Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 11 (2): 70-76, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.70.76

Physiological Characterization of Two Tropical Epiphyte Species: *Dyckia distachya* and *Vriesea platynema* (Bromeliaceae)

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Abstract: Leaf extracts of two species of bromeliads (*Vriesea platynema* and *Dyckia distachya*) differing in their phenotype were investigated in this study. Physiological and biochemical changes combined with the photosynthetic behavior were investigated. *Dyckia distachya* showed superiority in chlorophylls, carotenoids and total carbohydrates. *Vriesea platynema* showed higher starch content, light and CO_2 flow curves following a quadratic model. We find out that *Dyckia distachya* has an efficient mechanism for surviving adverse environmental factors.

Key words: *Dyckia distachya*, *Vriesea platynema*, carotenoids, photosynthesis, starch

INTRODUCTION

The Bromeliads are plants with different growth habits (epiphytic and terrestrial) that can be found in almost all ecosystems, particularly in tropical rainforests but may also occur in dry climates. Both C_3 and CAM (crassulacean acid metabolism) metabolisms are present in epiphytic plants (Monteiro *et al*., 2009). The metabolism allows CAM species a remarkable physiological flexibility, allowing adjustment to increase the capacity of plant metabolism to environmental variations (Cushman and Borland, 2002). They have a variety of adaptive mechanisms to water supply, juiciness and specific structures such as leaf trichomes (Endres and Mercier, 2001). The leaves are considered the most important vegetative parts of these bromeliads due to the ability to absorb and assimilate nutrients through the trichomes (Takahashi and Mercier, 2011).

The family includes several genera and species occurring in an endemic form in the subtropics of the Americas and some in West Africa and 40% of the known species are present in Brazil which ranks it among the most important in terms of diversity of the family (Tamaki *et al*., 2011). Many bromeliads have been decreasing due to various human activities and constant climate changes which put them at risk of extinction. In the state of Santa Catarina have been described 31 species of *Vriesea* sp. that occur in endemic forms (Alves *et al*., 2006; Dal Vesco and Guerra, 2010). There are several studies describing different species of the family, however, ecophysiological correlations to understand the metabolism of these species are still scarce, especially in *Vriesea platynema* and *Dyckia distachya* with enough occurrences in the State of Santa Catarina (Southern Brazil).

The photosynthetic capacity may differ between species and even between individuals, depending on environmental conditions. In many species of bromeliads the knowledge about their metabolism is lacking in the literature. In this way physiological and biochemical studies are necessary to generate basic knowledge and later, to have more details over some specific points that may help preserve the species. The aim of this study was to assess the differential capacity of *Dyckia distachya* and *Vriesea platynema* in carbon assimilation, carbohydrates, starch, chlorophyll and carotenoid content, as well as determine the light saturation curve, important physiological parameters in metabolism analysis of those species.

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MATERIALS AND METHODS

Plant material: Leaves of *Vriesea platynema* and *Dyckia distachya* were collected from plants growing in a greenhouse under controlled conditions at the Center for Agricultural Sciences (CCA/UFSC), Federal University of Santa Catarina, Santa Catarina State, southern Brazil. Briefly, segments of leaves were collected in healthy middle leaves for analysis while for CO_2 and light flow curves were used without removing the leaves from the plants. The moisture content of the material was determined by drying the material in an oven with forced air circulation at 30°C to constant weight and calculated following the equation:

$$U(\%) = \frac{Ca - Cv}{a} \times 100 \quad (1)$$

where, U is moisture content (%), Ca is weight of crucible with sample, Cv is weight of crucible without sample and a is weight of sample.

Chlorophyll content: The chlorophyll content was determined according to Lichtenthaler and Buschmann (2001). Briefly, about 1.0 g of fresh weight of leaves was steeped in ceramic crucible with liquid nitrogen and added 3 mL of cold acetone. Each sample was filtered through a funnel lined with filter paper previously moistened with cold acetone. The filtrate was collected in 50 mL volumetric flasks. Then, each volume collected was stored on ice in test tubes that were capped with aluminum foil and PVC (polyvinyl chloride) film. The samples were read in a spectrophotometer calibrated with pure acetone. The chlorophyll a absorbance was read at 622 nm and chlorophyll b at 645 nm. The chlorophyll content was carried out according to the equations defined below and values expressed in μg g^{-1} of dry weight as (Mean±Standard deviation):

$$Chl_a = 11.24A_{(662\ nm)} - 2.04A_{(645\ nm)} \quad (2)$$

$$Chl_b = 20.13A_{(645\ nm)} - 4.19A_{(622\ nm)} \quad (3)$$

$$Chl_{total} = Chl_a + Chl_b \quad (4)$$

Total carotenoid content: About 1 g of flour of dry leaves was added 10 mL of Hexane: acetone (1:1 v/v) containing 100 mg L^{-1} of BHT (butylated hydroxytoluene). Samples were incubated for 30 min in the dark at room temperature. After, the samples were filtered through a cellulose support (0.45 μm) under vacuum, the extract was collected and washed 3 times with distilled water (10 mL each wash) to remove hydrophilic compounds and then concentrated under nitrogen gas flow (Aman *et al*., 2005). The concentrated residue was dissolved in hexane (3 mL), followed by reading the absorbance (450 nm) in a spectrophotometer (Spectrum Gold lab 53). The results were expressed as (μg g^{-1} of dry weight). To quantify total carotenoids in the extracts was used the Lambert-Beer formula below and the molar extinction coefficient of lutein (2348 M^{-1} cm^{-1}) (Britton, 1992). All experiments were performed in triplicate:

$$A = \varepsilon.c.l \quad (5)$$

where, ε is molar extinction coefficient of lutein, c is concentration, l is width of the cuvette and A is absorbance. The values were converted into μg g^{-1} using the equation below:

$$CTA(\mu g\ g^{-1}) = \left(\frac{A}{\varepsilon}\right).\left(\frac{v}{1000}\right).PM.\left(\frac{1}{PA}\right).10^{6} \quad (6)$$

where, CTA is total carotenoid (μg g^{-1}), A is absorbance, ε is molar extinction of lutein (2348 1 M^{-1} cm^{-1}), v is final volume (3 mL), PM is molecular weight of lutein (568, 88 g mol^{-1}) and PA is weight of sample (g).

Total soluble carbohydrates: Total carbohydrates were determined using phenol-sulfuric method (DuBois *et al*., 1956). Briefly, 1.0 g of fresh material was macerated in a mortar with liquid nitrogen to obtain a fine powder. The macerated material was transferred to Falcon tubes which were added 5 mL of 80% ethanol in water bath at 100°C for 5 min. This process was performed three times. After each boiling, the extracts were centrifuged at 3000 rpm for 10 min. The extracts were centrifuged, filtered through glass microfiber and placed in test tubes by adjusting the volume with 80% ethanol to 10 mL. In test tubes of 10 mL were pipetted 50 μL of the extract and diluted to 500 μL with distilled water. Were then added 0.5 mL of 5% phenol and 2.5 mL of concentrated sulfuric acid 96% and the absorbance read at 490 nm. The total carbohydrate content was calculated with the help of a standard curve ($y = 0.005923 + 0.08819x$, $r^2 = 0.9792$) determined using solutions with known concentrations of glucose (10, 20, 40, 60, 80, 100 mL of glucose).

Starch content: The precipitate resulting from the extraction of total soluble carbohydrates (previously described) were added 10 mL of cold distilled water, 13 mL of 52% perchloric acid for 15 min stirring occasionally. Then was added 20 mL of distilled water and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the residue was added 5 mL of cold distilled water and 6.5 mL of 52% perchloric acid with stirring for 15 min and

centrifuged again (3000 rpm/15 min). The supernatant was decanted and starch extracted in 100 mL beaker, joining all the fractions of starch. The solution was homogenized and filtered through glass wool discarding the initial 5 mL. The starch dosage followed the steps described in section 2.4 and external standard curve determined previously were used (McCready *et al*., 1950).

Light and CO_2 flow curves: To determine the light curve and the rate of CO_2 assimilation was used portable analyzer, working with infrared to measure the flow of light and CO_2 (IRGA) coupled with an air chamber and data processor, engaging the equipment in the intermediate leaves of two species of bromeliads.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) and t-test was used to compare averages at 5% of significance. Experiments were done in triplicate.

RESULTS AND DISCUSSION

Chlorophyll content: Chlorophylls are the most abundant natural pigments in photosynthetic plants. Chlorophyll a is used in the photochemical step in photosynthesis while the others are accessory pigments (Da Cruz *et al*., 2007). As a component of the photosynthetic machinery, it absorbs sunlight and is involved in energy transfer during photosynthesis (Reinbothe *et al*., 2010). The results of chlorophyll content (Fig. 1) showed higher levels for *Dyckia distachya* (5.91 μg g^{-1}) compared to *Vriesea platynema* (2.39 μg g^{-1}), with statistically significant differences ($p<0.05$, t-test). The results are not surprising, as reported by Da Cruz *et al*. (2007), the presence and abundance of chlorophyll vary according to species, the extraction method, the equipment used as the solvent. According to Hiscox and Israelstam (1979), the method of acetone extraction has limited capacity when compared with DMSO (dimethyl sulfoxide) as a way of example to illustrate several other factors that may influence the levels of chlorophyll such as environmental conditions such as saline conditions (Nivas *et al*., 2011) and drought stress (Helaly and Hosieny, 2011). In agreement with the results, the highest amount of chlorophyll found in the specie *Dyckia distachya* reveals a greater need of light for photosynthesis than *Vriesea platynema*.

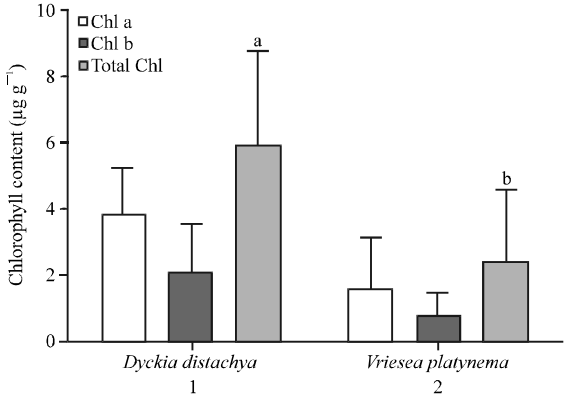

Fig. 1: Total chlorophyll content in leaf extracts of *Vriesea platynema* and *Dyckia distachya* obtained in a spectrophotometer at 622 and 645 nm and determined with the help of the formula described by Lichtenthaler (1987), expressed as (Mean±Standard deviation) of 4 replicates. Same letters above the bars indicate no significant differences between the averages and t-test at 5% of probability

Carotenoid content: Carotenoids represent one of the most widely distributed groups of natural pigments occurring mostly in plants, microorganisms and animals. They act as precursors of vitamin A and are reported to act as scavengers of reactive oxygen species (Aman *et al*., 2005). According to Uarrota *et al*. (2011a), these pigments represent a chemical interface between plants and the surrounding environment. Its synthesis is often affected by environmental conditions. The results of our study on total levels (Fig. 2) as well as scan spectra (Fig. 3) showed higher levels of carotenoids to *Dyckia distachya* (e.g., 320 μg g^{-1}) compared with *Vriesea platynema* (115 μg g^{-1}) statistically significant by t-test ($p<0.05$). In fact, the results confirm those found on the chlorophyll content. The high content of carotenoids

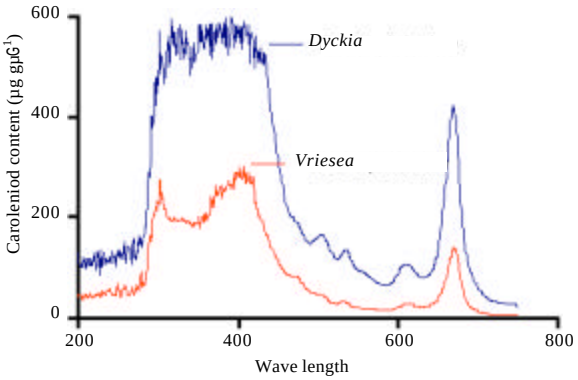

Fig. 2: UV-vis profiles of carotenoid scanning (200-750 nm) of leaf extracts for *Vriesea platynema* and *Dyckia distachya* by spectrophotometer (Spectrum Gold lab 53) based on the mean concentration, 3 and 2 repetitions for *Dyckia* and *Vriesea*, respectively

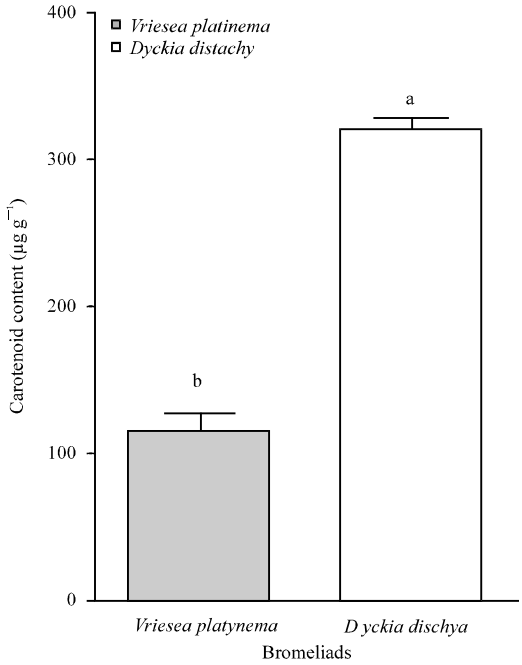

Fig. 3: Total carotenoid content in extracts of leaf of *Vriesea platynema* and *Dyckia distachya* obtained in a spectrophotometer at 450 nm and determined with the help of the Beer-Lambert law, expressed as (Mean±Standard deviation) of 3 and 2 repetitions for *Dyckia* and *Vriesea*, respectively, letters above the bars indicate significant differences between the averages and t-test at 5% of probability

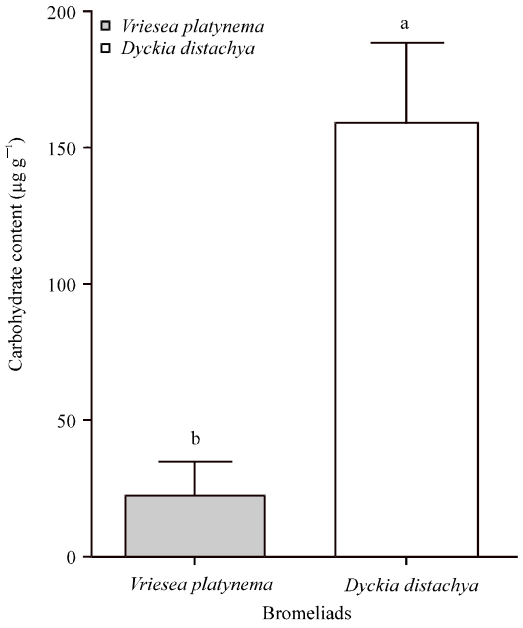

Fig. 4: Total carbohydrate content in leaf extracts of *Vriesea platynema* and *Dyckia distachya* obtained in a spectrophotometer at 490 nm (UV Spectrophotometer SP 2000) by the phenol-sulfuric method (DuBois *et al*., 1956) and determined with the help of an external standard curve of glucose ($y = 0.005923+0.08819\ x$, $r^2 = 0.9792$), values are in µg g^{-1} (Mean±Standard deviation), expressed as glucose equivalents resulting from four replicates, Letters above the bars indicate significant differences between the averages and t-test at 5% probability

suggests that *Dyckia distachya* need greater protection of the photosynthetic apparatus against light, one of the functions straightened by these pigments. According to Takahashi and Badger (2011), sunlight damages the photosynthetic apparatus, initially in photosystem II (PSII) and can cause photoinhibition which may limit the photosynthetic activity of the plant. Under conditions of excess light, there is accelerated production of reactive oxygen species in PSI and PSII in chloroplasts and to prevent oxidative stress plants produce various metabolic compounds, such as carotenoids which are potent antioxidants. The results also revealed that the specie *Dyckia distachya* has higher metabolic activity and has a different survival strategy that *Vriesea platynema.* Hala *et al.* (2005) showed that soil conditions may also affect the carotenoid content and all together with genetic factors will influence the plant growth (Uarrota, 2010).

Total soluble carbohydrates: The photosynthesis-the conversion of carbon dioxide into organic compounds by sunlight occurs in three pathways in terrestrial plants, the most common being via the C_3 and CAM pathways being derived from C_4 and C_3 metabolism (West-Eberhard *et al*., 2011). According to Sage (2002), the C_4 pathway is the major route that concentrates more carbon to offset carbon dioxide limitations. The carbon assimilates in the leaves is exported to other parts to support the growth of various organs (Peng *et al*., 2011). The quantification of the total carbohydrate content (Fig. 4) in this study revealed higher levels for *Dyckia distachya* (158.98 µg g^{-1}) than *Vriesea platynema* (22.87 µg g^{-1}) with statistically significant differences (t-test, 5% of probability) once again confirming the previous results. The specie concentrates higher levels of carbohydrates to maintain the survival of other plant organs and as a regulatory mechanism of photosynthesis. As reported by Merchant *et al*. (2010), high levels of carbohydrates can also be seen as a defense mechanism of plants against the

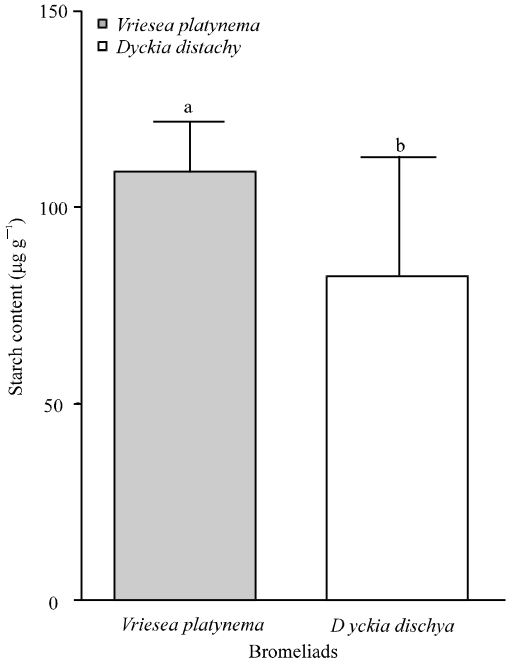

Fig. 5: Carbohydrate content in leaf extracts of *Vriesea platynema* and *Dyckia distachya* obtained in a spectrophotometer at 490 nm (UV Spectrophotometer SP 2000) by the phenol-sulfuric method (DuBois *et al*., 1956) and determined with the help of an external standard curve of glucose (y x = 0.005923+0.08819, r^2 = 0.9792), values are in µg g^{-1} (Mean±Standard deviation), expressed as glucose equivalents resulting from four replicates. Same letters above the bars indicate no significant differences between the averages and t-test at 5% of probability

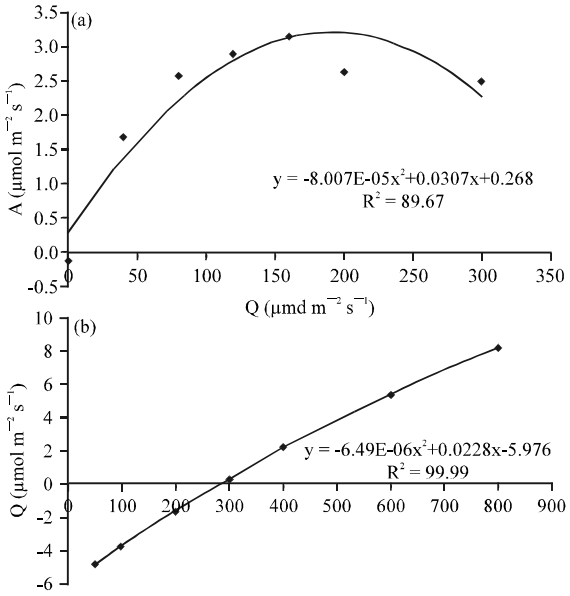

Fig. 6(a-b): Light curve (A) and CO_2 (B) of *Vriesea platynema* obtained in portable processor, IRGA meter portable photosynthesis. Because of the morphology of the species *Dyckia distachya* leaf (leaves very thick), the data obtained by this equipment were not consistent and therefore, will not be shown here

deficiency or excess water. It should be noted also that the carbohydrate content can be affected by various biotic and abiotic factors. Uarrota *et al*. (2011b) in their research in maize landraces related that the diversity of soils, climatic conditions and management practices may affect the carbohydrate content and plant healthy (Hassan *et al*., 2007; Sinha and Srivastava, 2010).

Starch content: The plant uses solar energy, carbon dioxide and water to synthesize starch, an important reserve of chemical energy that the technology explores in the form of a polymer with many functional properties. Due to the biological diversity and environmental, starch content may vary depending on the species (Uarrota, 2011). Starch consists basically of two polymers of α-D-glucose: amylose and amylopectin and has wide applicability not only in the vegetable kingdom, as well as serving in the food industry as ingredients of the basic components of various products. The analysis of starch content in the present study (Fig. 5) was contradictory to the assumptions previously discussed in previous sections. High starch content was observed for specie *Vriesea platynema* (109.59 µg g^{-1}) than *Dyckia distachya* (83.04 µg g^{-1}) with statistically significant differences between these two species ($p<0.05$, t-test). The starch content can be affected by the extraction method used, the content of amylose and amylopectin present in the species being analyzed. It is important to note that photosynthesis may be regulated by several processes and can occur in the leaves with the synthesis of starch, sucrose and amino acids. For better understanding of these factors, it requires an understanding in the broadest sense of the environment surrounding the plant and enzymes involved in metabolic regulation mechanisms (Paul and Pellny, 2003). According to Uarrota *et al*. (2011a), the carbon-nitrogen balance in plants occurs in reverse for the synthesis of carbon as in most cases, this could be explained as changes in the availability of carbon and nitrogen (such as photosynthesis and nitrogen fertilization) or always leads to similar changes in the

levels of carbon-rich metabolites, such as starch but the reverse can occur. Other work may be needed to better understand what may have caused the results on the starch content.

Light curves and rate of CO_2: Plants need carbon dioxide for photosynthesis. Our results of photosynthesis and CO_2 are shown in Fig. 6a and b, respectively for the specie *Vriesea platynema.* Although, it was not possible to better illustrate how the tendency of saturation of light in this specie due to a few points of analysis used, we can see a trend of flow of carbon dioxide of emitted light. It was not possible to obtain light curves and carbon dioxide for *Dyckia distachya* due to the morphology of the leaves are quite thick, measuring forced the equipment (IRGA) or flow chamber to be always open, resulting in inconsistent data. The present results are promising and require further detailed studies to better describe the photosynthetic strategies used by these two species of bromeliads. The trend of decline in carbon dioxide may be affected by several factors. Cornic (2000) comments that when the plant is stressed may occur a decline of carbon dioxide due to the closure of the stomata. Morphological and biochemical aspects together also create pumps of CO_2 around Rubisco which suppresses photorespiration, a phenomenon resulting from the oxygenase activity of Rubisco, causing release of CO_2 and energy loss (Singh *et al*., 2008; Besnard *et al*., 2009). Currently, the evidence is indirect on photosynthesis and is derived from studies of whole photosynthetic chain of leaves and oxygen measurement. However, the closing of the stomata have important role in the decline of photosynthesis.

CONCLUSIONS

The physiological characterization of the two species *Vriesea platynema* and *Dyckia distachya* allowed us to conclude that the two species have differences in the metabolic pathways of photosynthesis especially in relation to carbon assimilation, the presence of soluble carbohydrates, starch, carotenoids, chlorophyll and photosynthesis observed by light curve and carbon dioxide, important physiological parameters in the analysis of the metabolism of these species. The specie *Dyckia distachya* has physiological mechanism more responsive which can be a survival strategy in adverse environment. The findings need further studies to better understand details of the biochemical mechanisms involved in photosynthesis, however, are extremely important as they provide a basis for further studies aimed at better understanding the physiological processes of these two species. The understanding of these physiological parameters may contribute to the design of conservation strategies most appropriate for these species of bromeliads.

ACKNOWLEDGMENTS

This study was conducted under financial support in the form of scholarships, by various programs: CNPq-Brazil-MCT-Mozambique, CNPq and CAPES. The authors would like to thank the Laboratory of Plant Morphogenesis and Biochemistry, Laboratory of Developmental Physiology and Genetics, Laboratory of Seeds and Nucleus of studies in Ecophysiology, where the experiments were carried out. The greenhouse of Agricultural Sciences Center, for providing the plant materials.

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