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UV-B radiation as an elicitor of secondary metabolite production in plants of the genus *Alternanthera*

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ABSTRACT

Ultraviolet B radiation has been described as a potential elicitor agent of the synthesis of secondary metabolites in plants. Thus, the present study aimed to investigate the production of betalains and total flavonoids, as well as the antioxidant activity, of *Alternanthera sessilis*, *A. brasiliana*, *A. tenella* and *A. philoxeroides* exposed to different periods of UV-B radiation (280–315 nm). Plants of these four species were exposed to UV-B radiation for 0, 2, 4, 6, and 8 hours, which amounts to 0, 10, 20, 30, and 40 J cm^{-2} of radiation, respectively. Significant increases in betacyanin and betaxanthin levels were observed in *A. sessilis* and *A. brasiliana* during the period of UV-B exposure, while no differences were observed for the others species. The highest estimated flavonoid levels were for *A. sessilis* exposed to UV-B radiation for 8 h, followed by a 24 h recovery period. In conclusion, the action of UV-B radiation as an inducer of defence responses in plants is influenced by increasing exposure periods followed by a recovery period. Both increase the levels of these composts, yet this increase is different among the four *Alternanthera* species, having a greater influence on the species *A. sessilis* and *A. brasiliana*.

Keywords: abiotic elicitor, antioxidants, flavonoids, medicinal plants, pigments, ultraviolet radiation

Introduction

Even though several chemical substances are synthetically produced nowadays, plants still represent an important source of chemical compounds used in the pharmaceutical industry, adding great economic value. It is estimated that 25% of medicines prescribed are directly or indirectly derived from plants (Zhang & Björn 2009).

The use of both biotic and abiotic elicitors is a strategy used to increase the production of secondary metabolites with commercial application. Among abiotic elicitors, UV-B radiation (290–320 nm) may induce different changes in plant metabolism (Schreiner *et al.* 2009), including the production of secondary metabolites mostly involved in the plant defence system, such as alkaloids and flavonoids (Zhang & Björn 2009; Schreiner *et al.* 2012).

Even though plants are more tolerant to UV-B radiation than other organisms, it causes physiological changes such as increased quantities of some phenolics in plant tissues as well as an improvement in pigmentation levels (Flint *et al.* 2003; Caldwell *et al.* 2007). Studies have reported that, along with their antioxidant activity, flavonoids are ideal compounds for protection from UV radiation (Jansen *et al.* 2008). The first evidence of the role that flavonoids play in protecting against UV radiation appeared with mutants in the biosynthetic route for this compound, resulting in hypersensitive phenotypes to UV radiation (Winkel-Shirley 2002).

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Besides flavonoids, UV-B radiation also influences the levels of betalains, as observed in the plant order Caryophyllales (Ibdah *et al.* 2002; Sharma & Guruprasad 2009); these pigments are metabolites with desirable nutritional and pharmacological characteristics, and the increased production of such compounds is economically interesting. Studies with different cell lines have demonstrated the potential of betalains in the chemoprevention of cancer (Sreekanth *et al.* 2007). In humans, the plasma concentration of betalains after ingestion is sufficiently high to promote their incorporation into low-density lipoprotein (LDL) and red blood cells, protecting them from oxidative damage and haemolysis (Tesoriere *et al.* 2005).

Within Caryophyllales, the family Amaranthaceae stands out. Within this family, there is the genus *Alternanthera*, which comprises approximately 80 species, 30 of which can be found in Brazil. In this genus, four species deserve special attention due to their economic and social importance: *Alternanthera sessilis*, *Alternanthera brasiliana*, *Alternanthera tenella*, and *Alternanthera philoxeroides*. These four species have the ability to store secondary metabolites of great medicinal interest and chemical diversity (Salvador & Dias 2004).

Studies have shown that compounds from the secondary metabolism of the plant order Caryophyllales are changed in the face of UV-B radiation. In a study on *Mesembryanthemum crystallinum*, it was noticed that UV-B radiation increases the levels of flavonoids and betacyanins (Ibdah *et al.* 2002), whereas in *Amaranthus caudatus*, a lack of UV-B results in the inhibition of betacyanin synthesis (Sharma & Guruprasad 2009). However, there are no reports of studies regarding the effect of UV-B radiation on *A. sessilis*, *A. brasiliana*, *A. tenella*, and *A. philoxeroides*.

Taking into account previous studies, the present study aimed to investigate the elicitor effect of UV-B radiation on the production of betalains and total flavonoids, as well as quantifying antioxidant activity in the species *A. sessilis*, *A. brasiliana*, *A. tenella*, and *A. philoxeroides* so that they can be used in further studies as an alternative to obtain plants with increased levels of natural pigments and improved antioxidant capacity.

Materials and methods

Plant material and culture conditions

Althernanthera sessilis, *A. tenella*, *A. philoxeroides*, and *A. brasiliana* plants were previously cultured *in vitro* for 30 days in MS medium (Murashige & Skoog 1962) with no growth regulator in a chamber room kept under a photoperiod of 16 h, photon flux density of 48 μmol m^{-2} s^{-1}, and temperature of 23 ± 2 °C.

Then, plant roots were washed in tap water to remove any trace of medium and subsequently transferred individually to 200 mL plastic pots containing vermiculite as a substrate. The plants were acclimatized for 50 days in a semi-automated greenhouse, with average temperature ranging from 16 to 28 °C, and a photon flux density of about 1,000 μmol m^{-2} s^{-1}. The plants were irrigated every two days with Hoagland nutrient solution (50 %) (Hoagland & Arnon 1950) up to the 50th day.

Afterwards, plants with 10 to 15 pairs of leaves were subjected to UV-B radiation (30 cm distant from plants) for 0, 2, 4, 6, and 8 h, characterizing 0, 10, 20, 30, and 40 J cm^{-2} of radiation, respectively. Thus, the longer the exposure time to radiation, the higher the radiation level to which the plant was subjected. The radiation was measured at the apex of the plants with an Instrutherm ultraviolet radiometer, model MRU-201. Immediately after exposure to UV-B, half of the plants from each treatment were collected, while the other half remained for another 24 h under natural light, to characterize its permanence as a metabolic recovery period. Control plants remained in the greenhouse under natural light throughout this period in the same conditions as reported previously. Right after all collections, plants were stored in an ultrafreezer at –80 °C until subsequent biochemical analyses. The UV-B chamber was equipped with two Philips UVB Broadband TL 40 W/12 RS SLV light bulbs with a peak at 302 nm.

Statistical analysis

The experimental design was completely randomized in a 4 × 5 × 2 factorial pattern, with four species, five periods of exposure to UV-B radiation, and two collection periods (immediately after each period of radiation, and after 24 h following exposure to UV-B radiation – recovery period). Ten plants of each species were used; after exposure to radiation, five were immediately collected, and five remained for another 24 h under natural light. The results were subjected to analysis of variance (ANOVA) and comparison of means by Tukey test at 5 % error probability using the statistical software SAS v.9.3 (SAS Institute Inc., Cary, NC) (SAS 2003).

Quantification of total betacyanins

The extraction of betacyanins (betanidin and betanin) was carried out with two different extraction buffers. For betanidin, we used a mixture of sodium acetate buffer 10 mM and methanol, 70 % and 30 %, respectively, added to sodium ascorbate at a concentration of 10 mM (pH 5.0); meanwhile, for betanin, extraction was performed using a 10 mM potassium phosphate buffer added to 10 mM sodium ascorbate (pH 6.0), with no addition of organic solvent. For both analyses, we used 125 mg of shoot dry matter which had been ground in a mortar; the extract was filtered in gauze and centrifuged at 10,000 *g* for 20 min at 4 °C, as described by Gandía-Herrero *et al.* (2005). The molar extinction coefficients used to calculate betanidin and

betanin were ϵ = 54,000 M^{-1} cm^{-1} and ϵ = 65,000 M^{-1} cm^{-1}, respectively, at a wavelength of 536 nm (Schliemann *et al.* 1999).

Quantification of betaxanthins

For the extraction of betaxanthins, we used 125 mg of shoot dry matter ground in a mortar with 10 mM phosphate buffer, pH 6.0, added to 10 mM sodium ascorbate. The homogenate was subsequently filtered in gauze and centrifuged at 10,000 *g* for 20 min at 4 °C (Gandía-Herrero *et al.* 2005). The betaxanthin concentration was determined by taking into account a coefficient of molar extinction for miraxanthin of ϵ = 48,000 M^{-1} cm^{-1} at 480 nm, and the results were expressed in milligrams of miraxanthin per gram of fresh matter (FM) (Schwartz & Elbe 1980).

Quantification of total flavonoids

The level of flavonoids was also measured by using acetate/methanol buffer as extraction buffer. The results for flavonoids were expressed in µmol of quercetin per gram of FM. The readings were carried out in a spectrophotometer at 330 nm (Gandía-Herrero *et al.* 2005; Reis *et al.* 2015).

Quantification of antioxidant activity

Antioxidant activity was analysed with the 2,2-diphenylpicrylhydrazyl (DPPH) method as described by Brand-Williams *et al.* (1995), which is based on capture of the radical DPPH by antioxidants, decreasing absorbance at 515 nm. In order to assess the free radical scavenging activity, we measured the percentage of DPPH inhibition in relation to the control (acetate/methanol + DPPH 60 µM), by the following equation: % DPPH inhibition = [(A0 – A1)/ A0 × 100], where A0 = control absorbance and A1 = sample absorbance (Molyneux 2004).

Results

The levels of betanidin after exposure to UV-B radiation showed significant interaction between the factors period and species as well as between species and recovery period.

The average level of betanidin differed significantly as the period of exposure to UV-B radiation increased for the species *A. sessilis* and *A. brasiliana*. It was observed that *A. sessilis* had the highest level of this pigment in the control treatment, and after undergoing radiation for 8 h, there was a 39 % increase in this level. For *A. brasiliana*, the levels in the control treatment were lower than those for *A. sessilis*; however, it was noticed that from 2 h of exposure to radiation on, the level increased by 45 % in relation to the control, reaching a 52 % increase after 8 h. No significant differences were found for the species *A. tenella* and *A. philoxeroides* in the face of exposure to UV-B radiation (Fig. 1A).

For interaction between the factors species and recovery period, the species *A. sessilis* showed the highest level of betanidin, corresponding to 27.1 mg betanidin g^{-1} FM shortly after receiving radiation, and 0.317 mg betanidin g^{-1} FM after 24 h of recovery, making up a total increase of 16 %. *A. brasiliana* also showed different levels of betanidin, with an increase of 11 % after the recovery period, which differed from those for *A. tenella* and *A. philoxeroides* whose levels of betanidin did not differ during the recovery period (Fig. 1B).

The ANOVA results for variation in betanin showed a significant difference for the interactions period × species and species × recovery period. The same was not observed for the other possible interactions.

Regarding the interactions period × species, *A. sessilis* showed the highest level of betanins both in control plants and in irradiated plants. This corresponds to 51 % more than estimated in the control. The species *A. brasiliana* showed lower values in comparison to *A. sessilis*, but there was an increase from 2 h of exposure to radiation, reaching up to 24 % more than that found in control plants after 6 h of radiation. *A. tenella* and *A. philoxeroides* plants did not differ significantly according to the period of exposure to UV-B radiation (Fig. 1C).

With respect to the interaction species × recovery period for the betanin level, the species *A. sessilis* and *A. brasiliana* showed the most significant results in relation to this variable, regardless of whether or not there was a recovery period. There was a 14 % increase in the betanin level for *A. sessilis* and a 33 % increase for *A. brasiliana* in the recovery period when compared to plants analysed immediately after radiation. The species *A. tenella* and *A. philoxeroides* had constant values in relation to the control regardless of whether there was a recovery period or not (Fig. 1D).

The effects of UV-B radiation were significant for the levels of betaxanthins, with a significant effect on the interactions period × species, species × recovery period, and period × recovery period.

The species *A. sessilis* exhibited the highest level of these pigments, even when there was no exposure to radiation; in the face of UV-B radiation, the level increased considerably soon after 2 h of exposure, reaching 55 % more than the control after 8 h. For *A. brasiliana*, the period of exposure to radiation (2, 4, 6, and 8 h) also showed significant results in relation to the control. The values estimated for 4 h of exposure are approximately 40% higher than those found in non-irradiated plants. The species *A. tenella* and *A. philoxeroides* showed no differences between treatments when compared to the control (Fig. 2A).

The values obtained when analysing the interaction species × recovery period were more significant for *A. sessilis* which differed significantly from the other species both in the analysis shortly after radiation (0 h) and in that

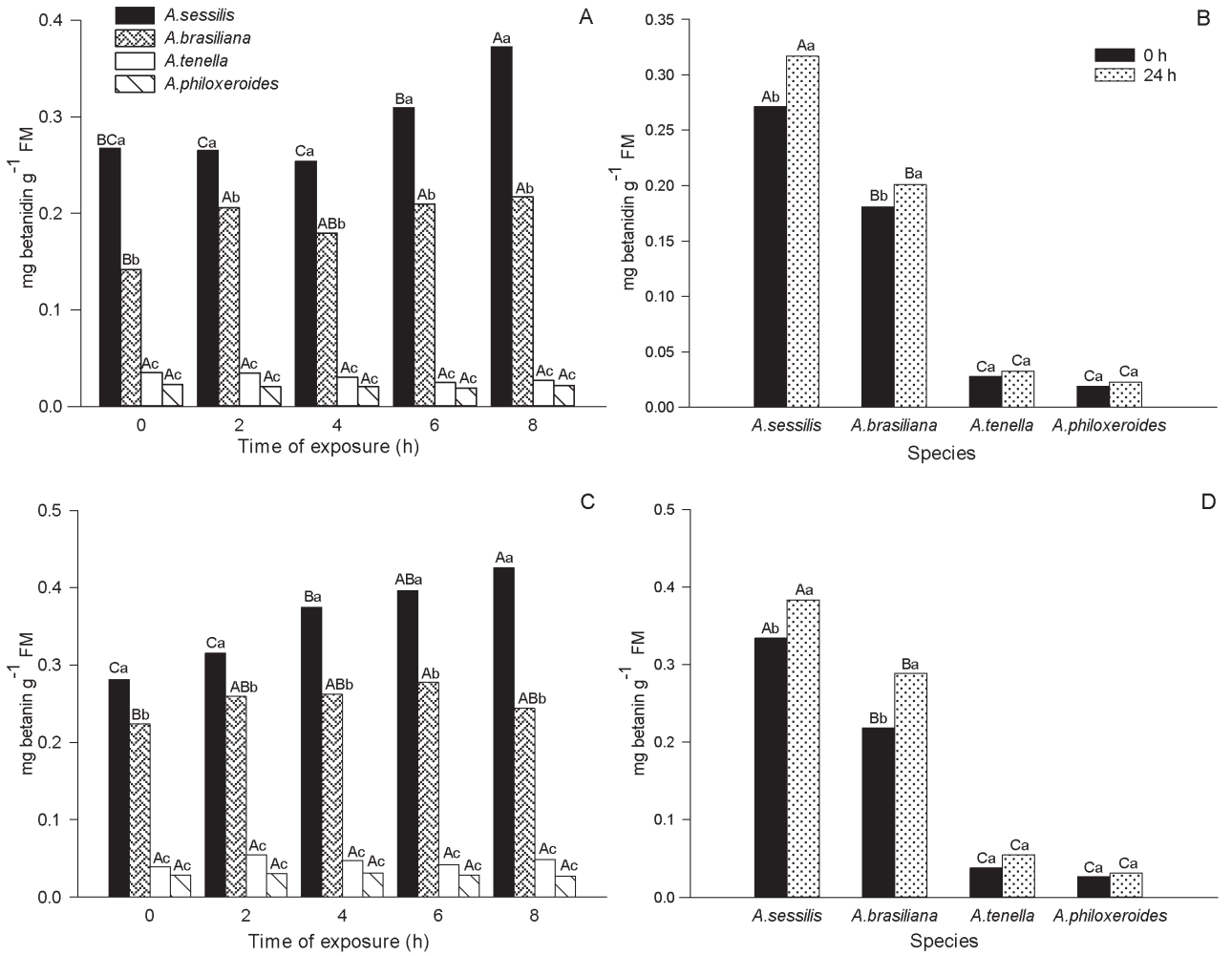

Figure 1. Level of betanidin (**A**) and betanin (**C**) in shoots of four *Alternanthera* species exposed to UV-B radiation for 0, 2, 4, 6, and 8 h and level of betanidin (**B**) and betanin (**D**) in the four species shortly after radiation (0 h) and after a 24-h recovery period. Means followed by distinct upper-case letters differ between them for period of exposure to radiation of each species and lower-case letters for species at each period (**A, C**). Means followed by distinct upper-case letters differ between them for species at the different recovery periods and lower-case letters for recovery within each species (**B, D**), according to Tukey's test ($p<0.05$). FM = fresh mass.

carried out a 24-h recovery period. *A. sessilis* and *A. brasiliana* plants increased production of betaxanthins by 28 % and 31 %, respectively, after 24 h of recovery when compared to those analysed shortly after exposure to radiation (0 h). For plants of the species *A. tenella* and *A. philoxeroides*, there was no significant increase, whether they had a 24-h recovery period or not (0 h) (Fig. 2B).

In analysis of the interaction period × recovery period, an increased level of total betaxanthins, which differed significantly from that in control plants, was observed at 2, 4, 6, and 8 h of exposure. For exposure followed by a recovery period, the increase was more pronounced when compared with the levels for the corresponding exposure time alone, and there was a 26 % increase at 6 h in relation to the control with recovery. For all exposure periods, including the control, there was a greater accumulation of betaxanthins in plants subjected to the recovery period, reaching maximum values of an increase by 36 % at 4 h and by 47 % after 6 h. The significant difference between control plants, non-recovered, and recovered control plants is explained by an increase in this pigment in plants from different species in the recovery period.

Measurement of flavonoids and antioxidant activity

For flavonoids, the interactions period × species, species × recovery period, and period × recovery period showed significant results in the face of UV-B radiation.

Among the species studied, the one which presented the highest level of total flavonoids in control plants was *A. sessilis*. This species had an increased synthesis of flavonoids when exposed to UV-B radiation, beginning at 6 h and reaching 51 % more than the control at 8 h. The species *A. philoxeroides* presented the second highest level of total

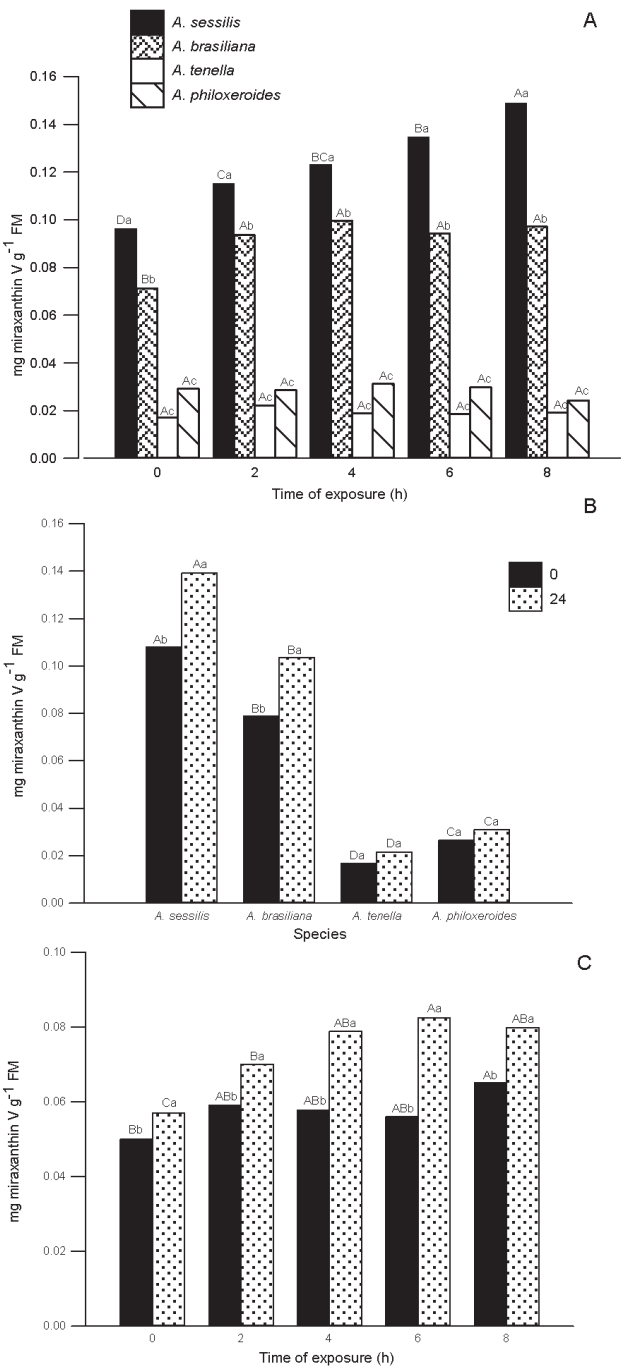

Figure 2. Level of total betaxanthin in shoots of four *Alternanthera* species exposed to UV-B radiation for 0, 2, 4, 6, and 8 h (**A**); Level of total betaxanthin in the different species shortly after radiation (0 h) and after a 24-h recovery period (**B**), and level of total betaxanthins at the different periods of exposure: shortly after radiation (0 h) and after 24-h recovery (**C**). Means followed by distinct upper-case letters differ between them for period of exposure and lower-case letters differ between them for species (**A**); Means followed by distinct upper-case letters differ between them for species and lower-case letters differ between them for recovery period (**B**). Means followed by upper-case letters differ between them for the period of exposure and lower-case letters differ between them for the recovery period (**C**) according to Tukey's test ($p<0.05$). FM = fresh mass.

flavonoids in control plants, and it remained constant throughout all radiation periods. At 8 h, *A. brasiliana* showed a 62 % increase, but this increase differs statistically only from the control. For *A. tenella*, average values of 0.78 µmol of total flavonoids g^{-1} FM were estimated for all periods, and did not differ from the control (Fig. 3A).

The greatest amount of total flavonoids after the recovery period was estimated in *A. sessilis*. The same was observed in *A. tenella* and *A. philoxeroides*, for which the recovery period increased the level of flavonoids by 100 % and 40 %, respectively, compared to plants that had no recovery period. *A. sessilis* had a 20 % increase in total flavonoid level after the recovery period while *A. brasiliana* had no significant increase of this level in comparison to the treatment with no recovery (Fig. 3B).

With respect to period × recovery period, the treatments with 24 h of recovery had a significant increase, and exposure to radiation for 8 h presented the highest level of flavonoids, corresponding to a 63 % increase in relation to the control. Within the different exposure periods, there were significant differences between treatments with and without a recovery period from 2 h on; the greatest difference was observed at 6 h, with 57 % more in plants that had a recovery period in comparison to plants which did not have the same exposure period to UV-B radiation (Fig. 3C).

Regarding antioxidant activity, *A. sessilis* was the species that provided the greatest DPPH inhibition even in control plants (68.78 %). And for this species, as well as for *A. brasiliana* and *A. philoxeroides*, there were no significant differences between exposure periods. For *A. tenella*, a 30 % increase in relation to the control was observed at 4 h of exposure, which did not differ from that for the other periods (Fig. 3D).

Discussion

In the present study, four species of *Alternanthera* (*A. sessilis*, *A. brasiliana*, *A. tenella*, and *A. philoxeroides*) were exposed to UV-B radiation (0, 10, 20, 30, and 40 J cm^{-2}) in order to investigate changes in the betalain (betacyanins and betaxanthins) and total flavonoid content as well as its possible beneficial effect on antioxidant activity after this exposure.

Overall, our results showed a significant alteration in the content of different pigments and total flavonoids, but no significant increase in antioxidant activity (by the method used) in the four *Alternanthera* species subjected to different levels of UV-B radiation.

The species *A. sessilis* and *A. brasiliana* had a significantly increased content of total betacyanins and betaxanthins in relation to the other species studied; this may be related to a possible photo-protective activity that these pigments perform in the face of UV-B radiation in these species. On the other hand, it is known that, regardless of the presence of radiation, the species *A. tenella* and *A. philoxeroides* naturally

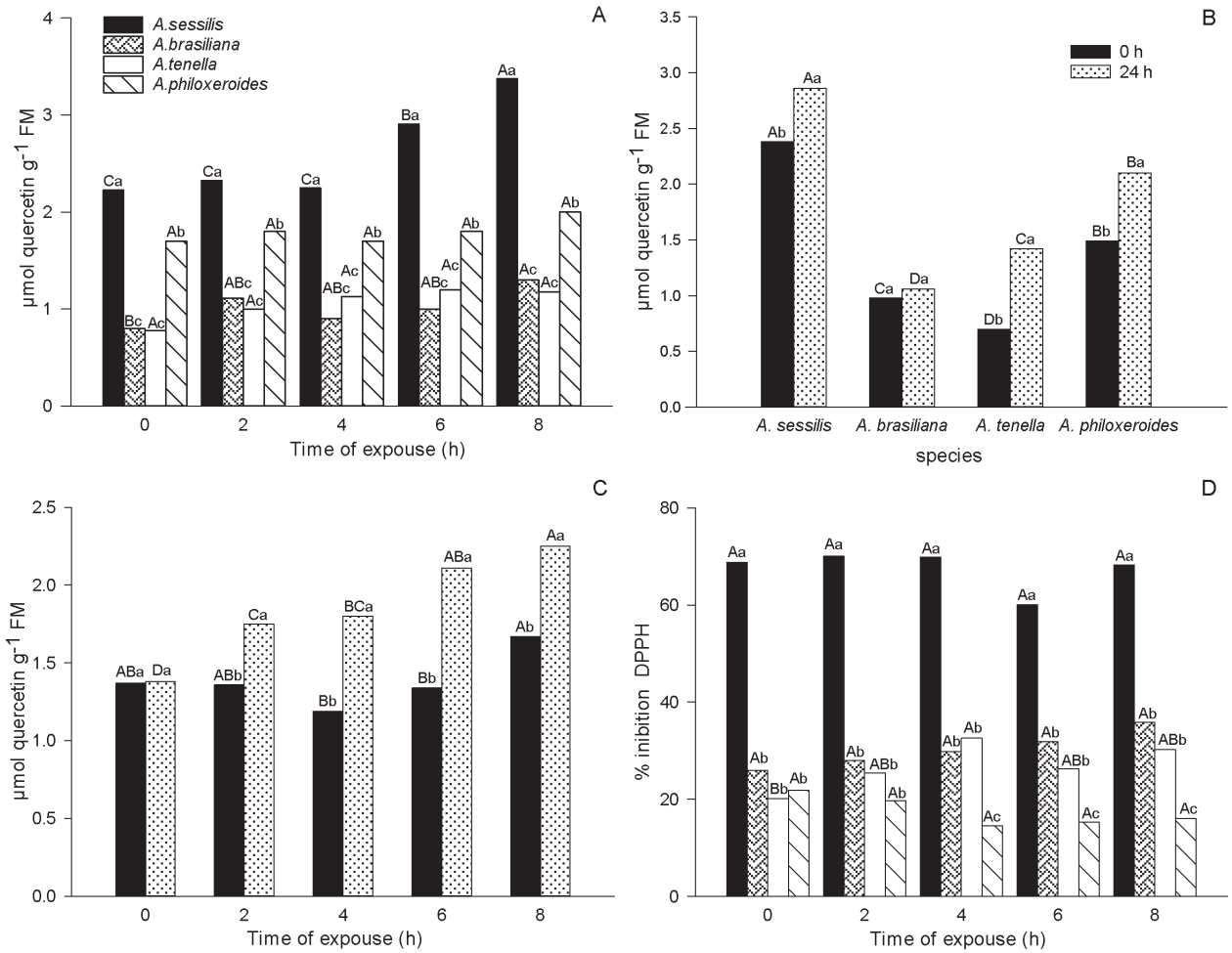

Figure 3. Level of total flavonoids and antioxidant activity of shoots of four *Alternanthera* species exposed to UV-B radiation for 0, 2, 4, 6, and 8 h (**A, D**); level of total flavonoids in four species shortly after UV-B radiation (0 h) and after 24 h of recovery (**B**). Level of total flavonoids at different exposure periods shortly after UV-B radiation (0 h) and after 24-h recovery (**C**). Means followed by different upper-case letters differ between them for period and lower-case letters differ between them for species (**A, D**). Means followed by distinct upper-case letters differ between them for species and lower-case letters differ between them for recovery period (**B**). Means followed by upper-case letters differ between them for exposure period and lower-case letters differ between them for recovery period (**C**), according to Tukey's test ($p<0.05$). FM = fresh mass; DPPH=2.2-diphenyl-1-picryl-hydrazyl.

have less purple colouration in their leaves than *A. sessilis* and *A. brasiliana*, and that different levels of exposure to radiation are not effective in promoting an increase in these pigments.

Studies have reported that UV-B radiation may change the composition of pigments, as plants seek to reduce radiation levels in their tissues (Delgado-Vargas *et al.* 2000); this seems to be species-specific, and the association of pigments with other compounds such as copigments may attenuate the oxidative damage caused by UV-B radiation. So, pigment synthesis in plants has been proven to be a consequence of exogenic stress or senescence and of ecological adaption to changing environments like excess radiation (Delgado-Vargas *et al.* 2000).

In a study realized by Reis *et al.* (2015), the betacyanin levels in *A. sessilis* plants cultured *in vitro* under different light wavelengths (blue, white, and red) were quantified as over 0.4 mg betanidin and betanin g^{-1} FM, with no significant increase among the different wavelengths. In the present study, in analysis of the betanin and betanidin content in control plants of *A. sessilis*, the value estimated was similar when compared to the study with different light wavelengths. Yet, the increase observed in this study during exposure to UV-B radiation, especially in recovered plants, demonstrates that this type of radiation has a positive influence on the production of total betacyanins for the species *A. sessilis* and *A. brasiliana*.

Still, in a study on *Amaranthus caudatus* L., it was also observed that betacyanins are induced by UV-B, and that a lack of this type of radiation results in inhibition of the synthesis of these pigments (Sharma & Guruprasad 2009). In a study on *M. crystallinum* by Ibdah *et al.* (2002), it was

found that the accumulation of total betacyanins under a longer exposure time to radiation starts by the second day of treatment and reaches saturation after five days of treatment. In general, the effect of UV-B radiation on the secondary metabolism of plants is dose-dependent. However, the real dose that is perceived by plant tissues depends on a series of factors, including the morphological structure of the plant organ (Schreiner *et al.* 2012).

Plants are predominantly characterized by the presence of chlorophylls that are crucial for photosynthetic activity; however, pigmentation other than chlorophylls, such as anthocyanins and betalains (betacyanins and betaxanthins), is important to create contrasting hues for the attraction of animals, both for pollination and seed dispersal, and especially for its importance as a food colouring.

There are no reports in the literature on the production of betaxanthins in plants exposed to UV-B radiation, although in our study, UV-B radiation increased the levels of betacyanins after 24 h of recovery; the same was observed for betaxanthin levels at all exposure times. These results encourage us to believe that UV-B radiation is an eliciting agent for the species *A. sessilis* and *A. brasiliana*, and that exposure to it could be an easy way of optimizing the production of these compounds for commercial use of these pigments.

Alternanthera tenella and *A. philoxeroides* presented no differences in the levels of total betacyanins and betaxanthins after radiation, possibly because they triggered other defence mechanisms that have not been studied in this work, or even because they have a tolerance mechanism.

The health benefits of natural pigments have been the focus of many works, especially those on carotenoids and anthocyanins, whose antioxidant properties have been extensively studied. Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments. These findings have helped to motivate utilization of betalains as food colourants. An increase in the production of these compounds is of economic interest because they are chemically stable over a long pH range, wider than that for anthocyanins, in addition to being compared to flavonoids, being potent antioxidants (Pavokovic & Krsnik-Rasol 2011).

As anthocyanins (pigments belonging to the flavonoid class) are absent in the order Caryophyllales, and from the increase in betalain levels observed with an increase in the exposure period to UV-B radiation, it may be inferred that these pigments may play a similar role to anthocyanins, resulting in plants having a 'sunscreen' ability.

An effect of UV-B radiation on the biosynthetic route of flavonoids different to that for betalains is widely reported; UV-B radiation has been strongly related to the regulation and biosynthesis of key enzymes in the biosynthetic route of flavonoids such as phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS). Greenberg *et al.* (1997) have shown that when exposed to UV-B radiation, most plants increase transcriptional levels of these enzymes. Possibly, such a response is related to the important role of flavonoids as photo-protectors; they play a key role in plant UV-B protection, having both antioxidant and UV-screening properties (Agati & Tattini 2010), and therefore contribute to the prevention of UV-B stress and stress-mediated morphogenesis.

Flavonoids are present in betalain-producing species, and functional genes have been identified for the flavonoid biosynthetic enzymes CHS, dihydroflavonol 4-reductase, and anthocyanidin synthase. This suggests that the lack of anthocyanin production in betalain-producing species may be due to a lack of transcriptional activation of all the necessary biosynthetic genes, although a hypothesis based on repressive interaction between anthocyanin and betalain metabolites and biosynthetic enzymes has also been suggested (Shimada *et al.* 2005; Brockington 2011). Anthocyanin and betalain pigments are initially derived from the shikimic acid pathway (anthocyanins from phenylalanine, and betalains from tyrosine) (Tanaka *et al.* 2008).

The present study shows that the flavonoid content in the four species studied is different, and that *A. sessilis* has a higher content than the others; this may be important to focus studies on species that have a greater antioxidant power, due to the potential use of these plants as medicinal products. Still, it may be inferred that the increase in the production of total flavonoids in the species *A. sessilis* may be due to the activation of key enzymes of the route by UV-B radiation.

In this study, exposure periods to UV-B followed by a 24-h recovery period induced a better response in the accumulation of the compounds studied. It is believed that the perception of the elicitor signal initiates a signal transduction network leading to the activation of transcriptional factors as an early response, and subsequent expression of genes involved in the production of secondary metabolites (Zhao *et al.* 2005). The compounds studied in this study are present in plant cell compartments, although, in the presence of the elicitor, plants may have triggered transcription of genes involved in the biosynthesis route of betacyanins; this mechanism might induce an increase in their levels over time which would explain the higher levels in plants after the recovery period.

UV-B radiation has the potential to damage macromolecules, including DNA, to generate reactive oxygen species (ROS) and impair cell processes (Jenkins 2009). In our study, no significant effect of radiation exposure on total antioxidant capacity was found, when measured by DPPH, indicating that free radical elimination activity is not affected by UV-B radiation. Regarding the different species, *A. sessilis* showed the greatest inhibition of DPPH, but it did not differ from that in control plants that were not exposed to radiation. For the other species, no significant

change in the percentage of DPPH inhibition was observed for any of the radiation doses.

It can be assumed that betalains and flavonoids, in addition to their UV-absorbing capacity, may have increased their concentrations, acting as potent antioxidants that quickly neutralize radicals. The antioxidant activity was greater in species that showed increased production of secondary metabolites in this study, allowing us to infer that the increased antioxidant activity may be related to increased synthesis of betalains and flavonoids, since these compounds have high levels of antioxidant activity (Gandía-Herrero *et al.* 2005). Likewise, antioxidant activity is highly correlated with increased levels of UV-B and flavonoids in apple fruits (Hagen *et al.* 2007; Huyskens-Keil *et al.* 2007). However, an increase in antioxidant activity is not only determined by the concentrations of flavonoids and betalains, but also related to induction of enzymes with free radical scavenging properties by UV-B radiation, such as peroxidases (Eichholz *et al.* 2012).

We conclude that the elicitor action of UV-B radiation is influenced by increasing exposure periods followed by a recovery period. Both increase the levels of total betacyanins, betaxanthins, and flavonoids, yet this increase is different among the four *Alternanthera* species, having a greater influence on the species *A. sessilis* and *A. brasiliana*.

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