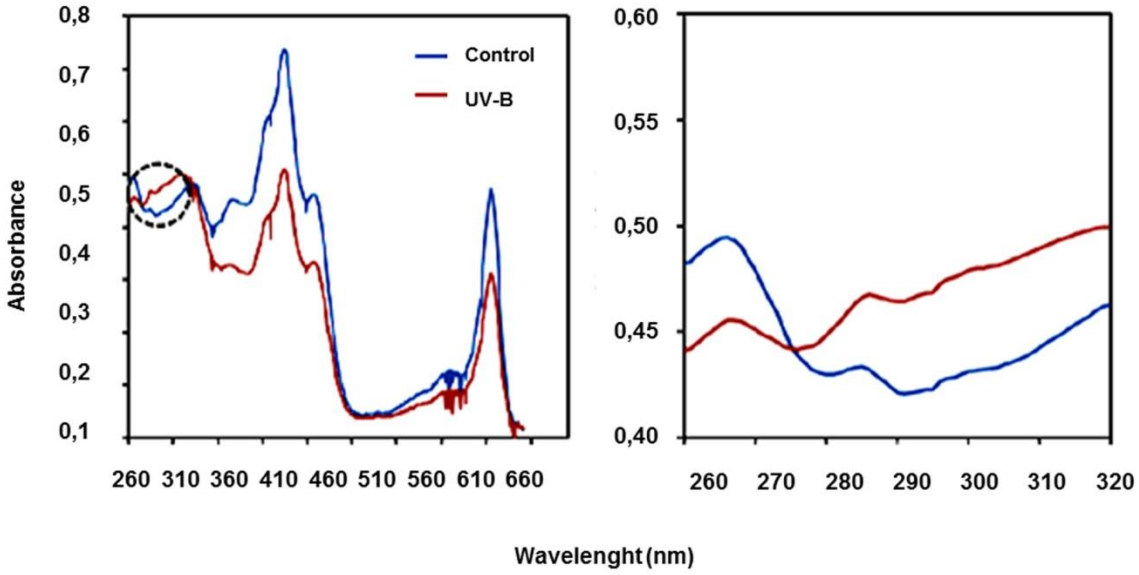

Figure 2 - Effect of UV-B radiation on the UV-visible absorption spectra of the foliar ethanolic extract of *P. vulgaris* plants. The figure on the right represents an enlargement of the graph highlighted within the dotted area in the figure on the left.