Ciência Rural
ISSN: 0103-8478
cienciarural@mail.ufsm.br
Universidade Federal de Santa Maria
Brasil

Rodrigues-Brandão, Isabel; Moraes Kleinowski, Alítcia; Millech Einhardt, Andersom; Conceição Lima, Milene; do Amarante, Luciano; Peters, José Antonio; Bolacel Braga, Eugenia Jacira
Salicylic acid on antioxidant activity and betacyan in production from leaves of Alternanthera tenella
Ciência Rural, vol. 44, núm. 10, octubre, 2014, pp. 1893-1898
Universidade Federal de Santa Maria
Santa Maria, Brasil

Ciência Rural, Santa Maria, v.44, n.10, p.1893-1898, out, 2014 http://dx.doi.org/10.1590/0103-8478cr20130873
ISSN 0103-8478

Salicylic acid on antioxidant activity and betacyan in production from leaves of *Alternanthera tenella*

Ácido salicílico sobre a atividade antioxidante e produção de betacianinas em folhas de *Alternanthera tenella*

Isabel Rodrigues-Brandão[I] Alítcia Moraes Kleinowski[I] Andersom Millech Einhardt[I] Milene Conceição Lima[II] Luciano do Amarante[II] José Antonio Peters[I] Eugenia Jacira Bolacel Braga[I*]

ABSTRACT

*This research investigates effects of salicylic acid (an abiotic elicitor) on the antioxidant activity and betacyan production from leaves of **Alternanthera tenella** cultured* in vitro *was evaluated. Plants were grown in a liquid MS medium and vermiculite substrate. After 35 days salicylic acid was added to the medium. Content of betacyanins, total phenols and flavonoids and non-enzymatic antioxidant capacity were determined in leaves of **A. tenella** after 0, 12, 36 and 48h of treatment. After 36h, concentration of betacyanins and total phenols increased. On the other hand, the increase of the treatment time caused a slight decrease in total flavonoids and reduced the DPPH free radical activity. As result the antioxidant activity of the leaves of **A. tenella** is promoted by salicylic acid and can be attributed to the increase in betacyanin content, which are compounds with recognized antioxidant action.*

Key words: ***Alternanthera tenella**, DPPH, total flavonoids, phenolic compounds, betacyanins.*

RESUMO

*Este trabalho investiga o efeito do ácido salicílico (um elicitor abiótico) sobre a atividade antioxidante e produção de betacianinas em folhas de **Alternanthera tenella** cultivada* in vitro*. As plantas foram cultivadas em meio MS líquido e vermiculita como substrato. Após 35 dias, o ácido salicílico foi adicionado ao meio. Conteúdo de betacianinas, fenóis totais e flavonoides e a capacidade antioxidante não-enzimática foi determinada em folhas de **A. tenella** após 0, 12, 36 e 48h de tratamento. Após 36h, a concentração de betacianinas e fenóis totais aumentaram. Em contrapartida, o aumento no tempo de exposição causou uma ligeira diminuição nos teores de flavonoides totais e inibição da atividade do radical livre DPPH. Como resultado, a atividade antioxidante de folhas de **A. tenella** é promovida pelo ácido salicílico e pode ser atribuída ao aumento do conteúdo de betacianina, os quais são compostos com ação antioxidante reconhecida.*

Palavras-chave: ***Alternanthera tenella**, DPPH, flavonoides totais, compostos fenólicos, betacianina.*

INTRODUCTION

The production of free radicals in living organisms is controlled by various antioxidant compounds of endogenous origin, from diet or other sources. The radicals formed from antioxidants are not subject to the chain propagation reaction, but are neutralized by reaction with another radical, forming stable products or being recycled by another antioxidant (ATOUI et al., 2005).

In this context, many studies have shown that phenolic compounds from plants exhibit high antioxidant potential and, in addition to antioxidant enzymes, directly capture reactive oxygen species (ANDRADE et al., 2007). Natural antioxidants, such as phenolic compounds, inhibit lipid peroxidation and lipooxygenase *in vitro* (SOARES, 2002). The activity of the phenolic compounds is mainly due to their reducing properties. These properties are important in neutralization and capture of free radicals and chelation of transition metals, acting in the initiation and propagation stages of oxidation. The intermediaries formed by the action of phenolic antioxidants are relatively stable due to high electronic delocalization on the π-system of the aromatic ring (CHUN et al., 2005). Interest in betacyanins has also grown since the characterization of their anti-radical activity. These compounds have been widely used as a

[I]Instituto de Biologia, Departamento de Botânica, Universidade Federal de Pelotas (UFPel), 96010-900, Pelotas, RS, Brasil. E-mail: isabel.brandao@posgrad.ufla.br. *Autor para correspondência.
[II]Centro de Ciências Químicas, Farmacêutica e de Alimentos, UFPel, Pelotas, RS, Brasil.

Received 06.26.13 Approved 03.04.14 Returned by the author 07.11.14
CR-2013-0873.R3

food additive and constituent in pharmaceuticals and cosmetics due to their natural colorant properties and absence of toxicity (STRACK et al., 2003).

Betacyanins are found in a number of plants, including ***Alternanthera tenella*** Colla, an herbaceous plant commonly found in Brazil (popularly known as "*apaga-fogo*") (FERREIRA et al., 2003). This species has antibacterial and antifungal activities and infusions are orally taken for the treatment of inflammations and infections (BIELLA et al., 2008). This species is considered a weed (SIQUEIRA, 1995), usually eliminated in plantations, and thus becoming as endangered one (DECRETO 42.099, 2002).

Species of the genus ***Alternanthera*** contain various biologically active compounds, including betalains (betacyanins and betaxanthins), ecdysteroids, flavonoids, saponins, and triterpenes (FERREIRA & DIAS, 2000).

The micropropagation of medicinal plants is a widely used technique for the production of active compounds and natural products for the pharmaceutical industry. Compared with traditional methods, *in vitro* techniques have many advantages, such as independence of seasonal variation for massal propagation, identification and production of clones with the desired characteristics, and the ability to manipulate the microenvironment to increase production of the metabolite of interest (EL-TARRAS et al., 2012). The use of elicitors in *in vitro* culture has been one of the most effective strategies to increase the formation of secondary metabolites, including the application of certain hormones associated with stress in plants (JALLEL et al., 2009; PEROTTI et al., 2010).

Salicylic acid is a phenolic phytohormone and considered as efficient chemical elicitor. This compound is involved in the signaling systems, inducing enzymes to catalyze the formation of defense compounds such as polyphenols and alkaloids (VAN LOON, 1997). When applied exogenously, elicitors can systemically trigger the expression of a defense genes set that is naturally activated by pathogen infection, stimulating the synthesis of various plant metabolites (ATSUSHI et al., 2007). This work describes the influence of salicylic acid on the production of secondary metabolites and antioxidant capacity of leaves of ***Alternanthera tenella*** cultured *in vitro*.

MATERIALS AND METHODS

A. tenella was collected in the City of Pelotas (Brazil). Its taxonomy was confirmed by the ***Amaranthaceae*** identification key and was cataloged in the PEL Herbarium under the number 25.26. Leaves of the plant were established *in vitro* on MS medium (MURASHIGE & SKOOG, 1962), without growth regulators, and were sub-cultured to obtain a sufficient number of individuals for the experiment. Nodal segments of approximately 1cm in length were removed and inoculated in flasks containing liquid MS and vermiculite – used as a substrate to enable increased absorption of the elicitor. This procedure was performedin a laminar flow chamber under aseptic conditions. The flasks were then placed in the growth room at 25±2°C, under a 16h photoperiod and photon flux density of 48μmol m^{-2} s^{-1}. After 35 days, 15mL of elicitor (salicylic acid) was added to the vermiculite at a concentration of 400μM. Leaves were collected at time periods of 0, 12, 36, and 48h of the addition of the elicitor, and were stored in an ultrafrezeer (-70°C) for later analysis of content of betacyanins, total phenolics, and total flavonoids and antioxidant capacity. The choice of concentration was based on experiments conducted in parallel (data not shown).

To determine betacyanin content, of fresh leaves (100mg) were macerated with celite in distilled (5mL) water and centrifuged at 13632*g* and 4°C for 25min. The assays were performed with the supernatant and absorbances were registered at 536 and 650nm in a spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences®), as described by CAI et al. (1998). Betacyanin concentration was calculated taking into account the coefficient of molar extraction for amaranthine ($5.66x10^4$) and the results were expressed as mg of amaranthine 100g FM^{-1}.

Total flavonoid concentration was determined by the colorimetric method described by ZOU et al. (2004). The absorbance reading was performed in a spectrophotometer at 510nm, using aluminum chloride at 5% (m/v) in methanol (WU & NG, 2008). The analysis was performed using methanol extract (25μg mL^{-1}) prepared from dry leaf matter (250mg), which remained in of 70% methanol (10mL) for 24h.

Content of phenolic compounds was determined by Folin-Ciocalteu method, modified according to JENNINGS (1981). Fresh leaf material (100g) was macerated, added to methanol:chloroform:water (12:5:3) solution (4mL). The mixture was then placed into centrifuge tubes placed in the dark for 24h. Samples were centrifuged for 10min at room temperature at 7000g. The supernatant was then collected and the precipitate again centrifuged with methanol:chloroform:water (12:5:3) solution (4mL) under the same conditions. The collected supernatant was joined to the first centrifuging and, to each extract

(4mL), chloroform (1mL) and water (1.5mL) were added, followed by further centrifugation. Ultrapure water (500µL) and 1N Folin-Ciocalteu reagent (500µL) were added to supernatant (500µL). After 15min, alkaline reagent (5mL), consisting of sodium hydroxide (0.1N) and sodium carbonate (0.1N), were allowed to stand for 60min. After this period, readings were performed in a spectrophotometer at 760nm, using water as a blank. Phenic acid was used as standard for the construction of the analytical curve at concentrations of 0-200µg mL^{-1}, at intervals of 20µg. The results were expressed in µg of phenic acid gFM^{-1}.

Antioxidant activity was analyzed by the DPPH method (BRAND-WILLIAMS et al., 1995), which is based on the capture of the DPPH radical (2.2-diphenyl-1-picryl-hydrazyl) by antioxidants, producing a decrease in absorbance at 515nm. DPPH methanolic solution (60µM) was prepared to exhibit present absorbance at 515nm between 0.6 and 0.7: 3.9mL DPPH solution and 0.1mL of the methanolic extracts at 25µg mL^{-1} were added to test tubes. To assess the free radical scavenging activity, the percent inhibition of DPPH in relation to the control sample (methanol + DPPH 60µM) was calculated by the following equation: % inhibition of DPPH = [(A0 - A1) / A0 x 100], where: A0 = absorbance of control and A1 = absorbance of the sample (MOLYNEUX, 2004).

The experimental design was completely randomized, consisting of four treatments (exposure time to salicylic acid), with three replicates. The experimental unit was represented by five flasks containing four explants per flask. The results were assessed by analysis of variance (ANOVA) and the means compared by Tukey's test at 5% error probability using the statistical software WinStat (MACHADO & CONCEIÇÃO, 2002).

RESULTS AND DISCUSSION

Salicylic acid affects the secondary metabolism of plants (BOONSNONGCHEEP et al., 2010; KORSANGRUANG et al., 2010) and has been widely studied in relation to signaling mechanisms and responses to pests and diseases (FUJITA et al., 2006). In the present study, exposing ***A. tenella*** to 400µM salicylic acid significantly influenced the betacyanin content of the leaves. Time periods of 36 and 48h were sufficient to stimulate the accumulation of these compounds (14.6 and 13.9mg of amaranthine $100gMF^{-1}$, respectively) when compared to the control (3.7mg of amaranthine $100gMF^{-1}$) (Figure 1A). However, the observed concentrations of betacyanins were much lower than those found by KLEINOWSKI et al. (2014) in the stems of the same species: 36.95mg of amaranthine 100g MF^{-1} with tyrosine elicitation (75µM). This response might be due to the type of plant material and elicitor used in the analyses. For example, betacyanins may be preferentially stored in the stem of ***A. tenella***.

The success of an elicitor depends on the type and duration of treatment and concentration and kind of elicited compound. For example, VAKIL & MENDHULKAR (2013) investigated the effect of two elicitors, salicylic acid and chitosan, on cultured cells of the medicinal plant ***Andrographis paniculata***. A stronger response was observed in the production of secondary metabolites using chitosan in the treatment, although the exact mechanism underlying this metabolic effect was unable to determine.

In the present study, salicylic acid elicited an increase in betacyanin synthesis in ***A. tenella***. Nevertheless, increased exposure to the elicitor did not increase the concentration of flavonoids, possibly because the common precursor of both compounds, tyrosine, was preferentially converted into betacyanins.

Betacyanin is a type of betalains, hydrophilic pigments responsible for the coloration of fruits, flowers, roots and leaves of plants belonging to the order Caryophyllales (CASTELLANOS-SANTIAGO &YAHIA, 2008). Betalains and anthocyanins (flavonoids) are two different chemical classes of pigments and are never found together in the same plant (GANDÍA-HERRERO et al., 2005). Such exclusiveness may have an explanation at the biochemical level - the relevant enzymes for the production of anthocyanins are not certainly expressed in betalain-producing plants (BROCKINGTON et al., 2011).

The highest means of flavonoid contents were observed in the control plants and plants after 12h exposure to the elicitor (6088.7 and 5932.9µg of quercetin g MF^{-1}, respectively), with a significant decrease (18%) for plants experiencing longer periods of elicitation (36 and 48h) (Figure 1B). This is in contrast to YU et al. (2006), who recorded a gradual increase in total flavonoids over time, reaching 96% at 48h of treatment when compared to control. However, the concentration of the elicitor was lower than that used in the current work. HOUHUA et al. (2007) observed significant increases in the synthesis of phenylpropanoids in ***Malus domestica*** treated with salicylic acid. These results suggest that salicylic acid induces the accumulation of compounds derived from phenylpropanoids due to the increase in activity of the phenylalanine ammonia-lyase (PAL) enzyme. PAL is known to play an important role in the transduction process, stimulating the synthesis of these compounds and regulating the expression of defense genes (YU et al., 2006).

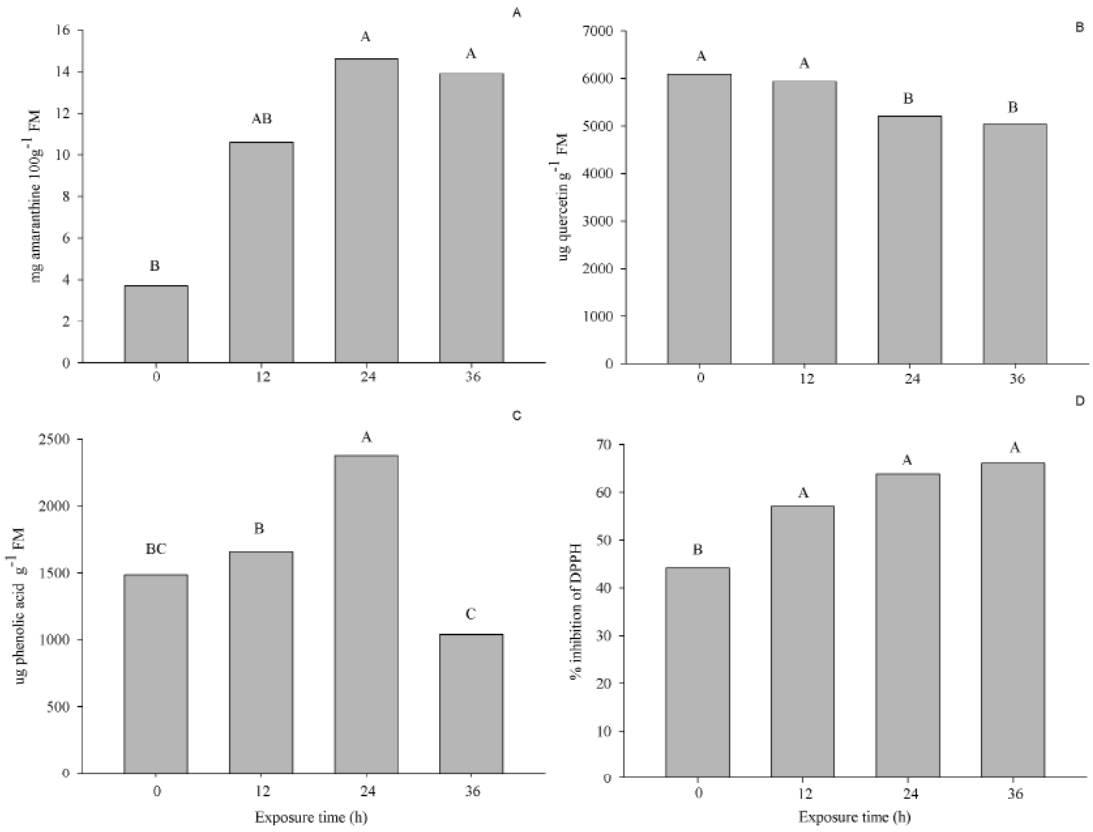

Figure 1 - Betacyanins content (A), total flavonoids content (B), total phenolic compounds content (C) and inhibition of the DPPH radical by methanolic extracts (D) from plants of ***Alternanthera tenella*** subjected to different exposure times to salicylic acid. Time periods labeled with the same letters do not differ significantly.

Alternanthera tenella probably deviates from their secondary metabolite synthesis, where the shikimate acid pathway would lead to increased production of tyrosine. The accumulation of this amino acid would trigger the synthesis of betacyanin compounds with confirmed antioxidant activity.

A decrease in phenolic compound content was observed after 48h exposure to the elicitor (1039.8µg g FM^{-1}), having the maximum synthesis after 36h of elicitation (2378.2µg of phenic acid g FM^{-1}) with 400mM salicylic acid. However, after 12h of exposure there was no significant difference in phenolic compound content between the treatment and the control (1657µg of phenic acid g FM^{-1}) (Figure 1C). These results are somewhat different to those of a recent study in ***Solanum melongena*** L. using different types of elicitors and different exposure times (MANDAL, 2010). The previous showed that salicylic acid was the most efficient elicitor at triggering phenolic compound synthesis after 48h exposure - although a gradual decrease in synthesis was observed over longer time periods (MANDAL, 2010).

An assay of DPPH radical capture is a very useful tool for screening compounds for antioxidant potential. The analysis of antioxidant activity of ***A. tenella*** by the DPPH method indicated that exposure to the elicitor significantly increased the antioxidant potential of the extract. However, no statistical difference was observed between exposure times, indicating that plant extract has good antioxidant activity (Figure 1D).

The detoxifying effect of phenolic compounds on the DPPH radical has been well described in literature (e.g. HEIM et al., 2002). For example, LEUNG & SHUI (2002) used the DPPH method to characterize the antioxidant activity of 27 different fruits (with extracts rich in phenolic compounds) found in the public markets of Singapore. A detoxifying effect on the DPPH radical was also observed in extracts from 33 Chinese medicinal plants containing phenolic compounds (TAN et al., 2004).

The increase in antioxidant activity observed in the present study is related to the increased synthesis of betacyanins, since these compounds show

high antioxidant activity - as already reported in studies with sugar beet (STINTZING & CARLE, 2004). Indeed, betacyanins are among the ten most powerful antioxidants due to their characteristic structural conformation. Betacyanins have even been linked to the prevention of some types of cancer, including skin and liver cancer, presumably as a consequence of their antioxidant properties (LILA, 2004).

CONCLUSION

A. tenella has a significant pharmacological potential. Antioxidant activity of leaves of ***A. tenella*** can be significantly increased by the addition of an elicitor, such as salicylic acid, to the culture medium.

REFERENCES

ANDRADE, C.A. et al. Determinação do conteúdo fenólico e avaliação da atividade antioxidante de ***Acacia podalyriifolia*** A. Cunn ex. Don ***Leguminosae-Mimosoidae***. **Revista Brasileira de Farmacognosia**, v.17, p.231-235, 2007. Available from: <http://www.scielo.br/scielo.php?pid=S0102-695X2007000200017&script=sci_arttext>. Accessed: Nov. 10, 2011. doi: 10.1590/S0102-695X2007000200017.

ATSUSHI, O. et al. Elicitor induced activation of the methyterythritol phosphate pathway toward phytoalexins biosynthesis in rice. **Plant Molecular Biology**, v.65, p.177-187, 2007. Available from: <http://www.springerlink.com/content/5128776012668444/>. Accessed: Nov. 12, 2011. doi: 10.1007/s11103-007-9207-2.

BIELLA, C.A. et al. Evaluation of immunomodulatory and anti-inflammatory effects and phytochemical screening of ***Alternanthera tenella*** Colla (***Amaranthaceae***) aqueous extracts. **Memórias do Instituto Oswaldo Cruz**, v.103, p.569-577, 2008. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762008000600010>. Accessed: Dec. 04, 2011. doi: 10.1590/S0074-02762008000600010.

BOONSNONGCHEEP, P. et al. Growth and isoflavonoid accumulation of ***Pueraria candollei*** var. candollei and ***P. candollei*** var. mirifica cell suspension cultures. **Plant Cell Tissue and Organ Culture**, v.101, p.119-126, 2010. Available from: <http://www.springerlink.com/content/n346215h7578w538/>. Accessed: Dec. 15, 2011.doi: 10.1007/s11240-010-9668-x.

BRAND-WILIAMS, W. et al. Use of a free radical method to evaluate antioxidant activity. **Food Science and Technology**, v.28, p.25-30, 1995. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643895800085>. Accessed: Oct. 10, 2011. doi: 10.1016/S0023-6438(95)80008-5.

BROCKINGTON, S.F. et al. Complex pigment evolution in the Caryophyllales. **New Phytologist**, v.190, p.854-864, 2011. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21714182>. Accessed: Nov. 01, 2013.

CAI, Y. et al. Characterization and quantification of betacyanin pigments from diverse ***Amaranthus*** species. **Journal of Agricultural and Food Chemistry**, v.46, p.2063-2070, 1998. Available from: <http://pubs.acs.org/doi/abs/10.1021/jf9709966>. Accessed: Oct. 27, 2011. doi: 10.1021/jf9709966.

CASTELLANOS-SANTIAGO, E.; YAHIA, E.M. Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high-performance liquid chromatography and electrosprayionization mass spectrometry. **Journal of Agricultural and Food Chemistry**, v.56, p.5764-5778, 2008. Available from:<http://pubs.acs.org/doi/abs/10.1021/jf800362t>. Accessed: Nov. 01, 2013.doi: 10.1021/jf800362t.

CHUN, S.S. et al. Phenolic antioxidants from clonal oregano (***Origanum vulgare***) with antimicrobial activity against Helicobacter pulori. **Process Biochemistry**, v.40, p.809-816, 2005. Available from: <http://www.sciencedirect.com/science/article/pii/S0032959204001189>. Accessed: Nov. 15, 2011. doi: 10.1016/j.procbio.2004.02.018.

DECRETO N.42.099, de 31 de dezembro de 2002. **Declara as espécies da flora nativa ameaçadas de extinção no estado do Rio Grande do Sul e dá outras providências**. Porto Alegre: Palácio Piratini, 2002.

EL-TARRAS, A. et al. *In vitro* multiplication of the important medicinal plant, harmal. **Journal of Medicinal Plants Research**, v.6, p.3586-3590, 2012. Available from: <http://www.academicjournals.org/journal/JMPR/article-abstract/F6A8D3F21276>. Accessed: Nov. 04, 2013. doi:10.5897/JMPR12.315.

FERREIRA, E.A. et al. Estudos anatômicos de folhas de espécies de plantas daninhas de grande ocorrência no Brasil. IV – ***Amaranthus spinosus***, ***Alternanthera tenella*** e ***Euphorbia heterophylla***. **Planta Daninha**, v.21, p.263-271, 2003. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-83582003000200012>. Accessed: Oct. 02, 2011. doi: 10.1590/S0100-83582003000200012.

FERREIRA, E.O.; DIAS, D.A. A methyllenedioxyflavonol from aerial parts of ***Blutaparon portulacoides***. **Phytochemistry**, v.53, p.145-147, 2000. Available from: <http://www.sciencedirect.com/science/article/pii/S0031942299003908>. Accessed: Oct. 10, 2011.doi: 10.1016/S0031-9422(99)00390-8.

FUJITA, M. et al. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. **Current Opinion in Plant Biology**, v.9, p.436-442, 2006. Available from: <http://www.sciencedirect.com/science/article/pii/S1369526606000884>. Accessed: Oct. 05, 2011.doi: 10.1016/j.pbi.2006.05.014.

GANDÍA-HERRERO, F. et al. Betaxanthins as pigments responsible for visible fluorescence in flowers. **Planta**, v.222, p.586-593, 2005. Available from: <http://link.springer.com/article/10.1007%2Fs00425-005-0004-3?LI=true#>. Accessed: Oct. 13, 2011.doi: 10.1007/s00425-005-0004-3.

HEIM, K.E. et al. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationship. **Journal of Nutritional Biochemistry**, v.13, p.572-584, 2002. Available from: <http://www.aseanfood.info/articles/11014997.pdf>. Accessed: Oct. 28, 2011. doi: 10.1016/S0955-2863(02)00208-5.

HOUHUA, L. et al. Maize Lc transcription factor enhances biosynthesis of anthocyaninas, distinct pro anthocyanidins, and phenylpropanoids in apple (***Malus domestica*** Bords). **Planta**, v.226, p.1243-1254, 2007. Available from: <http://link.springer.

com/article/10.1007/s00425-007-0573-4>. Accessed: Oct. 25, 2011.doi: 10.1007/s00425-007-0573-4.

JENNINGS, A.C. The determination of dihydroxy phenolic compounds in extracts of plant tissues. **Anal Biochemistry**, v.118, p.396-398, 1981. Available from: <http://www.sciencedirect.com/science/article/pii/000326978190600X>. Accessed: Jun. 21, 2011. doi: 10.1016/0003-2697(81)90600-X.

KLEINOWSKI, A.M. et al. Pigment production and growth of ***Alternanthera*** Plants cultured *in vitro* in the presence of tyrosine. **Brazilian Archives Biology and Technology**, v.57, p. 253-260, 2014. Available from: <http://www.scielo.br/pdf/babt/v57n2/cd_1037.pdf>. Accessed: Apr. 05, 2014.doi: 10.1590/S1516-89132013005000012.

KORSANGRUANG, S. et al. Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in ***P. candollei*** var. ***candollei*** and ***P. candollei*** var. ***mirifica*** cell suspension cultures. **Plant Cell Tissue and Organ Culture**, v.103, p.333-342, 2010.Available from: <http://rd.springer.com/article/10.1007/s11240-010-9785-6>. Accessed: Sept. 05, 2011.doi: 10.1007/s11240-010-9785-6.

LEUNG, L.P.; SHUI, G. An investigation of antioxidant capacity of fruits in Singapore markets. **Food Chemistry**, v.76, p.69-75, 2002. Available from: <http://www.sciencedirect.com/science/article/pii/S0308814601002515>. Accessed: Sept. 04, 2011.doi: 10.1016/S0308-8146(01)00251-5.

LILA, M.A. Plant pigments and human health. In: DAVIES, K.S. (Ed.). **Plant pigments and their manipulation**. 3.ed. Austrália:Blackwell Publishing Ltd, 2004. Cap. 14, p.248-274.

MACHADO, A.; CONCEIÇÃO, A.R. **Programa estatístico WinStat Sistema de Análise Estatístico para Windows**. Versão 2.0. Pelotas: UFPel, 2002.

MANDAL, S. Induction of phenolics, lignin and key defense enzymes in eggplant (***Solanum melongena*** L.) roots in response to elicitors. **African Journal of Biotechnology**, v.9, p.8038-8047, 2010. Available from: <http://www.academicjournals.org/ajb/PDF/pdf2010/22Nov/Mandal.pdf>. Accessed: Nov. 17, 2011.doi: 10.5897/AJB10.984.

MOLYNEUX, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. **Journal of Scienceand Technology**, v.26, p.211-219, 2004. Available from: <http://rdo.psu.ac.th/sjst/journal/26-2/07-DPPH.pdf>. Accessed: Sept. 27, 2011.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, v.15, p. 473-497, 1962. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1962.tb08052.x/>. Accessed: Jun. 11, 2011.doi: 10.1111/j.1399-3054.1962.tb08052.x.

PEROTTI, J.C. et al. Produção de betacianina em erva-de-jacaré cultivada *in vitro* com diferentes concentrações de sulfato de cobre. **Ciência Rural**, v.40, p.1874-1880, 2010. Available from: <http://www.scielo.br/scielo.php?pid=S0103-84782010000900004&script=sci_arttext>. Accessed: nov. 04, 2013.doi:10.1590/S0103-84782010000900004.

SIQUEIRA, J.C. **Fitogeografia das *Amaranthaceae* Brasileiras**. São Leopoldo: IAP, 1995. 131p.

SOARES, S.E. Ácidos fenólicos como antioxidantes. **Revista de Nutrição**, v.15, p.71-81, 2002. Availablefrom: <http://www.scielo.br/scielo.php?pid=S1415-52732002000100008&script=sci_abstract&tlng=pt>. Accessed: Nov. 01, 2013.doi: 10.1590/S1415-52732002000100008.

STINTZING, F.C.; CARLE, R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. **Trends in Food Science and Technology**, v.5, p.19-38. 2004. Available from: <http://www.sciencedirect.com/science/article/pii/S0924224403001638>. Accessed: Oct. 22, 2011. doi: 10.1016/j.tifs.2003.07.004.

STRACK, D. et al. Recent advances in betalain research. **Phytochemistry**, v.62, p.247-269, 2003. Available from: <http://www.sciencedirect.com/science/article/pii/S0031942202005642>. Accessed: Nov. 12, 2011.doi: 10.1016/S0031-9422(02)00564-2.

TAN, S.Y. et al. Caracterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. **Free Radical Biology & Medicine**, v.15, p.1575-1587, 2004. Available from: <http://www.sciencedirect.com/science/article/pii/S0891584904002783. Accessed: Oct.02, 2011. doi: 10.1016/j.freeradbiomed.2004.03.017.

VAKIL, M.M.A.; MENDHULKAR, V.D. Salicylic acid and chitosan mediated abiotic stress in cell suspension culture of A***ndrographi spaniculata*** (burm.f.) nees. for and rographolide synthesis. **International Journal of Pharmaceutical Sciences and Research**, v.4, p.3453-3459, 2013. Available from: <http://www.ijpsr.com/V4I9/21%20Vol.%204,%20Issue%209,%20September%202013,%20IJPSR,%20RA%202604,%20Paper%2021.pdf>. Accessed: Nov. 01, 2013 doi:10.13040/IJPSR.0975-8232.4(9).3453-59.

VAN LOON, L.C. Induced resistance in plants and the role of pathogenesis-related proteins. **European Journal of Plant Pathology**, v.103, p.753-765, 1997. Available from: <http://igitur-archive.library.uu.nl/bio/2001-1220-112648/UUindex.html>. Accessed: Sept. 27, 2011.

WU, S.J.; NG, L.T. Antioxidant and free radical scavenging activites of wild bitter melon (***Momordica charantia*** Linn. var. ***abbreviate*** Ser.) in Taiwan. **LWT- Food Science and Technology**, v.41, p.323-330, 2008. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643807001181>. Accessed: Sept. 27, 2011. doi: 10.1016/j.lwt.2007.03.003.

ZOU, Y.P. et al. Antioxidant activity of a flavonoid-rich extract of ***Hypericum perforatum*** L. *in vitro*. **Journal of Agricultural and Food Chemistry**, v.52, p.5032-5039, 2004. Available from: <http://pubs.acs.org/doi/abs/10.1021/jf049571r?mi=xg2m3d&af=R&pageSize=20&publication=40026026&searchText=PMS-NADH>. Accessed: Nov. 09, 2011.doi: 10.1021/jf049571r.

YU, Z.Z. et al. Salicylic acid enhances jaceosidin and syringin production in cell cultures of **S*aussurea medusa***. **Biotechnology Letters**, v.28, p.1027-1031, 2006. Available from: <http://link.springer.com/article/10.1007%2Fs10529-006-9035-5?LI=true>. Accessed: out. 04, 2011.doi: 10.1007%2Fs10529-006-9035-5.