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ARTICLE

Biochemical activity of plants of the genus *Alternanthera* after UV-C radiation exposure

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ABSTRACT: (Biochemical activity of plants of the genus *Alternanthera* after UV-C radiation exposure). UV-C radiation interferes with the physiological processes of plants. The increased production of secondary metabolites, such as flavonoids and betalains, as well as higher enzymatic activities can explain the behaviour of plants exposed to radiation. The objective of this study was to analyse the possible changes in the metabolism of *Alternanthera sessilis*, *Alternanthera brasiliana* and *Alternanthera philoxeroides* exposed to different periods of UV-C radiation. Plants from *in vitro* cultures were acclimatized in a greenhouse for 60 days prior to exposure to UV-C radiation for 0, 5, 10, 15 and 20 minutes. We evaluated the production of total betanidin, betanin, betaxanthins, flavonoids and the response of antioxidant enzymes as a result of exposure to UV-C radiation. We observed an increase in secondary metabolism compounds in the species *A. sessilis*, with maximum values after 10 and 12 minutes of exposure. The species *A. brasiliana* showed an increased betanin content after 16 minutes of exposure. In terms of superoxide dismutase (SOD), there was no significant difference among the species at different exposure times, while catalase (CAT) activity was significantly higher in *A. philoxeroides*. We also observed a higher activity of ascorbate peroxidase (APX) in *A. philoxeroides* after 20 minutes of exposure to UV-C radiation. Malondialdehyde (MDA) contents increased in *A. philoxeroides* and *A. sessilis* as a response to UV-C radiation. The different species used different mechanisms as protection against UV-C radiation.
Keywords: abiotic stress, betalains, protection mechanisms, oxidative stress.

RESUMO: (Atividade bioquímica em espécies do gênero *Alternanthera* quando expostas a radiação UV-C)**.** A radiação UV-C interfere em processos fisiológicos das plantas. O incremento de metabólitos secundários como flavonoides e betalaínas, bem como atividades enzimáticas, podem esclarecer o comportamento de plantas diante da radiação. O objetivo deste estudo foi analisar as possíveis alterações no metabolismo de plantas de *Alternanthera sessilis*, *Alternanthera brasiliana* e *Alternanthera philoxeroides*, quando expostas a diferentes tempos de exposição à radiação UV-C. Plantas provenientes do cultivo *in vitro* foram aclimatizadas em casa de vegetação durante 60 dias e posteriormente foram expostas durante 0, 5, 10, 15 e 20 minutos à radiação UV-C. Análises bioquímicas da produção de betanidina, betanina, betaxantinas, flavonoides totais e a resposta de enzimas antioxidantes em decorrência da exposição à radiação UV-C, foram realizadas ao final deste estudo. Os resultados mostraram que houve incremento destes compostos na espécie *A. sessilis*, e os valores máximos foram alcançados após 10 e 12 minutos de exposição. A espécie *A. brasiliana* apresentou aumento do conteúdo de betanina após 16 minutos de exposição. Para a atividade da enzima superóxido dismutase (SOD), não houve diferença significativa entre as espécies nos diferentes tempos de exposição e para a catalase (CAT), a espécie *A. philoxeroides* mostrou maior atividade, diferindo significativamente das demais. Foi observado também para essa espécie maior atividade da ascorbato peroxidase (APX) aos 20 minutos de exposição à radiação UV-C. O conteúdo de manolildialdeído (MDA) aumentou em *A. philoxeroides* e *A. sessilis* diante da radiação UV-C. Conclui-se que as espécies estudadas acionam mecanismos de proteção diferentes diante da radiação UV-C.
Palavras chave: estresse abiótico, betalaínas, mecanismos de defesa, estresse oxidativo.

INTRODUCTION

Ultraviolet radiation (UV) is a small part of the solar radiation reaching the Earth's surface, but with a significant impact on living organisms, including plants. According to the International Commission on Illumination, this wavelength region is divided into UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm) (Katerova *et al*. 2009).

The negative effect of UV radiation is greater the shorter the wavelength is; therefore, due to its greater energy, UV-C radiation rapidly causes high levels of lesions and is more damaging to living organisms (Hollósy 2002, Katerova *et al.* 2012). On the other hand, low doses of UV-C can trigger acclimatisation responses in plants, including the activation of enzymatic and non-enzymatic defence systems (Jansen *et al*. 2008, Lavola *et al*. 2003, Katerova *et al*. 2009, Katerova & Todorova 2009, Katerova & Todorova 2011, Rai *et al*. 2011).

In plants, a common response to unfavourable environmental conditions is the occurrence of oxidative stress, with increased levels of reactive oxygen species (ROS) (Mewis *et al*. 2012). Thus, plants can activate antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), thereby mitigating the UV-C induced damage (Rai *et al*. 2011). Another

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defence mechanism responsible for a decrease of free radicals is the production of photo-protective pigments such as the phenol compounds flavonoids and betalains (Solovcheco & Merzylak 2008). Flavonoids are a group with a wide range of biological functions, including roles in stress protection.

Betalains are water-soluble nitrogen pigments and, based on their structure, can be divided into two major groups: betacyanins (red-violet) and betaxanthins (yellow) (Cai *et al*. 2005). Betacyanins can be further classified by their chemical structure into, e.g., glycosylated (betanin) and aglycones (betanidins) (Gandia-Herrero *et al*. 2010).

The genus *Alternanthera* Forssk. (Amaranthaceae), composed of 80 species of plants, 30 of them being found in Brazilian territory, is characterised by the presence of flavonoids and betalains, pigments suggested to replace anthocyanins in species of the order Caryophyllales (Brochado *et al*. 2003, Salvador & Dias, 2004, Salvador *et al*. 2006, Brockington *et al*. 2011). Among these species, we highlight *Alternanthera sessilis* (L.) R. Br. Ex DC., *Alternanthera brasiliana* (L.) Kuntze and *Alternanthera philoxeroides* (Mart.). Griseb. because they are native to Brazil and due their medicinal properties which have already been described (Preetha *et al*. 2018).

Alternanthera sessilis, popularly known in Brazil as violet or rubra, has chemical components such as β-sitosterol and stigmasterol. In addition, it has been used as an antidote to snake and scorpion stings (Jalalpure *et al*. 2008) and has antioxidant properties (Shymala *et al*. 2005).

Alternanthera brasiliana is the most widely used species in Brazil and is commonly known as penicillin, terramycin, doril or carrapichin (Macedo *et al*. 1999). It is widely found in the states of Santa Catarina and Rio Grande do Sul (Pereira 2007) (Lorenzi & Matos 2008); its inflorescences are used for headaches, colds and flu, the leaves as antipyretic and the roots against diarrhoea (Agra *et al*. 2007).

Alternanthera philoxeroides, popularly known as alligator weed, is used as anti-inflammatory and diuretic (Rattanathongkom *et al*. 2009). It is composed of glycosylated flavonoids, saponins and betalains, showing antitumor and antiviral action against HSV (1 and 2), cytomegalovirus, measles virus, MUMPS virus and HIV (human immunodeficiency viruses) (Fang *et al*. 2007; 2009, Rattanathongkom *et al*. 2009).

Previous studies have demonstrated that exposure of species of the genus *Althernanthera* to ultraviolet radiation (Rudat & Goring 1995, Silva *et al*. 2005, Sharma & Guruprasad 2009) interferes with the production of photo-protective pigments such as betacyanins and flavonoids. Although studies have reported the effect of UV-C radiation on the secondary metabolism of some plants (e.g. Rai *et al*. 2011), UV-C radiation has not been studied in the medicinal plants *A. sessilis, A. brasiliana* and *A. philoxeroides*.

In this context, we hypothesised that the production of protective compounds and the onset of a secondary metabolism are encouraged when plants of the genus *Alternanthera* are exposed to UV-C radiation, resulting in increased levels of flavonoids, betacyanins and betaxanthines as well as activation of antioxidants. The objective of this study was therefore to evaluate the influence of UV-C radiation on the production of secondary metabolites and the activity of antioxidant enzymes of three medicinal species of the genus *Alternanthera*: *A. sessilis, A. brasiliana* and *A. philoxeroides*.

MATERIAL AND METHODS

Plant material and growing conditions

Individuals of *A. sessilis, A. brasiliana and A. philoxeroides*, established for 30 days *in vitro* on MS medium (Murashige & Skoog 1962) without growth regulator in the Bank of Plants of the Plant Tissue Culture Laboratory at Federal University of Pelotas (UFPEL), were acclimatised for 60 days in a greenhouse. For this purpose, we removed the plants from the culture medium and rinsed their roots under running water to remove any traces of medium; subsequently, the plants were transferred individually to plastic cups containing vermiculite as substrate. Watering was carried out every 2 days with 50% Hogland nutrient solution (Hoagland & Arnon 1938) until day 60. After this period, plants with 10 to 15 pairs of leaves were subjected to UV-C radiation for 0 (no exposure-control), 5, 10, 15 and 20 minutes. For this, a camera with Philips TUV 30 W-C / Holland G30T8 254 nm UV-C lamps, placed at a distance of 35 cm from the plants, corresponding to a radiation of 1.7, 3.5, 5.2 and 6.9 kJ m^{-2} day^{-1}, respectively, was used. Radiation was measured with a radiometer (Instrutherm LTDA, MRUR-203 model) in the upper part of the tree canopy.

Experimental design

The experimental design was completely randomized with a 3 x 5 factorial scheme, with three species and five exposure times to UV-C radiation. Each treatment consisted of five replications, with each replication representing one plant, resulting in a total of 75 plants. For enzyme activity and lipid peroxidation, we used a 3 x 2 factorial scheme, with three species and two times of exposure to UV-C radiation (0 and 20 minutes). Each treatment consisted of five replications, with each replication representing one plant, totalling 30 plants. The collected material was stored in an ultrafreezer at -80° C for further biochemical analyses.

The results for total betacyanins, betaxanthins and flavonoids were subjected to analysis of variance and polynomial regression, using the statistical software package Winstat. The maximum point of each curve was calculated by the coefficients of second order equation (-b/2a). The data for enzymes and lipid peroxidation were subjected to analysis of variance and means were compared by Tukey´s test at 5% error probability, using Winstat (Machado & Conceição 2002).

Biochemical analysis

Quantification of total betacyanins

Extraction of total betacyanins was performed with the use of different buffers; potassium phosphate buffer (pH 6.0) was used for the extraction of betanin and betaxanthins, while acetate/methanol buffer (pH 5.0) was applied to stabilize bethanidine (Gandia-Herrero *et al*. 2005, 2007, 2010).

For bethanidine extraction, we used 10 mM acetate buffer and methanol (70/30%), to which we added sodium ascorbate at a concentration of 10 mM. For betanin extraction, we used 10 mM phosphate buffer, pH 6.0, added to 10 mM sodium ascorbate, without the addition of organic solvents. For both analyses, 125 mg of fresh shoot mass were used, previously macerated in a mortar, filtered through cheesecloth, and centrifuged at 10,000 g for 20 min at 4 °C, as described by Gandia-Herrero *et al*. (2007). The molar extinction coefficient used for the calculation of bethanidine and betanin was ϵ = 54,000 and 65,000 $M^{-1}cm^{-1}$, as reported by Schwartz and von Elbe (1980), at a wavelength of 536 nm. Readings were performed in a UV/VIS spectrophotometer model Ultrospec 7000 mark Ge Healthcare.

Quantification of betaxanthins

The concentration of betaxanthin was determined taking into account the molar extinction coefficient for miraxantin of ϵ = 48,000 $M^{-1}cm^{-1}$ at a wavelength of 480 nm, and the result was expressed in mg miraxantin 100 g^{-1} MF (Schliemann *et al*. 1999).

Quantification of flavonoids

The flavonoid content was also determined by using acetate/methanol buffer as solvent extractor; flavonoid concentrations were expressed in µmol of quercetin per gram of fresh mass. Readings were performed in a spectrophotometer, as described previously, at 330 nm (Gandia-Herrero *et al*. 2007, Reis 2013).

Enzymatic activity

To assess the activity of the antioxidant enzymes superoxide dismutase (SOD EC1.15.1.1), ascorbate peroxidase (APX EC1.11.1.11) and catalase (CAT EC1.11.1.6), approximately 200 mg of fresh shoot mass were homogenised in 2.5 mL of extraction buffer containing 100 mM potassium phosphate (pH 7.0), 0.1 mM EDTA and 10 mM ascorbic acid. The homogenate was centrifuged at 13,000 g for 10 min at 4 °C, and the supernatant was collected to determine enzyme activities.

The SOD activity was evaluated by the enzyme's ability to inhibit the photoreduction of nitro blue tetrazolium (NBT), according to Giannopolitis & Ries (1977), in a reaction medium containing 50 mM potassium phosphate (pH 7.8), methionine 14 mM EDTA, 0.1 µM, 75 µM NBT and 2 µM riboflavin. The tubes with the reaction medium and the sample (100 µL for *A. sessilis* and 50 µL for the other species), with a final volume of 2 mL, were placed for 7 minutes into a box fitted with a 20 W fluorescent lamp. For the control, the same reaction medium, but without the sample, was lighted, and as blank, a tube with the reaction medium kept in the dark was used; this tube was also used to reset the spectrophotometer. Readings were taken at 560 nm. One unit of SOD was regarded as the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under the assay conditions.

The CAT activity was determined as described by Azevedo *et al*. (1998) with modifications, where the author describes that the activity was monitored for 2 minutes. In this work, the activity was monitored via the decrease in absorbance at 240 nm for 1.5 minutes in the reaction medium (extract volume was 100 µL for *A. sessilis* and of 50 µL for the other species, amounting to a final volume of 4 mL) incubated at 28 °C, containing 100 mM potassium phosphate buffer (pH 7.0) and 12.5 mM of H_2O_2.

The APX activity was measured according to Nakano & Asada (1981) by monitoring the oxidation rate of ascorbate at 290 nm. The incubation buffer consisted of 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbic acid and 0.1 mM H_2O_2 (volume of the extract of 100 µL for *A. sessilis* and 50 µL and for the other species, amounting to a final volume of 4 mL).

Determination of malondialdehyde (MDA) content

Lipid peroxidation was determined from protocol described by Velikova *et al*. (2000) using thiobarbituric acid (TBA), determining MDA as a final product of lipid peroxidation. Approximately 300 mg of fresh shoot mass were homogenised in 2.5 mL of 0.1% trichloroacetic acid (TCA) (w/v) and the solution was centrifuged at 12,000 g for 15 minutes at 4 °C. For the species *A. sessilis*, a reaction medium was prepared using 200 µL of the supernatant added of 300 µL of 0.1% TCA and 1 mL of a solution containing 0.5% (w/v) of thiobarbituric acid (TBA) and 10% (w/v) of TCA. For the other species, in addition to 1 mL of 0.5% (w / v) of thiobarbituric acid (TBA) and 10% (w/v) TCA, 400 µL of supernatant were used by adding 100 µL of 0.1% TCA, resulting in a reaction mixture of 1.5 mL.

After the preparation of the reaction mixtures, the samples were incubated in a water bath at 90 °C for 20 minutes. The reaction was stopped by rapid cooling on ice, and the readings were performed in a spectrophotometer at 535 and 600 nm. Lipid peroxidation was expressed in mmol of malonaldehyde (MDA) per gram of fresh mass, and the molar extinction coefficient was used to calculate 155 $mM^{-1}cm^{-1}$.

RESULTS AND DISCUSSIONS

Quantification of bethanidine, betanin, betaxanthins and total flavonoids

The values obtained for the variables bethanidine, betanin, betaxanthin and total flavonoids and for the three

species under study when subjected to different exposure times to UV-C are depicted in Figure 1. Bethanidine contents were greater for the species *A. sessilis* when compared to the other species, and a reaction time of 9.8 minutes resulted in the maximum value. In this time range, a 42% increase was observed in relation to the control (time zero), amounting to 29.4 mg of bethanidine per 100 g fresh mass. For *A. brasiliana* and *A. philoxeroides*, different radiation exposure times did not result in significant differences between betanidine contents. The highest values found for those species were 19.6 mg and 2.7 (20 and 15 min) of bethanidine per 100 g fresh mass, respectively (Fig. 1A).

Betanin levels showed a significant interaction between radiation exposure time and species (Fig. 1B). Among the species studied, *A. sessilis* presented the highest betanin content (25 mg of betanin per 100 g fresh weight), estimated at 12 minutes of exposure to UV-C radiation, an increase which corresponds to 61% relative to the control. When compared with the control, for *A. brasiliana*, we observed an increase of 44% in the production of betanin, estimated at 14 minutes of exposure, reaching a maximum value of 23 mg of betanin per 100 g fresh mass, whereas in the species *A. philoxeroides*, there was no significant increase in relation to the exposure time, with an estimated maximum value of 2.6 mg of betanin per 100 g fresh mass.

In the present study, the betaxanthine content (Fig. 1C) did not change with increasing radiation times for the species *A. brasiliana* and *A. philoxeroides*, with average contents of 6.0 to 2.0 mg of betaxanthins per 100 g of fresh mass, respectively. In *A. sessilis,* betaxanthin levels increased gradually with increasing radiation, with 10.7 mg of betaxanthins per 100 g of fresh mass evaluated after 12 minutes, corresponding to an increase of 72% relative to the control.

The oxidative burst caused by UV-C exposure must be counteracted by antioxidants and protective pigments, such as flavonoids, carotenoids and nitrogen molecules, to prevent cell damage (Surjadinata *et al*. 2017). On the other hand, the species type and the nature of the radiation are factors that influence the quantity and quality of the formed compounds (Carletti *et al*. 2003) and the way they protect the plant. In this work, species of the same genus synthesized different compounds as a response to UV-C radiation.

According to our results, the species *A. sessilis* increased the contents of total betacyanins (bethanidine and betanin) and betaxanthins in response to UV-C radiation with different exposure times. Similar results have also been reported by other authors who worked with the species *Mesembryanthemum crystallinum* L.*, Chenopodium quinoa* Willd and *Amaranthus caudatus* L. (Ibdah *et al*. 2002, Htlal *et al*. 2004, Sharma & Guruprasad 2009), all belonging to the same family of the genus *Alternanthera.*

The species *A. philoxeroides* showed the lowest amounts of bethanidine, betanin and betaxanthins after radiation. Nevertheless, against the background of other elicitors such as tyrosine, the increase in total betacyanin content was proportional to the highest concentration of tyrosine in *A. philoxeroides* cultivated *in vitro* for 40 days, with a total betacyanin content in leaves higher than that found in this study (15.32 mg per 100 grams of fresh mass) (Kleinowski *et al*. 2013). In general, the effect of radiation on secondary metabolites is dependent on the dose applied, and the actual dose perceived by plant tissues depends on a number of factors, such as the morphological structure of the plant as well as genetic and biochemical aspects (Schreiner et al. 2009, Schreiner et al. 2012). In addition, it has been documented that plants respond differently to UV exposure times (Frohnmeyer & Staiger 2003).

Although it has been reported that UVC radiation induces the synthesis of these compounds from secondary metabolism, the levels of betacyanins presented in this study were relatively low compared to data from other species of the same genus, such as in Perotti *et al*. (2009), who studied *A. philoxeroides*, elicited with copper sulphate, and obtained values close to 40 mg amaranthine 100 g^{-1} MF. The response to a particular elicitor can vary between species, and it is therefore crucial to determine appropriate doses and times to optimize the production (Namdeo 2007).

The efficacy of an elicitor under in vitro cultivation conditions as a tool to increase the synthesis of natural products in plants is subject to a complex network of biochemical and physiological interactions between the elicitor used and the secondary metabolism of the plant. It is important to emphasise that the way the elicitor acts on plants, influencing or not influencing the synthesis of molecules of the secondary metabolism, depends on the species, stage of development and the plant organ (Vasconsuelo & Boland 2007).

The UV-C light produces photons that may be absorbed by organic molecules such as betalains, which possess conjugated bonds and aromatic rings responsible for the colour (Woo *et al*. 2011). In the presence of UV-C light, such pigments may be degraded, which could explain the reduction in the content of these pigments after an exposure period of 20 minutes in the species *A. sessilis*. Guerrero-Beltrán *et al*. (2009) reported that treatment with UV-C light in fruits caused discoloration of the pigments.

Besides the action of these pigments in UV radiation absorption, previous studies have also demonstrated the ability of betalains to eliminate free radicals by acting as antioxidant compounds (Kanner 2001, Cai *et al*. 2003). The relationship between the structure and the chemical activity of betalains is such that the free radical scavenging action generally increases with the number of hydroxyl groups and the glycosylation of aglycone and betalain molecules (bethanidine).

The C-5 position of the hydroxyl groups in betalain molecules significantly improves the antioxidant activity (Cai *et al*. 2005). In this sense, it can be assumed that the different species can modify the structure of the

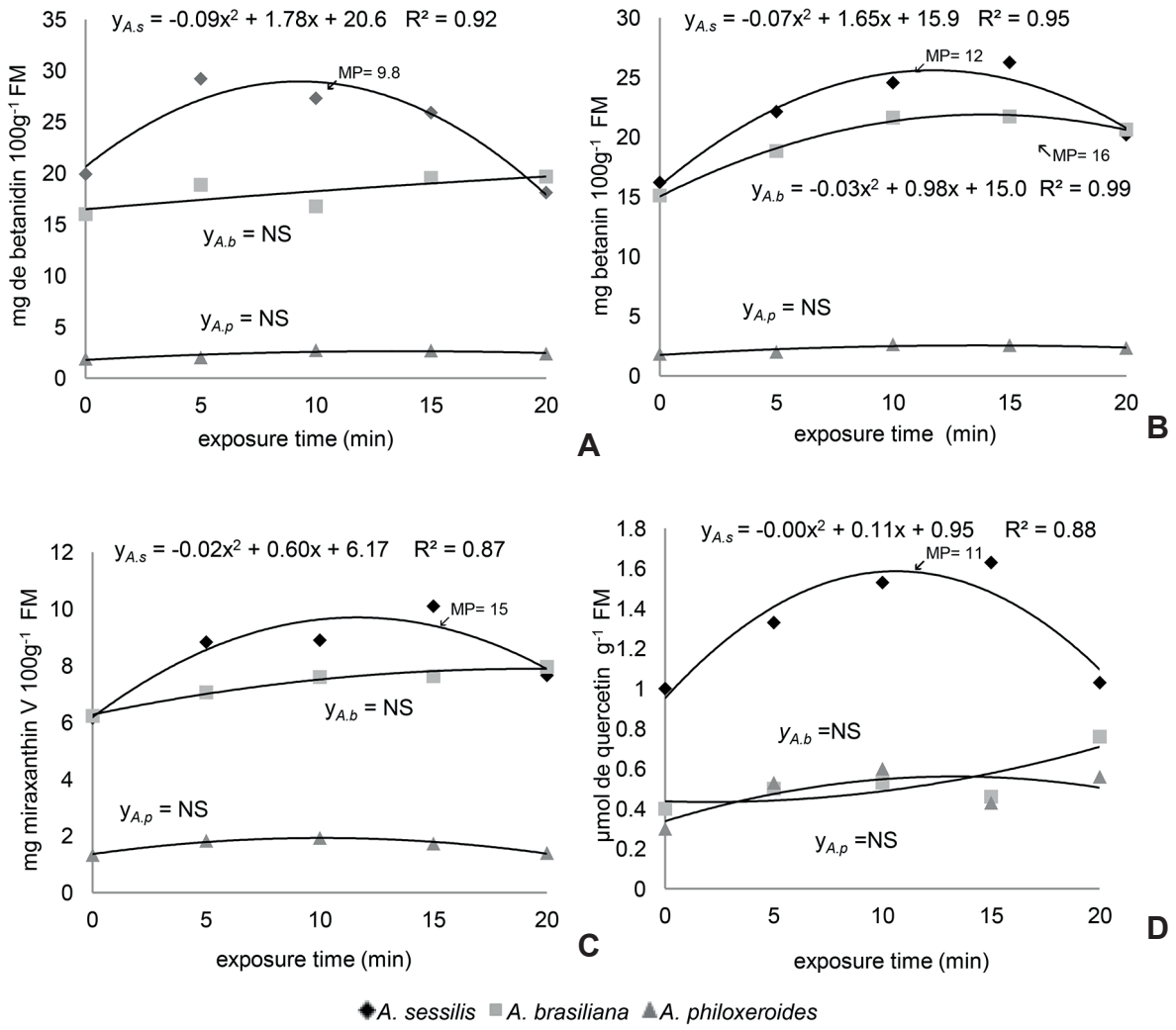

Figure 1. Contents of betanidin (**A**), betanin (**B**) betaxanthin (**C**) and total flavonoids (**D**) quantified in the shoots of three species of the genus *Alternanthera* exposed to UV-C radiation for 0, 5, 10, 15 and 20 minutes. Arrows indicate the maximum point relative to each curve; NS means that the straight-line equation was not significant at the level of 5% of error probability. MF = fresh mass.

compounds as a result of exposure to UV-C radiation, which would justify the estimated results for betanin (glycosylated betacyanin), which were higher in both *A. sessilis* and *A. brasiliana*, indicating that the plants have stronger defence mechanisms.

The average values of total flavonoids in UV-C-treated plants indicate a significant interaction between exposure time and species (Fig. 1D). Higher amounts of total flavonoids were found in *A. sessilis* after 11 minutes of exposure, with 1.56 µM per gram of fresh mass, corresponding to an increase of 79% relative to the control. The species *A. philoxeroides* and *A. brasiliana* presented maximum contents between 0.7 and 0.6 µmol of total flavonoids per gram of fresh mass, respectively, although these differences were not significant.

The highest levels of metabolites were observed for radiation times between 10 and 12 minutes (3.8 and 4.1 Kj m^{-2} day^{-1}, respectively) for the species *A. sessilis*. Comparing the contents of bethanidine, betanin, betaxanthins and total flavonoids in the species *A. sessilis* in relation to *A. brasiliana*, increases of 50, 8.7, 78, and 122% were observed, respectively. In *A. sessilis,* we observed a slight reduction in the contents of these metabolites after 12 minutes of exposure to radiation.

Phenolic compounds are excellent oxygen radical scavengers because the electron reduction potential of phenolics is lower than that of oxygen radicals; in fact, phenoxyl radicals are generally less reactive than oxygen radicals (Grace 2005). Among the phenolic compounds with antioxidant properties, flavonoids (Apel & Hirt 2004) stand out, and the characteristic absorption in the UV region has been considered as evidence for their role in UV protection (Winkel-Shirley 2002).

There is evidence that in leaves of wild-type Mitchell *Petunia* [*Petunia axillaris* x (*P. axillaris* x *P. hybrida*] and *Arabidopsis*, UV-C light induces the synthesis of flavonoids with high levels of hydroxylation. Hydroxylation does not affect the UV absorption properties of these compounds, but does affect the antioxidant capacity in the response to UV stress (Ryan *et al*. 2002). In our study, *A. sessilis* may have activated a defence mechanism in response to radiation, increasing the non-photosynthetic

pigments. These compounds can act by scavenging singlet oxygen produced by the oxidation of photons.

Certain amounts of total flavonoids and betalains were present in control plants of *A. sessilis*; however, prior to UV radiation, the biosynthesis of these compounds was increased, enhancing the elimination of ROS (reactive oxygen species) and the shielding of UV radiation (Jansen *et al*. 2008).

Previous studies have shown that photo-protective pigments possess a high photostability (Merzlyak & Solovchenko 2002, Wang *et al*. 2003). Therefore, a "photo-protective" screen could be formed in plants with a minimum expenditure of energy, enabling long-term natural protection (Pietrini *et al*. 2002). Plants with a good efficiency of this mechanism are less affected by environmental stresses (such as UV radiation), reducing the need for the enzyme systems to provide an adequate level of protection (Hoch *et al*. 2003). The results of this experiment showed that the species *A. brasiliana* and *A. sessilis* possess increased amounts of pigments and less enzymatic activity, which leads us to presume that betacyanins, betaxanthins and flavonoids could be acting as a natural defence barrier in these species.

Activity of the enzymes SOD, CAT, APX and lipid peroxidation

In terms of superoxide dismutase enzyme activity (SOD), there was no significant interaction among the tested sources of variation. The highest values of the activity of SOD were found in *A. brasiliana* (94.86 mg^{-1} Prot. at time zero and 93.08 mg^{-1} Prot. after 20 minutes), which did not differ statistically from those found for the other species (Fig. 2A).

The CAT enzyme activity in the species *A. philoxeroides* was higher than in the other species, irrespective of radiation, i.e. 190 and 297% higher than that for *A. sessilis* and *A. brasiliana*, respectively. When compared to the control, an exposure period of 20 minutes resulted in no significant differences between the species (Fig. 2B).

In terms of ascorbate peroxidase enzyme activity (APX), a significant interaction among the studied factors was found, with *A. philoxeroides* showing the highest activity (25.8 μmol ASA min^{-1} mg^{-1} Prot.) after 20 minutes of radiation exposure. This value significantly differed from those found for *A. sessilis* (10.13 μmol ASA min^{-1} mg^{-1} Prot.) and *A. brasiliana* (13.6 μmol ASA min^{-1} mg^{-1} Prot.) after 20 minutes, corresponding to increases by 154 and 89%, respectively (Fig. 2C).

In the present study, except for the species *A. brasiliana*, cell membrane damage due to UV-C radiation was evident by the increase in cellular MDA, which is a by-product of the peroxidation process. The MDA difference among the species was significant, with *A. philoxeroides* showing the highest value (27.7 mmol g^{-1} MF), followed by *A. sessilis* (13.96 mmol g^{-1} MF) and *A. brasiliana* (2.4 mmol g^{-1} MF) after 20 minutes of radiation. The highest value for *A. philoxeroides* corresponded to an increase by 98% compared to *A. sessilis* and *A. brasiliana.*

When comparing the effect of radiation after an exposure time of 20 minutes to the control without radiation, we observed a significant increase in cellular MDA by 57% in *A. philoxeroides* and 72% in *A. sessilis*, while for *A. brasiliana*, no differences between irradiated and non-irradiated plants were found (Fig. 2D).

Superoxide dismutase (SOD) is an enzyme that scavenges superoxide radicals in O_2 and H_2O_2, reducing cell damage; this represents an essential defence against the potent toxicity of oxygen (Chakraborty *et al*. 2009). Previous studies have shown that SOD activity upon exposure to UV-C radiation did not increase over 2 days of exposure in *Brassica oleracea* L. var. *alboglabra* (Chairat *et al*. 2013).

In the present study, SOD activity did not increase in response to radiation, which may be associated to the non-increase of the substrate of the enzyme during UV-C radiation. Furthermore, the flavonoids present in these plants also eliminate superoxide radicals (Apel & Hirt 2004).

Catalase (CAT) is a common enzyme living organisms and catalyses the decomposition of hydrogen peroxide into water and oxygen (Chakraborty *et al*. 2009). The fact that this enzyme has a constitutive feature, i.e. is present even in plants that are not subjected to stress, explains its greater activity in control plants of *A. philoxeroides*, possibly representing a natural defence mechanism. This leads us to infer that natural protection against oxidative stress in *A. sessilis and A. brasiliana* is provided by photo-protective pigments, while in *A. philoxeroides*, this protection is ensured via enzymatic activity.

An important factor in antioxidant activity is the affinity of the enzymes for the substrate. While CAT has a low affinity for H_2O_2, being active only at high concentrations, i.e. when the stress is more severe, APX and peroxidases, which also eliminate H_2O_2, have a high affinity, eliminating H_2O_2 at low concentrations. Additionally, CAT acts in peroxisomes, whereas APX basically acts in chloroplasts and cell walls (Gechev *et al*. 2006, Jaleel *et al*. 2009).

Ascorbate peroxidase (APX) is a key enzyme of the antioxidant metabolism and catalyses the decomposition of hydrogen peroxide (H_2O_2) into water, using ascorbate as an electron donor; H_2O_2 is a reactive oxygen species (ROS) produced by aerobic metabolism and under biotic or abiotic stress conditions (Ribeiro 2012).

A greater expression of APX in plants has been demonstrated under different stress conditions (Thind & Goyal 2012). Barka (2001) found that UV-C radiation at the dose of 3.7 kJ m^{-2} in tomato fruits increased ascorbate peroxidase activity, while the catalase activity remained unchanged. In a study with tobacco calluses (*Nicotiana tabacum L.*), Zacchini & Agazio (2004) reported an increased APX activity at 24 h after UV-C irradiation, but this was not the case for CAT activity. These results are in agreement with those found in our study for the species *A. philoxeroides*.

The increase in the overall activity of APX, observed in this study, reinforces the importance of this enzyme to protect the plant against damage caused by reactive

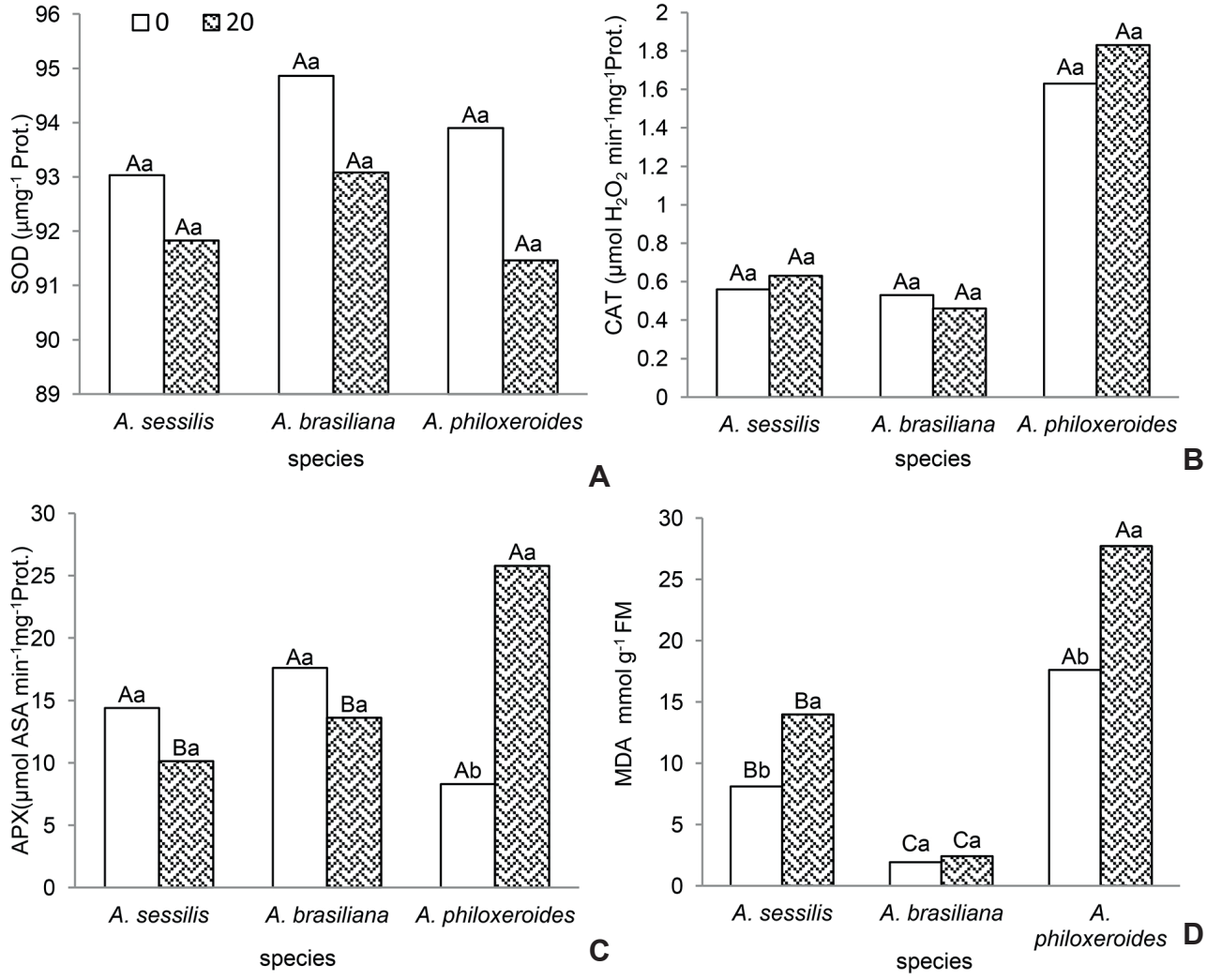

Figure 2. Specific activities of the enzymes SOD (**A**), CAT (**B**) and APX (**C**) and malondialdehyde content (MDA), (**D**) of three species of the genus *Alternanthera* exposed to UV-C radiation (0 and 20 minutes). Equal letters indicate no significant difference via Tukey´s test at 5% error probability; small letters compare the different exposure times within each species, while capital letters compare the species at the different exposure times.

oxygen species. The increased activity of this enzyme at increasing levels of UV-C can result in a better protection against ROS or in a reduction in H_2O_2 levels. The exposure of plants to UV-C radiation might have induced the increased production of hydrogen peroxide, which is mainly formed in chloroplasts and mitochondria and can potentially diffuse into the cytosol, where it is eliminated by APX , a cytosolic enzyme (Saurabh *et al*. 2011).

Our results also suggest that the enzyme activity is related to the period of exposure to UV-C radiation. According to Li *et al*. (2007), the activity of different antioxidant enzymes under UV-C radiation in wheat (*Oryza sativa* L.) and pea (*Pisum sativum* L.*)* first increased and then significantly decreased with increasing exposure time. This response demonstrates that enzyme activity under UV-C radiation varies in different plant species, which was also observed in the species studied here.

Even against the background of defence strategies of plants exposed to UV-C radiation, free radicals can initiate lipid peroxidation, leading to membrane damage (Sreenivasulu *et al*. 2007). In the present study, we observed an increase in the MDA content in *A. sessilis and A. philoxeroides*. Similarly, Du *et al*. (2003) also reported increased MDA levels in *Taxus cuspidata* (Siebold & Zucc.) exposed to UV-C radiation.

Hydroxyl radicals and singlet oxygen may react with lipids and form hydroperoxides (Hollósy 2002, Blokhina *et al*. 2003). The hydrogen peroxide radicals can abstract from other unsaturated fatty acids, leading to a peroxidation chain reaction. Peroxidation of the membrane lipids leads to the breakage of its structure and function (Hollósy 2002). In this study, the observed changes in MDA

levels demonstrate that UV-C radiation induced oxidative stress, with a greater intensity in *A. philoxeroides.*

In the present work, the increase in MDA in *A. sessilis* can be explained by the fact that although this plant has non-enzymatically activated defence mechanisms in stress situations, this mechanism was not sufficient for the protection of these plants. Furthermore, the non-activation of the enzyme system resulted in membrane damage. Brosché & Strid (2003) reported that plants first act by stimulating the non-enzymatic antioxidant defence system; subsequently, the enzymatic antioxidant defence system is activated, which is likely due to temporal gene expression patterns.

The species *A. philoxeroides* presented a greater activity of ascorbate peroxidase and higher MDA contents when compared with the other species after an exposure time of 20 minutes. A previous study has reported that under stress conditions, this species has a low betalain content when compared with *A. sessilis* and *A. brasiliana* (Reis *et al*. 2015). As these compounds act as antioxidants, their low concentrations in *A. philoxeroides* could partially explain the increased activity of ascorbate peroxidase and the high MDA content, representing compensatory defence mechanisms to stress as a result of UV-C radiation.

Revolutionary discoveries in the UV radiation perception field came with the identification of the UVR8 gene, which is sensitive to UV light. This mediates defence responses through the activation of a signalling pathway UVR8- COP1- hY5, which increases the pigments that act as solar protectors, in addition to the scavenging activity of reactive oxygen species on the UV radiation levels (Nawkar *et al*. 2013).

CONCLUSION

Our results suggest that UV-C radiation increases the production of secondary metabolites in the species *A. sessilis* and *A. brasiliana* and the activities of the CAT and APX enzymes in the species *A. philoxeroides*, besides increasing lipid peroxidation in *A. sessilis* and *A. philoxeroides*, indicating that the studied species use different protection mechanisms against UV-C radiation.

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