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Short note

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A preliminary investigation on developmental and biochemical responses of *Amsonia orientalis* to ultraviolet-C irradiation

A. Acemi [1] (*), Y. Avcı Duman [2], Y. Yüzügüllü Karakuş [1], F. Özen [1]

[1] *Department of Biology, Faculty of Sciences and Arts, Kocaeli University, 41380 Kocaeli, Turkey.*

[2] *Department of Chemistry, Faculty of Sciences and Arts, Kocaeli University, 41380 Kocaeli, Turkey.*

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(*) **Corresponding author:**
arda.acemi@kocaeli.edu.tr

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The present study aims to investigate the developmental and biochemical responses of ornamental *Amsonia orientalis* irradiated with ultraviolet-C (UV-C). Nodal explants of the species were exposed to UV-C irradiation on the first, 15th and the last days of the *in vitro* culture for 15 (3.47 kJ m^{-2}), 30 (6.94 kJ m^{-2}) and 60 min (13.87 kJ m^{-2}). In general, root lengths and numbers were negatively influenced by prolonged UV-C exposure. However, mean shoot numbers and lengths were slightly enhanced after 15 and 30 min of irradiation. High hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were observed due to the UV-C exposure. Activities of the antioxidant enzymes, POD (peroxidase) and CAT (catalase) were found to be enhanced whereas SOD (superoxide dismutase) was reduced. These results indicated that UV-C irradiation for shorter durations may be carefully used to improve *in vitro* shoot proliferation in *A. orientalis*. However, it should be noted that longer irradiation durations can trigger stress responses and lipid peroxidation-dependent cell membrane damage which will further result in the plant loss.

1. Introduction

Ultraviolet (UV) irradiation is present in sunlight in three different wavelengths which are classified as UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm). UV irradiation may affect growth and metabolic processes in plants due to its high quantum energy (Kobashigawa *et al.,* 2011). Since wavelengths below 280 nm are absorbed by the ozone layer they do not reach to the surface of the Earth (Alexieva *et al.,* 2001). However, reduction of stratospheric ozone and, a decrease in the ozone layer may lead to increase in UV-C irradiation reaching the biosphere.

Beside its germicidal activity, application of UV-C irradiation at 254 nm wavelength is used for several purposes including regulation of fruit ripening in tomato (Tiecher *et al.,* 2013), fruit quality stabilization during storage of fresh-cut watermelon (Artés-Hernández *et al.,* 2010) and retardation of fruit decay in strawberry (Erkan *et al.,* 2008). Its role in growth regulation and modulation of flowering time in ornamental plants was declared since UV-C light application increased branching and the number of flowers in *Salvia splendens* and *Viola tricolor* (Bridgen, 2016). Also, the antioxidative function was found to be enhanced after UV-C application in *Spinacia oleracea* (Kobashigawa *et al.,* 2011). However, the application of UV irradiation can be hazardous in higher doses. Therefore, to better understand the developmental and metabolic responses of horticultural plants to UV-C irradiation, UV-dose-dependent studies in controlled environmental factors should be also conducted on other plant species.

Amsonia orientalis Decne. [syn. *Rhazya orientalis* (Decne.) A. DC.] which is also known as European Blue star or Eastern Rhazya is an ornamental plant with medicinal properties. It is cultivated more commonly by gardeners in the USA than those in Europe since it is deer-resistant due to its slightly toxic latex content (Acemi *et al.,* 2016). However, the natural populations of the species were taken under conservation by the European Council in the frame of Bern Convention since they are quite limited in nature (Acemi *et al.,* 2017). In the current study, the effects of dose-dependent UV-C irradiation applications on *in vitro* development and antioxidative enzymes of *A. orientalis* were investigated to shed light the usability of this treatment in horticultural plant propagation.

2. Materials and Methods

Plant material preparation and in vitro UV-C treatments

Nodal explants were excised from mature individuals of *Amsonia orientalis* growing in the garden of Kocaeli University. The shoots were multiplied by following the protocol described by Acemi *et al.,* (2013 a). Nodal explants excised from *in vitro*-raised shoots were inoculated into the MS (Murashige and Skoog's) medium (1962) supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} of plant agar. The pH of the medium was set to 5.7, and the cultures were incubated for 30 days under the same conditions defined by Acemi *et al.,* (2013 a). The applied UV-C doses were determined from previous reports (López-Rubira *et al.,* 2005; Artés-Hernández *et al.,* 2010; Bridgen 2014; Castronuovo *et al.,* 2014; Bridgen, 2016). The cultures were subjected to UV-C irradiation at the first, 15th and the last days of the culture period. The irradiation was applied from 30 cm distance for 15, 30 and 60 min per application day by using Sylvania G15W UV-C lamp (λ_{max} 253.7 nm). UV-C treatments were applied from the top of the culture vessels in a sterile biosafety cabinet, and lids of the culture vessels were kept opened during treatments to ensure penetration of the irradiation energy. The total accumulated irradiation levels were estimated as 3.47, 6.94 and 13.87 kJ m^{-2} for 15, 30 and 60 min of UV-C irradiation, respectively (Kobashigawa *et al.,* 2011; Severo *et al.,* 2015).

Biochemical assays

The optimized method of Acemi *et al.* (2017) was followed to determine malondialdehyde (MDA) as lipid peroxidation product, and H_2O_2 contents. The crude extract for antioxidant enzyme activities' determination was prepared by homogenizing the tissue samples (shoots and roots together) in extraction buffer of 50 mM sodium phosphate (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). After centrifugation of the homogenate at 14,000 *g* for 15 min at 4°C, the resulting supernatants were collected and used for catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity assays. CAT activity was determined according to the method of Aebi (1974). POD activity was assayed using the pyrogallol oxidation method (Kar and Mishra, 1976) while SOD activity was determined by following the method of Dhindsa *et al.* (1981).

Data collection and statistical analysis

The morphometric evaluation was done using 30 explants in each repeat. All assays were repeated thrice and mean values were compared using Duncan's multiple range test at $p<0.05$ significance level. The enzyme assays and extract preparation were done on the same day to minimize the loss of enzymatic activities.

3. Results

Plant growth and organ development

At the end of the incubation period, the mean shoot length slightly increased in response to 15 and

30 min UV-C exposure while this parameter was negatively affected by 60 min of exposure. However, increments in the mean shoot length were not statistically different than the control group (Fig. 1A). The mean shoot numbers were influenced positively by 15 and 30 min UV-C exposure whereas only the result of 15 min exposure was found to be statistically different than the control group (Fig. 1B). The mean root lengths were found to be decreased due to UV-C application even at the shortest duration. This reduction in root lengths was between 62.5% (15 min) and 95% (60 min) compared to the control (Fig. 1A). In contrast to mean shoot numbers, the mean root numbers reduced gradually in response to the elevated UV-C exposure (Fig. 1B).

Lipid peroxidation and H_2O_2 content

Application of UV-C irradiation significantly changed the lipid peroxidation level of the plant. Fifteen and 30 min of UV-C exposures triggered MDA accumulations without statistical differences between them. The highest accumulation level was observed from the cultures exposed to UV-C for 60 min (Fig. 2A). H_2O_2 content increased following UV-C exposure maintaining similar levels within treatments (Fig. 2B).

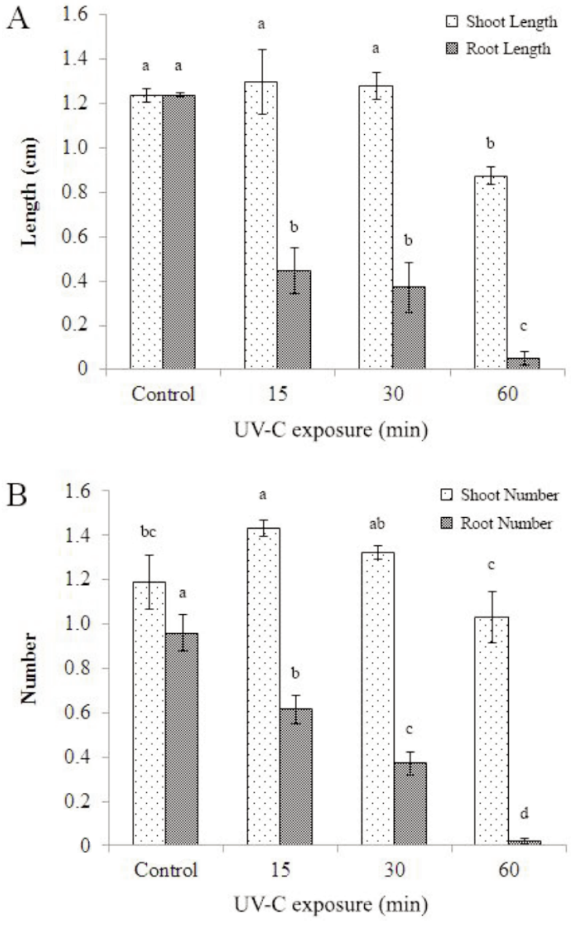

Fig. 1 - Effects of UV-C irradiation on *Amsonia orientalis* growth parameters. A= Length of shoot and root, B= Number of shoot and root. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

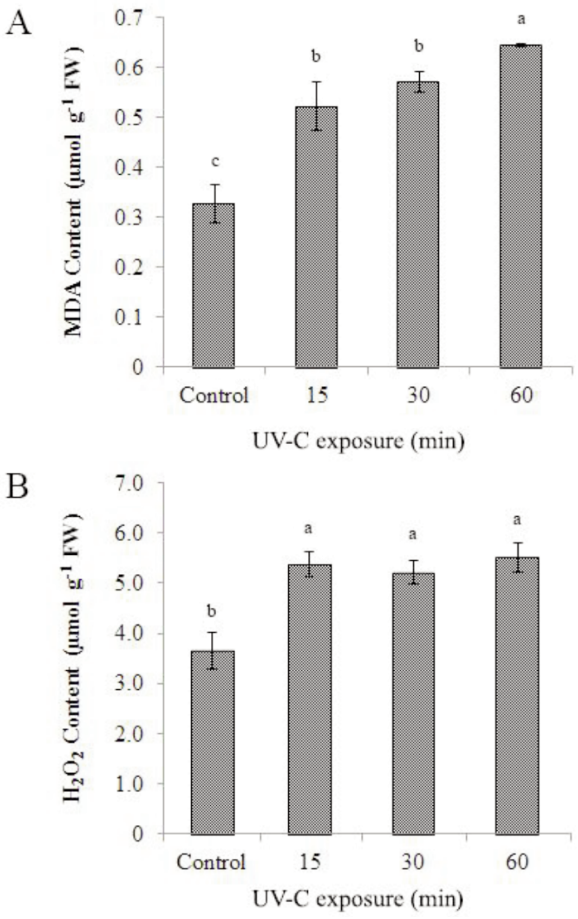

Fig. 2 - Oxidative effects of UV-C irradiation on *Amsonia orientalis*. A= MDA and B= H_2O_2 contents. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

Antioxidant enzyme activities

UV-C irradiation caused a decrease in SOD activity without statistical differences between treatments (Fig. 3A). POD activity significantly increased due to UV-C exposures. The highest POD activity was observed in cultures exposed to 15 min of UV-C followed by a significant decrease at 30 and 60 min of UV-C exposures, which were not significantly different (Fig. 3B). CAT activity exhibited a similar trend with POD activity. The shortest UV-C exposure caused the highest increase in the CAT activity while longer exposures induced statistically same results (Fig. 3C).

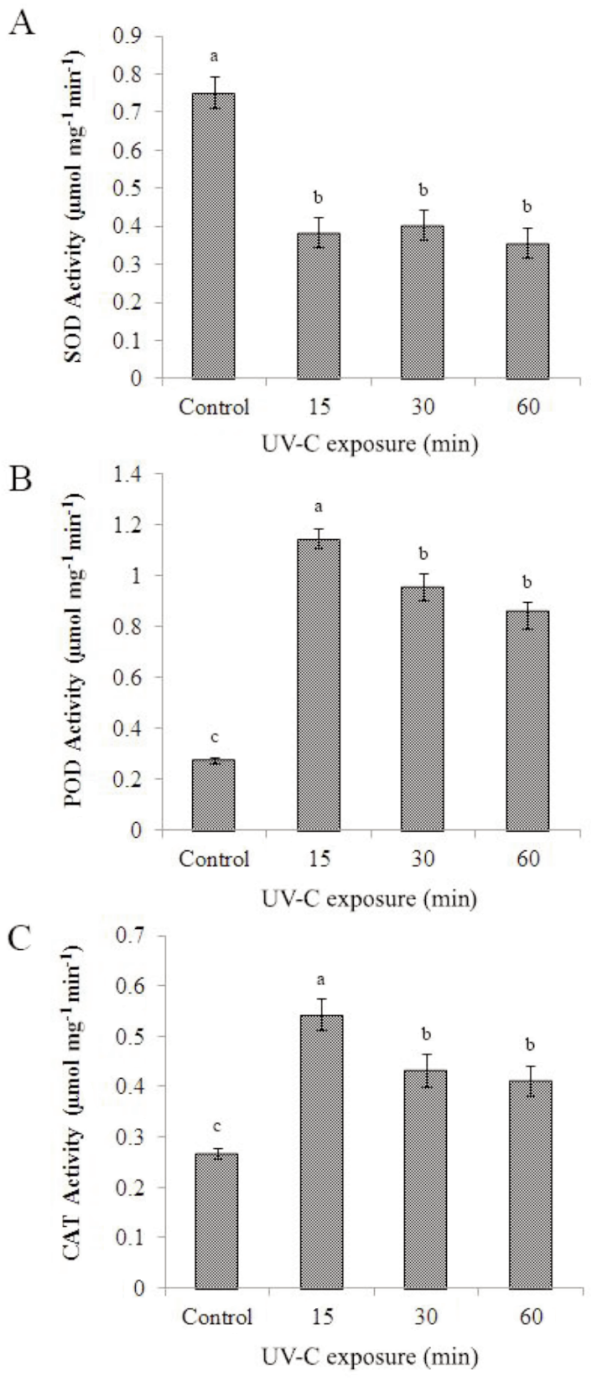

Fig. 3 - Effects of UV-C irradiation on enzymatic antioxidant activities in *Amsonia orientalis*. A= SOD, B= POD and C= CAT activities. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

4. Discussion and Conclusions

UV irradiation can damage DNA and affect plant growth through various regulatory and/or stress-mediated processes. As one of these processes, cell cycle modulation sourced by DNA damage may particularly slow down the progress from G_1 to S phase (Jiang *et al.,* 2011). Therefore, in our results the decrease in the mean shoot length after 60 min UV-C irradiations might be due to the UV-induced DNA damage and impaired cell cycle progress. Differently, mean shoot number was found to be increased in *A. orientalis* after 15 min of UV-C exposure, which was in accordance with the previous report that found an increment of axillary branching in *Viola tricolor* (Bridgen, 2016). Our results also showed adverse effects of UV-C on the root growth of *A. orientalis*. This negative effect can be explained by oxidative degradation and/or inhibited-synthesis of indole acetic acid (IAA); a phytohormone responsible for root proliferation (Berli *et al.,* 2013). This possible inhibition of IAA might also be a result of photo-oxidation of the plant growth regulator (Ciurli *et al.,* 2017). The oxidative degradation of IAA starts with a peroxidase-involved decarboxylation process on the side chain or oxidation of the indole ring (Normanly, 2010). In the enzyme assays, elevated peroxidase activity against UV irradiation supports this discussion which is still in need of further experiments to be clearly proven.

MDA content indicates reactive oxygen species (ROS)-mediated cellular damage considering damage to membrane lipids of stress-exposed plants. The observed increase in H_2O_2 levels coincided with enhanced MDA levels in *A. orientalis*. Also, POD and CAT activities were found to be increased to remove ROS to limit MDA production in *A. orientalis*. Degradation of the enzyme proteins and nucleic acids can be started after ROS-induced peroxidation of the cell membrane lipids. At the same time, H_2O_2 could activate mitogen-activated protein kinases (MAPKs) in plants, leading to an enhanced antioxidant defense system (Nie *et al*., 2013). However, excess accumulation of H_2O_2 causes cellular damage (Gong *et al.,* 2001). The increase in both H_2O_2 and MDA levels indicates cellular damage in *A. orientalis*. Excessive ROS in UV-C-exposed plants may be produced because of disruption in metabolic activities or increased activity of membrane-localized NADPH-oxidase (Kalbina and Strid, 2006). The increment in the activities of CAT and POD enzymes did not seem to limit ROS production in *A. orientalis* since H_2O_2 accumulation was consistently found at high levels.

As a defense mechanism against environmental stress factors, plants favor the production of antioxidant enzymes (Berli *et al.,* 2013). Maintenance of the

antioxidant defense system to cope with ROS plays a significant role in keeping the cell membranes stabilized. The antioxidant enzymes SOD, CAT, and POD are widely distributed in all higher plants and involved in decomposition of different forms of ROS (Foyer and Noctor, 2000). Excessive production of O^{2-} triggers SOD enzyme activity which converts superoxide radicals into either O_2 or H_2O_2 while the excess accumulation of H_2O_2 is prevented by catalase and/or the ascorbate-glutathione cycle enzymes (Ma *et al.,* 2014). Although the O^{2-} content was not determined in our study, the inhibition of SOD activity by UV irradiation might be due to increased O^{2-} content. POD is involved in such processes like lignification and tolerance to environmental stresses in higher plants. During UV exposure, elevated POD and CAT activities showed that activities of both enzymes can be triggered in *A. orientalis.* The bulk H_2O_2 removal activity of CAT in the cell is followed by the scavenging action of POD on the H_2O_2 which is not taken by CAT (Willekens *et al.,* 1997). In this report, this cooperation between both enzymes is also shown in *A. orientalis*.

Thanks to *in vitro* plant tissue culture technique, several conservation studies on *A. orientalis* were conducted and a high number of individuals were propagated thereby populations in Turkey were conserved (Acemi *et al.,* 2013 a, b). The present study has revealed that although short-term application of UV-C irradiation can enhance shoot induction, it limits root growth and triggers oxidative stress at extended exposure durations. Therefore, in further detailed studies on *A. orientalis* UV-C radiation should be applied for less than 30 min (6.94 kJ m^{-2}). However, it should be noted that short-term UV-C application can be considered as a promoter factor only in shoot multiplication phase in *A. orientalis*. The lower doses may be carefully applied to enhance branching in the horticultural industry. However, the possible developmental and biochemical responses given against UV-C irradiation would change species to species.

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References

ACEMİ A., DUMAN Y., KARAKUŞ Y.Y., KÖMPE Y., ÖZEN F., 2017 - *Analysis of plant growth and biochemical parameters in* Amsonia orientalis *after* in vitro *salt stress.* - Hortic. Environ. Biotechnol., 58(3): 231-239.

ACEMİ A., KIRAN R., ÖZEN F., BALCI S., BAYDEMİR S., İLMAZ Ç., 2013 b - *Influence of medium composition on* in vitro *propagation of* Amsonia orientalis *Decne.* - J. Anatolian Nat. Sci., 4(2): 1-6.

ACEMİ A., ÖZEN F., KIRAN R., 2013 a - In vitro *propagation of* Amsonia orientalis *Decne. from nodal segments of adult plants.* - Propag. Ornam. Plants, 13(1): 25-32.

ACEMİ A., TÜRKER-KAYA S., ÖZEN F., 2016 - *FT-IR spectroscopy based evaluation of changes in primary metabolites of* Amsonia orientalis *after* in vitro *6-benzylaminopurine treatment.* - Not. Bot. Horti. Agrobo., 44(1): 209-214.

AEBI H., 1974 - *Catalase,* pp. 673-684. - In: BERGMEYER H.U. (ed.) *Methods of enzymatic analysis*. Academic Press, New York, USA, pp. 1065.

ALEXIEVA V., SERGIEV I., MAPELLI S., KARANOV E., 2001 - *The effect of drought and ultraviolet irradiation on growth and stress markers in pea and wheat.* - Plant Cell Environ., 24: 1337-1344.

ARTÉS-HERNÁNDEZ F., ROBLES P.A., GÓMEZ P.A., TOMÁS-CALLEJAS A., ARTÉS F., 2010 - *Low UV-C illumination for keeping overall quality of fresh-cut watermelon.* - Postharvest Biol. Technol., 55: 114-120.

BERLI F.J., ALONSO R., BRESSAN-SMITH R., BOTTINI R., 2013 - *UV-B impairs growth and gas exchange in grapevines grown in high altitude.* - Physiol. Plantarum, 149(1): 127-140.

BRIDGEN M., 2014 - *Using ultraviolet-c light as a plant growth regulator*. - Acta Horticulturae, 1085: 167-169.

BRIDGEN M., 2016 - *Using ultraviolet-C (UV-C) irradiation on greenhouse ornamental plants for growth regulation*. - Acta Horticulturae, 1134: 49-56.

CASTRONUOVO D., TATARANNI G., LOVELLI S., CANDIDO V., SOFO A., SCOPA A., 2014 - *UV-C irradiation effects on young tomato plants: preliminary results.* - Pak. J. Bot., 46(3): 945-949.

CIURLI A., HUARANCCA REYES T., GUGLIELMINETTI L., 2017 - *Commercial advantages on basil architecture by ultraviolet-B irradiation.* - Adv. Hort. Sci., 31(3): 215-221.

DHINDSA R.S., PLUMB-DHINDSA P., THORPE T.A., 1981 - *Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase.* - J. Exp. Bot., 32: 93-101.

ERKAN M., WANG S.Y., WANG C.Y., 2008 - *Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit.* - Postharvest Biol. Technol., 48: 163-171.

FOYER C.H., NOCTOR G., 2000 - *Oxygen processing in photosynthesis: regulation and signaling.* - New Phytol., 146: 350-388.

GONG M., CHEN B., LI Z., GUO L., 2001 - *Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2.* - J. Plant Physiol., 158: 1125-1130.

JIANG L., WANG Y., BJÖRN L.O., LI S., 2011 - *UV-B-induced DNA damage mediates expression changes of cell cycle regulatory genes in* Arabidopsis *root tips.* - Planta, 233: 831-841.

KALBINA I., STRID Å., 2006 - *The role of NADPH oxidase and MAP kinase phosphatase in UV-B-dependent gene expression in* Arabidopsis. - Plant Cell Environ., 29(9): 1783-1793.

KAR M., MISHRA D., 1976 - *Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence.* - Plant Physiol., 57: 315-319.

KOBASHIGAWA C., TAMAYA K., SHIMOMACHI T., 2011 - *Effect of UV-C treatment on plant growth and nutrient contents.* - Acta Horticulturae, 907: 237-242.

LÓPEZ-RUBIRA V., CONESA A., ALLENDE A., ARTÉS F., 2005 - *Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C.* - Postharvest Biol. Technol., 37(2): 174-185.

MA H., XU X., FENG L., 2014 - *Responses of antioxidant defenses and membrane damage to drought stress in fruit bodies of* Auricularia auricula-judae. - World J. Microbiol. Biotechnol., 30: 119-124.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tobacco tissue cultures.* - Physiol. Plantarum, 15(3): 473-497.

NIE W.F., WANG M.M., XIA X.J., ZHOU Y.H., SHI K., CHEN Z.X., YU J.Q., 2013 - *Silencing of tomato RBOH1 and MPK2 abolishes brassinosteroid-induced H_2O_2 generation and stress tolerance.* - Plant Cell Environ., 36: 789-803.

NORMANLY J., 2010 - *Approaching cellular and molecular resolution of auxin biosynthesis and metabolism.* - Cold Spring Harb. Perspect. Biol., 2: 1-17.

SEVERO J., TIECHER A., PIRRELLO J., REGAD F., LATCHÉ A., PECH J.-C., BOUZAYEN M., ROMBALDI C.V., 2015 - *UV-C irradiation modifies the ripening and accumulation of ethylene response factor (ERF) transcripts in tomato fruit.* - Postharvest Biol. Technol., 102: 9-16.

TIECHER A., DE PAULA L.A., CHAVES F.C., ROMBALDI C.V., 2013 - *UV-C effect on ethylene, polyamines and the regulation of tomato fruit ripening.* - Postharvest Biol. Technol., 86: 230-239.

WILLEKENS H., CHAMNONGPOL S., DAVEY M., SCHRAUDNER M., LANGEBARTELS C., VAN MONTAGU M., INZÉ D., VAN CAMP W., 1997 - *Catalase is a sink for H_2O_2 and is indispensable for stress defence in C_3 plants.* - EMBO J., 16(16): 4806-4816.