

Leaf senescence in tomato mutants as affected by irradiance and phytohormones

R.F. CARVALHO¹, C.C. MONTEIRO¹, A.C. CAETANO², M.N. DOURADO², P.L. GRATÃO¹, C.R.B. HADDAD³, L.E.P. PERES², and R.A. AZEVEDO^{2*}

Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal, SP, 14884-900, Brazil¹

Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, SP, 13418-900, Brazil²
Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, 13083-970, Brazil³

Abstract

We explored the interaction between radiation of different wavelength and jasmonic acid (JA) or brassinosteroids (BR) on leaf senescence-induced oxidative stress. Three approaches were used: 1) *jasmonic acid insensitive1-1* (*jail-1*) and brassinosteroid-deficient [*dumpy* (*dpy*)] mutants were treated with red (R) or far-red (FR) radiation; 2) phytochrome-deficient *aurea* (*au*) and *high pigment-1* (*hp-1*) (radiation exaggerated response) mutants were treated with methyl jasmonate (MeJA) or epibrassinolide (epiBL); and 3) double mutants *au jail-1* and *au dpy* were produced. Leaf chlorophyll content, lipid peroxidation, and antioxidant enzyme activities were determined. After senescence induction in detached leaves, we verified that the patterns of chlorophyll degradation of hormonal and photomorphogenic mutants were not significantly different in comparison with original cv. Micro-Tom (MT). Moreover, there was no significant change in lipid peroxidation measured as malondialdehyde (MDA) production, as well as catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activities in the hormonal mutants. Exogenous BR increased CAT and APX activities in MT, *au*, and *hp-1*. As concerns the double mutants, severe reduction in H₂O₂ production which was not accompanied by changes in MDA content, and CAT and APX activities was observed during senescence in *au dpy*. The results suggest that JA and BR do not participate in light signaling pathway during leaf senescence-induced oxidative stress.

Additional key words: antioxidant enzymes, brassinosteroids, jasmonates, lipid peroxidation, oxidative stress, photomorphogenesis, *Solanum lycopersicum*.

Introduction

Leaf senescence is a genetically regulated process characterized by a network of molecular and biochemical mechanisms. The most evident events triggered during senescence are oxidative stress and chlorophyll and protein breakdowns (Khanna-Chopra 2012, Qian *et al.* 2012). Thus, an enhancement in the production of reactive oxygen species (ROS) may occur triggering a complex detoxification mechanism capable of preventing uncontrolled oxidation (Vitória *et al.* 2003, Andrade *et al.* 2009, Cia *et al.* 2012a). ROS-scavenging mechanisms include the action of some key enzymes (Gallego *et al.* 2012), such as superoxide dismutase (SOD, EC 1.15.1.1), which dismutates superoxide radical to H₂O₂, and a range

of peroxidases that break down H₂O₂, among others catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) (Gratão *et al.* 2005, Azevedo *et al.* 2012). In addition, for the detoxification of H₂O₂, phenolic compounds can act as antioxidants by donating electrons to guaiacol peroxidase (GPOX, EC 1.11.1.7; Gratão *et al.* 2005). The ascorbate-glutathione cycle is also important and allows the regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) by glutathione reductase (GR, EC 1.8.1.7) using NAD(P)H as a reducing agent (Ghelfi *et al.* 2011, Foyer and Noctor 2012, Jozefczak *et al.* 2012).

Nowadays, it is also known that the oxidative stress

Received 22 November 2012, accepted 18 February 2013.

Abbreviations: APX - ascorbate peroxidase; BR - brassinosteroids; CAT - catalase; Chl - chlorophyll; epiBL - epibrassinolide; FR - far red radiation; GR - glutathione reductase; JA - jasmonic acid; MDA - malondialdehyde; MeJA - methyl jasmonate; MT - cv. Micro-Tom; R - red radiation; ROS - reactive oxygen species; SOD - superoxide dismutase.

Acknowledgements: This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Grants Nos. 2008/53065-4 and 2009/54676-0).

* Corresponding author; fax: (+55) 3447 8620, e-mail: raa@usp.br

is strongly controlled by phytohormones (Gratão *et al.* 2012, Monteiro *et al.* 2011, 2012). They can interact with a wide range of other molecules, particularly those of light signaling pathways, such as phytochromes (Carvalho *et al.* 2010). In fact, several responses have been shown to be modulated by the interaction between phytochromes and phytohormones. For example, phytochromes interact with abscisic acid (ABA) and gibberellin (GA) on seed germination (Seo *et al.* 2006, Oh *et al.* 2007), with cytokinin on flowering (Zheng *et al.* 2006), and with ethylene (ET) (Li *et al.* 2011) and auxin (AUX) (Franklin *et al.* 2011) on stem elongation. In a jasmonic acid (JA)-deficient rice mutant *hebiba*, the failure to produce jasmonates resulted in an elevated content of phytochrome type A in its active form which was especially pronounced under continuous far-red (FR) radiation (Riemann *et al.* 2009). Robson *et al.* (2010) reported that the *coi1-16 Arabidopsis thaliana* mutant deficient in JA signaling was also deficient in a subset of FR responses. This mutant displayed exaggerated shade response to low, but not high, red (R)/FR ratio suggesting a role of JA in phytochrome signaling. The participation of BRs in light signaling has been investigated by Luo *et al.* (2010), Tang *et al.* (2010), Fan *et al.* (2012), and Jaillais and Vert (2012). For instance, the use of a BR biosynthesis-defective mutant of *Arabidopsis* (*det2*) as well as the use of exogenous BR revealed a fundamental role of BR in the expression of light-responsive genes (Song *et al.* 2009) which probably involves a phyto-

chrome-dependent network (Luccioni *et al.* 2002, Jeong *et al.* 2007).

Although it has been shown that radiation (Xu *et al.* 2009, Carvalho *et al.* 2011, Lopez *et al.* 2012), JA (Ge *et al.* 2010, Liu *et al.* 2012a), and BR (Hasan *et al.* 2011, Xia *et al.* 2011) can separately modulate plant growth under stress condition, the involvement of JA and BR in light signaling in the stress responses is still little understood. So far, some evidence shows that phytochromes are required for a JA-modulated wound response (He *et al.* 2005, Moreno *et al.* 2009, Robson *et al.* 2010, Cerrudo *et al.* 2012). In *Arabidopsis*, the expression of pyrroline-5-carboxylate synthase (P5CS, EC 2.7.2.11), which catalyze the rate-limiting steps of proline biosynthesis, can be induced by light but inhibited by BR (Abrahám *et al.* 2003). However, a more comprehensive molecular and biochemical understanding of how photoreceptors interact with JA or BR during stress response are still not available for many species and stress conditions.

In this study, three approaches were used to explore the interaction between radiation and hormones during oxidative stress induced by tomato leaf senescence: *jasmonic acid insensitive1-1* (*jai1-1*) and brassinosteroid-deficient (*dpy*) mutants were exposed to R or FR radiation; phytochrome-deficient *aurea* (*au*) and high pigment-1 (*hp-1*) (radiation exaggerated response) mutants were treated with exogenous JA or BR; and *au jai1-1* and *au dpy* double mutants were constructed.

Materials and methods

The tomato (*Solanum lycopersicum* Mill.) *au*, *hp-1*, *dpy*, and *jai1-1* mutants were obtained in the miniature cv. Micro-Tom (MT; Li *et al.* 2004, Carvalho *et al.* 2011) and the double mutants used were developed according to Weigel and Glazebrook (2008). The *au jai1-1* double mutant was selected initially for chlorotic aspect expressed by the *au* mutation (Fig. 1A) and the MeJA insensitivity expressed by *jai1-1* which was confirmed by PCR-based analysis as described by Li *et al.* (2004) for the *jai1-1* mutation. The *au dpy* double mutant was selected for chlorotic aspect and for the short height and curled leaves of the *dpy* (Fig. 1B; Koka *et al.* 2000). The MT was used as the wild-type counterpart.

Seeds were germinated in trays containing a commercial mix (*Plantmax HT*, *Eucatex*, Salto, Brazil) and *Vermiculite* supplemented with 0.001 g cm⁻³ NPK (1:1:1) and 0.004 g cm⁻³ lime. Ten days after germination, the seedlings were transferred to 150 cm³ pots containing the aforementioned substrate mix. The cultivation was carried out in a glasshouse under automatic irrigation (three times per day to field capacity; Medici *et al.* 2010) at temperature of 28 °C, a 13-h photoperiod, irradiance of 250 - 350 µmol m⁻² s⁻¹ and air humidity of 80 %.

The third leaves from 35-d-old plants were cut at the basis of the petioles with a sharp scalpel, placed inside

black boxes, and stored at 25 °C for 48 h under different hormonal or radiation treatments. Leaves of hormonal mutants (*dpy* and *jai1-1*) were treated each hour with 15 min R or FR radiations. Monochromatic R radiation was obtained from white fluorescent lamps (20 W) by filtration through a 3 mm red acrylic sheet (30 µmol m⁻² s⁻¹) whereas the FR radiation was obtained from incandescent bulb lamps (40 W) by filtration through blue and red acrylic filters (10 µmol m⁻² s⁻¹). Prior to leaf senescence induction, the 30-d-old photomorphogenic mutants (*au* and *hp-1*) were sprayed with 100 µM methyl jasmonate (MeJA) or 1 µM 24-epibrassinolide (epiBL). Leaves of *au* and *hp-1* single mutants treated with the hormones and the double mutant were kept in the dark during senescence. At the beginning (0 h) and after 48 h, leaves were used for chlorophyll measurements or stored at -80 °C for biochemical analyses.

Leaf discs (0.5 cm diameter) were used for chlorophyll spectrophotometric determination. The extraction was carried out by adding 2 cm³ of methanol (Holm-Hansen and Riemann 1978). The total chlorophyll content was calculated as described by Lichtenthaler (1987).

Lipid peroxidation was determined by estimating the content of thiobarbituric acid reactive substances (TBARS) following the method of Heath and Packer

(1968) and optimized by Cia *et al.* (2012b). The concentration of malondialdehyde (MDA) was calculated using a coefficient of absorbance of $155 \text{ mM}^{-1} \text{ cm}^{-1}$. The content of H_2O_2 was determined as described by Alexieva *et al.* (2001). Leaves were homogenized in 0.1 % (m/v) trichloroacetic acid, the homogenate centrifuged at 12 100 g and 4 °C for 15 min, and 0.2 cm^3 of the supernatant was added to 0.2 cm^3 of 100 mM K-phosphate buffer (pH 7.0) plus 0.8 cm^3 of 1 M KI. Absorbance was read at 390 nm.

For determination of enzyme activities, leaves were homogenized in a mortar with a pestle in 100 mM K-phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM dithiothreitol, and 5 % (m/v) insoluble polyvinylpyrrolidone at 4 °C (Azevedo *et al.* 1998). The homogenate was centrifuged at 10 000 g for 30 min and the supernatant was stored in separate aliquots at -80 °C prior to enzymatic analyses. Protein content for all samples was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Catalase (CAT) activity was assayed at 25 °C in a reaction mixture containing 1 cm^3 of 100 mM K-phosphate buffer (pH 7.5) and 0.0025 cm^3 of 30 % (v/v) H_2O_2 (Gratão *et al.* 2008). The reaction was initiated by the addition of 0.025 cm^3 of the plant extract and the activity determined by following the decomposition of H_2O_2 as a change in absorbance at 240 nm over 1 min.

Glutathione reductase (GR) activity was assayed at 30 °C in a mixture consisting of 1 cm^3 of 100 mM K-phosphate buffer (pH 7.5), 1 mM 5,5'-dithio-2-nitrobenzoic acid (DTNB), 1 mM oxidized glutathione (GSSG), and 0.1 mM NADPH (Martins *et al.* 2011). The reaction was started by the addition of 0.05 cm^3 of the enzyme extract. The rate of reduction of GSSG was followed in a spectrophotometer by monitoring the increase in absorbance at 412 nm over 1 min.

Ascorbate peroxidase (APX) activity was determined by the addition of 0.04 cm^3 of the plant extract to 1 cm^3 of the reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 0.1 mM H_2O_2 (Nakano and Asada 1981). APX activity was determined by monitoring the rate of ascorbate oxidation at 290 nm at 30 °C.

SOD activity was determined following the method of Giannopolitis and Rues (1977) by measuring the enzyme ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction solution (3 cm^3) contained 75 μM NBT, 2 μM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM K-phosphate buffer (pH 7.8), and 0.03 cm^3 of the enzyme extract. The reaction solutions in test tubes were irradiated by 15 W fluorescent lamps for 5 min. The enzyme activity was determined at 560 nm.

All analyses were performed in three replicates. Data were analyzed by ANOVA followed by the Tukey's test.

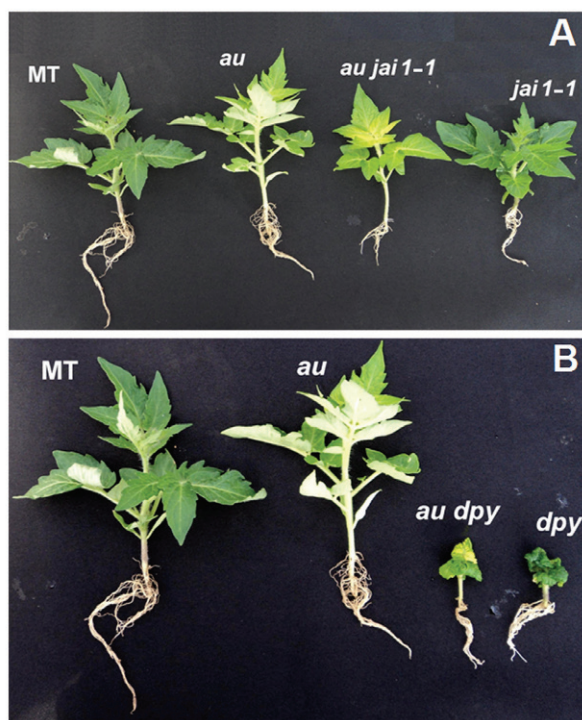


Fig. 1. Phenotypes of the different tomato mutants. A - the double mutant *au jai1-1* was selected initially for chlorotic aspect expressed by the mutant *au*, and MeJA insensibility displayed by the mutant *jai1-1* which was confirmed by PCR. B - the double mutant *au dpy* shows chlorotic aspect and elongated hypocotyl due to the *au* mutation, short stature, and leaf curled morphology expressed by the *dpy* mutation.

Results

Chlorophyll (Chl) degradation is the most obvious visible aspect during leaf senescence. At time zero, MT, *jail-1*, and *dpy* exhibited similar Chl content (Table 1). During senescence, these genotypes exhibited similar reduction of Chl content under R and FR radiation except the *dpy* mutant which exhibited higher Chl retention under R radiation (Table 1).

MDA content was lower in *jail-1* mutant than in MT at 0 h but did not differ from MT during the radiation treatments whereas the *dpy* mutant exhibited less induction of MDA under R radiation (Table 1). At 0 h, the activities of CAT, APX, and GR of MT, and the *jail-1* and *dpy* single mutants were similar (Table 1), nevertheless, after senescence induction, CAT and GR activities of the *jail-1* mutant under FR were higher when compared to MT (Table 1).

As concern the photomorphogenic mutants pretreated with MeJA or epiBL, *au* and *hp-1* leaves exhibited lower

and higher Chl content, respectively, than MT at 0 h. After 48 h, only the *hp-1* mutant exhibited higher Chl retention in water, MeJA, and epiBL treatments when compared to MT (Table 2). Although variations were observed for MDA production, only the *hp-1* mutant treated with epiBL exhibited slightly higher lipid peroxidation after senescence than MT (Table 2). When the activity of the antioxidant enzymes is concerned, variations were observed in response to the epiBL treatment, resulting in increased CAT, APX and GR activities for all genotypes indistinctively during senescence (Table 2).

We constructed *au jail-1* and *au dpy* double mutants to further investigate the hormonal-photomorphogenic control of oxidative stress. The most evident change observed was the reduction of the H₂O₂ content at 0 h observed in *jail-1* mutant when compared to MT and *au jail-1* (Table 3). After senescence induction,

Table 1. Chl content [$\mu\text{g cm}^2$ (f.m.)], MDA content [nmol g^{-1} (f.m.)], and specific activities [$\mu\text{mol mg}^{-1}(\text{protein min}^{-1})$] of CAT, APX, and GR in leaves of hormonal mutants at time zero (0 h) and after senescence (48 h). During senescence, leaves were treated with R or FR radiations. Means \pm SE, $n = 3$. Different letters in the same column indicate significant differences at $P < 0.05$.

Genotypes	Time [h]	Radiation	Chl	MDA	CAT	APX	GR
MT	0	-	5.53 \pm 0.14 ^{ab}	3.60 \pm 0.10 ^a	59.62 \pm 9.06 ^c	774.46 \pm 150.9 ^{ab}	0.44 \pm 0.06 ^{bc}
	48	R	1.95 \pm 0.33 ^{bc}	4.12 \pm 0.09 ^a	66.78 \pm 6.65 ^{bc}	847.79 \pm 60.17 ^{ab}	0.40 \pm 0.05 ^{bc}
	48	FR	1.99 \pm 0.03 ^{bc}	3.05 \pm 0.07 ^{ab}	73.15 \pm 4.64 ^{bc}	511.88 \pm 110.0 ^b	0.30 \pm 0.03 ^c
<i>jail-1</i>	0	-	5.63 \pm 0.09 ^{ab}	2.66 \pm 0.01 ^b	44.15 \pm 0.88 ^c	552.40 \pm 110.9 ^b	0.50 \pm 0.02 ^{bc}
	48	R	2.24 \pm 0.19 ^{bc}	3.33 \pm 0.01 ^{ab}	39.88 \pm 1.84 ^c	570.15 \pm 62.92 ^b	0.35 \pm 0.03 ^c
	48	FR	2.35 \pm 0.28 ^{bc}	3.63 \pm 0.01 ^a	133.98 \pm 18.1 ^a	605.17 \pm 69.84 ^b	0.94 \pm 0.09 ^a
<i>dpy</i>	0	-	6.20 \pm 0.41 ^a	2.93 \pm 0.07 ^{ab}	72.46 \pm 4.63 ^{bc}	742.96 \pm 33.88 ^{ab}	0.44 \pm 0.08 ^{bc}
	48	R	5.34 \pm 0.14 ^{ab}	2.31 \pm 0.06 ^b	47.12 \pm 9.21 ^{bc}	779.97 \pm 54.07 ^{ab}	0.34 \pm 0.04 ^c
	48	FR	3.46 \pm 0.40 ^{bc}	3.31 \pm 0.06 ^{ab}	90.28 \pm 12.7 ^{ab}	1098.32 \pm 73.20 ^a	0.67 \pm 0.06 ^{ab}

Table 2. Chl content [$\mu\text{g cm}^2$ (f.m.)], MDA content [nmol g^{-1} (f.m.)], and specific activities [$\mu\text{mol mg}^{-1}(\text{protein min}^{-1})$] of CAT, APX, and GR of leaves of photomorphogenic mutants at time zero control (0 h) and after senescence (48 h). During senescence, leaves were treated with H₂O (control), MeJA, or epiBL. Values are means \pm SE, $n = 3$. Different letters in the same column indicate significant differences at $P < 0.05$.

Genotypes	Time [h]	Hormones	Chl	MDA	CAT	APX	GR
MT	0	-	6.77 \pm 0.05 ^b	4.01 \pm 0.27 ^{abc}	62.54 \pm 3.33 ^{cd}	242.23 \pm 10.41 ^c	1.25 \pm 0.04 ^{abc}
	48	H ₂ O	2.13 \pm 0.10 ^c	4.86 \pm 0.41 ^{ab}	47.10 \pm 4.97 ^{cd}	226.59 \pm 10.55 ^c	1.19 \pm 0.08 ^{abc}
	48	MeJA	1.28 \pm 0.05 ^c	3.83 \pm 0.02 ^{bc}	26.37 \pm 2.49 ^d	230.06 \pm 27.43 ^c	0.90 \pm 0.08 ^{bc}
	48	epiBL	4.29 \pm 0.39 ^{bc}	5.28 \pm 0.02 ^{ab}	192.32 \pm 5.14 ^b	1707.11 \pm 21.70 ^a	1.88 \pm 0.24 ^{ab}
<i>au</i>	0	-	3.94 \pm 0.46 ^{bc}	5.42 \pm 0.62 ^{ab}	64.57 \pm 4.35 ^c	339.95 \pm 61.69 ^c	1.53 \pm 0.05 ^{abc}
	48	H ₂ O	3.48 \pm 0.33 ^{bc}	3.92 \pm 0.56 ^{bc}	56.25 \pm 9.22 ^{cd}	279.46 \pm 57.31 ^c	1.50 \pm 0.32 ^{abc}
	48	MeJA	3.00 \pm 0.34 ^{bc}	2.77 \pm 0.33 ^c	34.96 \pm 7.82 ^{cd}	312.08 \pm 33.52 ^c	0.77 \pm 0.19 ^c
	48	epiBL	3.29 \pm 0.37 ^{bc}	4.21 \pm 0.04 ^b	159.13 \pm 6.50 ^b	945.38 \pm 4.20 ^b	2.15 \pm 0.44 ^a
<i>hp-1</i>	0	-	9.92 \pm 0.26 ^a	4.18 \pm 0.77 ^{abc}	105.93 \pm 4.60 ^b	468.76 \pm 149.9 ^{bc}	1.14 \pm 0.30 ^{abc}
	48	H ₂ O	7.24 \pm 0.24 ^{ab}	3.82 \pm 0.04 ^{bc}	52.99 \pm 24.1 ^d	246.63 \pm 29.18 ^c	0.77 \pm 0.11 ^c
	48	MeJA	5.51 \pm 0.24 ^b	3.92 \pm 0.63 ^{bc}	122.72 \pm 19.4 ^b	441.79 \pm 20.48 ^{bc}	1.67 \pm 0.17 ^{abc}
	48	epiBL	4.87 \pm 0.12 ^b	6.29 \pm 0.31 ^a	263.73 \pm 0.54 ^a	1033.47 \pm 25.57 ^{ab}	1.69 \pm 0.02 ^{abc}

Table 3. H₂O₂ [$\mu\text{mol g}^{-1}(\text{f.m.})$] and MDA [$\text{nmol g}^{-1}(\text{f.m.})$] content, and specific activities of CAT, APX, GR [$\mu\text{mol mg}^{-1}(\text{protein}) \text{min}^{-1}$], and SOD [$\text{U mg}^{-1}(\text{protein})$] of leaves of double mutants at time zero control (0 h) and after senescence (48 h). Means \pm SE, $n = 3$. Different letters in the same column indicate significant differences at $P < 0.05$.

Genotypes	Time [h]	H ₂ O ₂	MDA	CAT	APX	SOD	GR
MT	0	0.24 \pm 0.05 ^{ab}	0.27 \pm 0.03 ^a	86.85 \pm 11.2 ^b	1045.28 \pm 103.7 ^{ab}	5.89 \pm 1.24 ^{abcd}	0.28 \pm 0.04 ^{ab}
	48	0.25 \pm 0.02 ^{ab}	0.15 \pm 0.01 ^{ab}	67.42 \pm 3.54 ^b	307.38 \pm 59.17 ^e	2.56 \pm 0.40 ^d	0.17 \pm 0.03 ^{bc}
<i>au</i>	0	0.14 \pm 0.02 ^{abc}	0.19 \pm 0.02 ^{ab}	75.10 \pm 6.26 ^b	673.80 \pm 20.26 ^{bc}	2.77 \pm 0.34 ^{cd}	0.22 \pm 0.04 ^{abc}
	48	0.19 \pm 0.03 ^{abc}	0.14 \pm 0.01 ^b	71.01 \pm 2.56 ^b	340.07 \pm 34.31 ^{de}	2.48 \pm 0.20 ^d	0.20 \pm 0.03 ^{abc}
<i>dpy</i>	0	0.10 \pm 0.03 ^{bc}	0.22 \pm 0.04 ^{ab}	138.88 \pm 19.8 ^a	461.51 \pm 93.02 ^{cde}	9.25 \pm 1.74 ^a	0.33 \pm 0.01 ^a
	48	0.27 \pm 0.01 ^a	0.21 \pm 0.01 ^{ab}	89.57 \pm 9.00 ^b	222.93 \pm 45.57 ^e	5.65 \pm 0.55 ^{abcd}	0.21 \pm 0.05 ^{abc}
<i>jail-1</i>	0	0.06 \pm 0.01 ^c	0.19 \pm 0.04 ^{ab}	76.84 \pm 11.5 ^b	506.40 \pm 83.38 ^{cde}	4.70 \pm 0.42 ^{bcd}	0.21 \pm 0.01 ^{abc}
	48	0.32 \pm 0.05 ^a	0.22 \pm 0.01 ^{ab}	71.88 \pm 3.45 ^b	255.54 \pm 35.90 ^e	4.96 \pm 0.76 ^{bcd}	0.11 \pm 0.03 ^c
<i>au dpy</i>	0	0.19 \pm 0.02 ^a	0.22 \pm 0.01 ^{ab}	99.10 \pm 6.17 ^{ab}	971.86 \pm 75.24 ^{ab}	6.78 \pm 0.41 ^{abc}	0.29 \pm 0.03 ^{ab}
	48	0.03 \pm 0.01 ^c	0.17 \pm 0.02 ^{ab}	97.57 \pm 11.8 ^{ab}	252.86 \pm 84.10 ^e	8.73 \pm 0.73 ^{ab}	0.18 \pm 0.01 ^{abc}
<i>au jail-1</i>	0	0.24 \pm 0.01 ^{ab}	0.19 \pm 0.02 ^{ab}	77.55 \pm 7.24 ^b	633.50 \pm 7.74 ^{cd}	5.06 \pm 0.89 ^{bcd}	0.24 \pm 0.03 ^{abc}
	48	0.27 \pm 0.05 ^{ab}	0.24 \pm 0.02 ^{ab}	105.62 \pm 6.47 ^{ab}	344.21 \pm 22.45 ^{de}	7.98 \pm 0.68 ^{ab}	0.24 \pm 0.01 ^{abc}

a severe reduction in H₂O₂ production was observed in *au dpy* when compared to MT as well as *dpy* simple mutant. However, MDA production did not differ among these genotypes at 0 and 48 h (Table 3). Enhanced CAT activity of *dpy* (Table 3) and reduced APX activity of *dpy*, *jail-1*, and *au jail-1* (Table 3)

were observed at 0 h. When senescence was induced, SOD activity of MT and *au* (Table 3), and GR activity of *jail-1* (Table 3) were reduced. Moreover, an abrupt reduction of APX activity for all the genotypes at 48 h was also detected.

Discussion

We have attempted three distinct approaches in order to explore how the interaction between radiation and hormones influences biochemical mechanisms during leaf senescence: 1) hormonal mutants were treated with R or FR radiations; 2) photomorphogenic mutants treated with hormones; and 3) double mutants. In fact, the use of double mutants is an additional useful tool in the study of signal transduction and changes to the regulation of metabolic pathways (Carvalho *et al.* 2010, Nole-Wilson *et al.* 2010).

The more recent literature on this topic revealed that phytochromes (Huq *et al.* 2004, Tang *et al.* 2012), JA (Jung 2004, Reinbothe *et al.* 2009), and BR (Saglam-Cag 2007, Fariduddin *et al.* 2008) can modulate Chl metabolism. However, in this study, Chl content of *jail-1* and *dpy* tomato mutants did not exhibit any differences from that of MT before senescence (Table 1). Moreover, R and FR radiations induced Chl degradation in *jail-1*, *dpy*, and MT besides the fact that the *dpy* mutant retained insignificantly more Chl under R radiation treatment. In accordance to this, photomorphogenic mutants treated with MeJA or epiBL did not exhibit different changes in the Chl content after senescence induction when compared with MT (Table 2). This includes the enhanced pigment accumulation of *hp-1* when compared to MT at 0 and 48 h which is due to the well documented light exaggerated responses normally exhibited by this mutant (Jarret *et al.* 1984, Wann *et al.* 1985). These observations indicate that although irradiance evidently controls Chl

biosynthesis in tomato, due to the Chl reduction in the phytochrome-deficient *au* mutant (Table 2), JA and BR as well as the interaction between irradiance and these hormones do not appear to modulate Chl metabolism during leaf senescence

On the first inspection of oxidative stress triggered by senescence, attention was drawn to the fact that the lipid peroxidation rate was reduced in *jail-1* at 0 h when compared to MT (Table 1), as well as H₂O₂ content at 0 h in the double mutants (Table 3). Although it could be argued that JA was involved in the modulation of lipid peroxidation, the MDA content of *jail-1* mutant did not differ from that of the wild type counterpart in the third experimental approach tested (Table 3). Although it has been shown that exogenous JA can stimulate lipid peroxidation estimated by MDA content (Fedina *et al.* 2009), we did not observe such a response when MeJA was used (Table 2). Although after senescence induction, an increase in MDA was observed in *jail-1* under FR when compared to 0 h (Table 1), such response does not appear to be dependent on radiation signaling, particularly the phytochrome system, since similar MDA content was observed for *jail-1* under R or FR treatments. In accordance to this, MDA content of *au* and *hp-1* at 48 h was similar for MeJA and H₂O treatments (Table 2), as well as the MDA content of the double mutant *au jail-1* did not differ from either single mutants *au* or *jail-1*.

It is interesting to note that the *au dpy* double mutant exhibited a severe reduction in H₂O₂ production

(Table 3) which could have led to a reduction in lipid peroxidation. Nonetheless, the MDA content of the *dpy* mutant was not reduced at 0 and 48 h (Table 3), apart from the fact that the content of MDA exhibited by this mutant under R and FR radiations (Table 1), as well as for *au* and *hp-1* treated with epiBL (Table 2), did not reveal any changes after senescence induction. Thus, our results suggest that, at least through MDA and H₂O₂ formation, JA appears not to be involved in radiation signaling during lipid peroxidation induced by leaf senescence in tomato.

Some parts of a complex biochemical pathway from ROS induction during leaf senescence to the response of antioxidant enzymes (Palma *et al.* 2006) were analyzed in this work. After senescence induction, the *jail-1* mutant under FR exhibited enhanced CAT and GR activities (Table 1). If such a response is mediated by phytochrome photoreversion, a change of CAT and GR activities in *au* mutant treated with MeJA can be expected when compared to MT. However, similar activities in MT and the *au* mutant treated with MeJA, as well as in MT and the double mutant *au jail-1* after senescence induction suggest a minor interaction between phytochrome and JA on CAT and GR activities. Additionally, JA does not appear to be involved in radiation signaling at least regarding the antioxidant enzymes analyzed in this work since MeJA treatment did not affect CAT, APX, and GR activities of *hp-1* at 48 h (Table 2). Nevertheless, the participation of other antioxidant enzymes in this process cannot be ruled out. On the other hand, epiBL strongly induced CAT and APX activities of MT and the *au* and *hp-1* single mutants (Table 2). In fact, exogenous BR has been shown to induce antioxidant enzyme activities in tomato (Ahmed *et al.* 2012, Hayat *et al.* 2012). Thus, a reduction in the MDA content under epiBL treatment could have been expected, although we did not verify H₂O₂ production in this approach. However, MDA production did not change among the genotypes tested suggesting that the effect of exogenous BR on antioxidant enzyme activities during leaf senescence is regardless of the genotypes and that this change is not enough to reduce or minimize the stress induced by senescence (Gratão *et al.* 2005). Therefore, no interpretation can be made regarding the interplay between radiation signaling and BR whereas the enhanced CAT of *hp-1*, when

compared to *au* and MT at 48 h, appears to be an additive effect of the exogenous BR and *hp-1* mutation since *hp-1* exhibited induced CAT activity at 0 h (Table 2).

Although in this research, the induction of CAT and APX activities by exogenous BR can point out to an important role of this hormone in the antioxidant mechanisms during leaf senescence from 0 to 48 h, the brassinosteroid-deficient mutant did not differ from the MT control (Tables 2 and 3). It is important to comment that although exogenous hormones were largely used for a long time, signaling can differ from that of endogenous phytohormones (Cia *et al.* 2012b). Mutants have been intensively used to unveil puzzling molecular and biochemical mechanisms related to plant growth and development (Campos *et al.* 2009, Carvalho *et al.* 2011). We are convinced that the *dpy* mutation can reflect the natural physiological changes observed in this work. So, the similarity in the response by CAT, APX, SOD, and GR activities before and after senescence induction by the *au dpy* double mutant suggests that no interaction between both mutations can support the BR participation in phytochrome signaling.

The radiation signaling depends on a wide range of photoreceptors which can interact with each other (Casal 2000, Hughes *et al.* 2012). In addition, several hormonal interactions are nowadays well elucidated, such as between BR and GA (Gallego-Bartolome *et al.* 2012) as well as between JA and ABA (Brossa *et al.* 2011). Thus, it is difficult to speculate on photoreceptor or hormone functions in many aspects of plant physiology. The nature or regulation of these interactions on the control of leaf senescence is still little understood, and hence, we cannot consider the hormone or photoreceptor responses or any interactions as isolated systems (Davies 2004, Liu *et al.* 2012b), specially because molecular and biochemical mechanisms of senescence control depend on environmental cues, such as temperature and irradiance (Xue *et al.* 2012) among others.

In conclusion, our results suggest that JA and BR may not take part in radiation signaling, particularly using phytochrome photoreceptors, for the control of oxidative stress during leaf senescence in tomato. However, it is clear that a complicated network of molecular and biochemical mechanisms is involved in the leaf senescence.

References

- Ábrahám, E., Rigó, G., Székely, G., Nagy, R., Konecz, C., Szabados, L.: Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. - *Plant mol. Biol.* **51**: 363-372, 2003.
- Andrade, S.A.L., Gratão, P.L., Schiavinato, M.A., Silveira, A.P., Azevedo, R.A., Mazzafera, P.: Zn uptake, physiological response and stress attenuation in mycorrhizal Jack bean growing in soil with increase Zn concentration. - *Chemosphere* **75**: 1363-1370, 2009.
- Ahmed, G.J., Gao, C.-J., Ogwen, J.O., Zhou, Y.-H., Xia, X.-J., Mao, W.-H., Shi, K., Yu, J.-Q.: Brassinosteroids induce plant tolerance against phenanthrene by enhancing degradation and detoxification in *Solanum lycopersicum* L. - *Ecotoxicol. environ. Safety* **80**: 28-36, 2012.
- Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E.: The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. - *Plant Cell Environ.* **24**: 1337-1344, 2001.
- Azevedo, R.A., Alas, R.M., Smith, R.J., Lea, P.J.: Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots

- of wild-type and a catalase-deficient mutant of barley. - *Physiol. Plant.* **104**: 280-292, 1998.
- Azevedo, R.A., Gratão, P.L., Monteiro, C.C., Carvalho, R.F.: What is new in the research on cadmium-induced stress in plants? - *Food Energy Security* **1**: 133-140, 2012.
- Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - *Anal. Biochem.* **72**: 248-254, 1976.
- Brossa, R., Lopez-Carbonell, M., Jubany-Mari, T., Alegre, L.: Interplay between abscisic acid and jasmonic acid and its role in water-oxidative stress in wild-type, ABA-deficient, JA-deficient and ascorbate-deficient *Arabidopsis* plants. - *J. Plant Growth Regul.* **30**: 322-333, 2011.
- Campos, M.L., De Almeida, M., Rossi, M.L., Martinelli, A.P., Litholdo Jr., C.G., Figueira, A., Rampelotti-Ferreira, F.T., Vendramim, J.D., Benedito, V.A., Peres, L.E.P.: Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. - *J. exp. Bot.* **60**: 4347-4361, 2009.
- Carvalho, R.F., Campos, M.L., Pino, L.E., Crestana, S.L., Zsogon, A., Lima, J.E., Benedito, V.A., Peres, L.E.P.: Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. - *Plant Meth.* **7**: 18, 2011.
- Carvalho, R.F., Quecini, V., Peres, L.E.P.: Hormonal modulation of photomorphogenesis-controlled anthocyanin accumulation in tomato (*Solanum lycopersicum* L. cv. Micro-Tom) hypocotyls: physiological and genetic studies. - *Plant Sci.* **178**: 258-264, 2010.
- Casal, J.J.: Phytochromes, cryptochromes, phototropin: photo-receptor interactions in plants. - *Photochem. Photobiol.* **71**: 1-11, 2000.
- Cerrudo, I., Keller, M.M., Cargnel, M.D., Demkura, P.V., De Wit, M., Patitucci, M.S., Pierik, R., Pieterse, C.M.J., Ballaré, C.L.: Low red/far-red ratios reduce *Arabidopsis* resistance to *Botrytis cinerea* and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. - *Plant Physiol.* **158**: 2042-2052, 2012.
- Cia, M.C., Capaldi, F.R., Carvalho, R.F., Gratão, P.L., Azevedo, R.A.: New approaches to study metal-induced stress in plants. - In: Ahmad, P., Prasad, M.N.V. (ed.): *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*. Pp. 413-427. Springer, New York 2012a.
- Cia, M.C., Guimarães, A.C.R., Medici, L.O., Chabregas, S.M., Azevedo, R.A.: Antioxidant responses to water deficit by drought-tolerant and -sensitive sugarcane varieties. - *Ann. appl. Biol.* **161**: 313-324, 2012b.
- Davies, P.J.: Regulatory factors in hormone action: level, location and signal transduction plant hormones. - In: Davies, P.J. (ed.): *Plant hormones: Biosynthesis, Signal Transduction, Action*. Pp. 16-35. Kluwer Academic Press, Dordrecht 2004.
- Fan, X.-Y., Sun, Y., Cao, D.-M., Bai, M.-Y., Luo, X.-M., Yang, H.-J., Wei, C.-Q., Zhu, S.-W., Sun, Y., Chong, K., Wang, Z.-Y.: BZS1, a B-box protein, promotes photomorphogenesis downstream of both brassinosteroid and light signaling pathways. - *Mol. Plant* **5**: 591-600, 2012.
- Fariduddin, Q., Hasan, S.A., Ali, B., Hayat, S., Ahmad, A.: Effect of modes of application of 28-homobrassinolide on mung bean. - *Turk. J. Biol.* **32**: 17-21, 2008.
- Fedina, I., Nedeva, D., Georgieva, K., Velitchkova, M.: Methyl jasmonate counteract UV-B stress in barley seedlings. - *J. Agron. Crop Sci.* **195**: 204-212, 2009.
- Foyer, C.H., Noctor, G.: Managing the cellular redox hub in photosynthetic organisms. - *Plant Cell Environ.* **35**: 199-201, 2012.
- Franklin, K.A., Lee, S.H., Patel, D., Kumar, S.V., Spartz, A.K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J.D., Wigge, P.A., Gray, W.M.: PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. - *Proc. nat. Acad. Sci. USA* **108**: 20231-20235, 2011.
- Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales, E.P., Zawoznik, M.S., Groppa, M.D., Benavides, M.P.: Unraveling cadmium toxicity and tolerance in plants: insight into regulatory mechanism. - *Environ. exp. Bot.* **83**: 33-46, 2012.
- Gallego-Bartolome, J., Minguet, E.G., Grau-Enguix, F., Abbas, M., Locascio, A., Thomas, S.G., Alabadi, D., Blazquez, M.A.: Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in *Arabidopsis*. - *Proc. nat. Acad. Sci. USA* **109**: 13446-13451, 2012.
- Ge, Y.-X., Zhang, L.-J., Li, F.-H., Chen, Z.-B., Wang, C., Yao, Y.-C., Han, Z.-H., Zhang, J., Shi, Z.-S.: Relationship between jasmonic acid accumulation and senescence in drought-stress. - *Afr. J. agr. Res.* **5**: 1978-1983, 2010.
- Ghelfi, A., Gaziola, S.A., Cia, M.C., Chabregas, S.M., Falco, M.C., Kuser-Falcão, P.R., Azevedo, R.A.: Cloning, expression, molecular modelling and docking analysis of glutathione transferase from *Saccharum officinarum*. - *Ann. appl. Biol.* **159**: 267-280, 2011.
- Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases: I. Occurrence in higher plants. - *Plant Physiol.* **59**: 309-314, 1977.
- Gratão, P.L., Polle, A., Lea, P.J., Azevedo, R.A.: Making the life of heavy metal-stressed plants a little easier. - *Funct. Plant Biol.* **32**: 481-494, 2005.
- Gratão, P.L., Monteiro, C.C., Carvalho, R.F., Tezotto, T., Piotto, F.A., Peres, L.E.P., Azevedo, R.A.: Biochemical dissection of *diageotropica* and *Never ripe* tomato mutants to Cd-stressful conditions. - *Plant Physiol. Biochem.* **56**: 79-96, 2012.
- Gratão, P.L., Monteiro, C.C., Peres, L.E.P., Azevedo, R.A.: The isolation of antioxidant enzymes from mature tomato (cv. Micro-Tom) plants. - *HortScience* **43**: 1608-1610, 2008.
- Hasan, S.A., Hayat, S., Ahmad, A.: Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. - *Chemosphere* **84**: 1446-1451, 2011.
- Hayat, S., Alyemeni, M.N., Hasan, S.A.: Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. - *Saudi. J. biol. Sci.* **19**: 325-335, 2012.
- Heath, R., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - *Arch. Biochem. Biophys.* **125**: 189-198, 1968.
- He, G.Z., Tarui, Y., Iino, M.: A novel receptor kinase involved in jasmonate-mediated wound and phytochrome signaling in maize coleoptiles. - *Plant Cell Physiol.* **46**: 870-883, 2005.
- Holm-Hansen, O., Riemann, B.: Chlorophyll *a* determination: improvements in methodology. - *Oikos* **30**: 438-447, 1978.
- Hughes, R.M., Vrana, J.D., Song, J., Tucker, C.L.: Light-dependent, dark-promoted interaction between *Arabidopsis* cryptochrome 1 and phytochrome B proteins. - *J. biol. Chem.* **287**: 22165-22172, 2012.
- Huq, E., Al-Sady, B., Hudson, M., Kim, C.H., Apel, M., Quail, P.H.: PHYTOCHROME-INTERACTING FACTOR 1 is a critical bHLH regulator of chlorophyll biosynthesis. -

- Science **305**: 1937-1941, 2004.
- Jaillais, Y., Vert, G.: Brassinosteroids, gibberellins and light-mediated signalling are the three-way controls of plant sprouting. - *Nature Cell Biol.* **14**: 788-790, 2012.
- Jarret, R.L., Sayama, H., Tigchelaar, E.C.: Pleiotropic effects associated with the chlorophyll intensifier mutations *high pigment* and *dark green* in tomato. - *Amer. Soc. hort. Sci.* **109**: 873-878, 1984.
- Jeong, D.H., Lee, S., Kim, S.L., Hwang, I., An, G.: Regulation of brassinosteroid responses by phytochrome B in rice. - *Plant Cell Environ.* **30**: 590-599, 2007.
- Jozefczak, M., Remans, T., Vangronsveld, J., Cuypers, A.: Glutathione is a key player in metal-induced oxidative stress defense. - *Int. J. Mol. Sci.* **13**: 3145-3175, 2012.
- Jung, S.: Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. - *Plant Physiol. Biochem.* **42**: 225-231, 2004.
- Khanna-Chopra, R.: Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. - *Protoplasma* **249**: 469-481, 2012.
- Koka, C.V., Cerny, R.E., Gardner, R.G., Noguchi, T., Fujioka, S., Takatsuto, S., Yoshida, S., Clouse, S.D.: A putative role for the tomato genes *dumpy* and *curl-3* in brassinosteroid biosynthesis and response. - *Plant Physiol.* **122**: 85-98, 2000.
- Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E., Howe, G.A.: The tomato homolog of CORONATINE-INSENSITIVE1 is required for maternal control of seed maturation, jasmonate-signaling defense responses, and glandular trichome development. - *Plant Cell* **16**: 126-143, 2004.
- Li, W., Song, Z., Neil Emery, R., Chinnappa, C.: Effects of day length, light quality and ethylene on *PHYTOCHROME B* expression during stem elongation in *Stellaria longipes*. - *Plant Growth Regul.* **63**: 291-300, 2011.
- Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. - *Meth. Enzymol.* **148**: 350-382, 1987.
- Liu, X., Chi, H., Yue, M., Zhang, X., Li, W., Jia, E.: The regulation of exogenous jasmonic acid on UV-B stress tolerance in wheat. - *J. Plant Growth Regul.* **31**: 436-447, 2012a.
- Liu, Y.D., Yin, Z.J., Yu, J.W., Li, J., Wei, H.L., Han, X.L., Shen, F.F.: Improved salt tolerance and delayed leaf senescence in transgenic cotton expressing the *Agrobacterium IPT* gene. - *Biol. Plant.* **56**: 237-246, 2012b.
- Lopez, L., Carbone, F., Bianco, L., Giuliano, G., Facella, P., Perrotta, G.: Tomato plants overexpressing cryptochrome 2 reveal altered expression of energy and stress-related gene products in response to diurnal cues. - *Plant Cell Environ.* **35**: 994-1012, 2012.
- Luccioni, L.G., Oliverio, K.A., Yanovsky, M.J., Boccalandro, H.E., Casal, J.J.: Brassinosteroid mutants uncover fine tuning of phytochrome signaling. - *Plant Physiol.* **128**: 173-181, 2002.
- Luo, X-M., Lin, W-H., Zhu, S., Zhu, J-Y., Sun, Y., Fan, X-Y., Cheng, M., Hao, Y., Oh, E., Tian, M., Liu, L., Zhang, M., Xie, Q., Chong, K., Wang, Z-Y.: Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in *Arabidopsis*. - *Dev. Cell* **19**: 872-883, 2010.
- Martins, P.F., Carvalho, G., Gratão, P.L., Dourado, M.N., Pileggi, M., Araújo, W.L., Azevedo, R.A.: Effects of the herbicides acetochlor and metolachlor on antioxidant enzymes in soil bacteria. - *Process Biochem.* **46**: 1186-1195, 2011.
- Medici, L.O., Rocha, H.S., Carvalho, D.F., Pimentel, C., Azevedo, R.A.: Automatic controller to water plants. - *Sci. Agr.* **67**: 727-730, 2010.
- Monteiro, C.C., Carvalho, R.F., Gratão, P.L., Carvalho, G., Tezotto, T., Medici, L.O., Peres, L.E.P., Azevedo, R.A.: Biochemical responses of the ethylene-insensitive *Never ripe* tomato mutant subjected to cadmium and sodium stresses. - *Environ. exp. Bot.* **71**: 306-320, 2011.
- Monteiro, C.C., Rolão, M.B., Franco, M.R., Peters, L.P., Cia, M.C., Capaldi, F.R., Carvalho, R.F., Gratão, P.L., Rossi, M.L., Martinelli, A.P., Peres, L.E.P., Azevedo, R.A.: Biochemical and histological characterization of tomato mutants. - *An. Acad. Bras. Cienc.* **84**: 573-585, 2012.
- Moreno, J.E., Tao, Y., Chory, J., Ballaré C.L.: Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. - *Proc. nat. Acad. Sci. USA* **106**: 4935-4940, 2009.
- Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - *Plant Cell Physiol.* **22**: 867-880, 1981.
- Nole-Wilson, S., Rueschhoff, E.E., Bhatti, H., Franks, R.G.: Synergistic disruptions in seuss cyp85A2 double mutants reveal a role for brassinolide synthesis during gynoecium and ovule development. - *BMC Plant Biol.* **10**: 198, 2010.
- Oh, E., Yamaguchi, S., Hu, J., Yusuke, J., Jung, B., Paik, I., Lee, H.-S., Sun, T.-p., Kamiya, Y., Choi, G.: PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in *Arabidopsis* seeds. - *Plant Cell* **19**: 1192-1208, 2007.
- Palma, J.M., Jiménez, A., Sandalio, L.M., Corpas, F.J., Lundqvist, M., Gómez, M., Sevilla, F., Del Río, L.A.: Antioxidative enzymes from chloroplasts, mitochondria, and peroxisomes during leaf senescence of nodulated pea plants. - *J. exp. Bot.* **57**: 1747-1758, 2006.
- Qian, C.L., Zhao, Y.Y., Mi, H.B., Chen, X.H., Guo, L.J., Mao, L.C.: Role of antioxidative system during the development and senescence of cucumber fruit. - *Biol. Plant.* **56**: 793-797, 2012.
- Reinbothe, C., Springer, A., Samol, I., Reinbothe, S.: Plant oxylipins: role of jasmonic acid during programmed cell death, defence and leaf senescence. - *Febs J.* **276**: 4666-4681, 2009.
- Riemann, M., Bouyer, D., Hisada, A., Müller, A., Yatou, O., Weiler, E., Takano, M., Furuya, M., Nick, P.: Phytochrome A requires jasmonate for photodestruction. - *Planta* **229**: 1035-1045, 2009.
- Robson, F., Okamoto, H., Patrick, E., Harris, S.-R., Wasternack, C., Brearley, C., Turner, J.G.: Jasmonate and phytochrome A signaling in *Arabidopsis* wound and shade responses are integrated through JAZ1 stability. - *Plant Cell* **22**: 1143-1160, 2010.
- Saglam-Cag, S.: The effect of epibrassinolide on senescence in wheat leaves. - *Biotechnol. Biotechnol. Equipment* **21**: 63-65, 2007.
- Seo, M., Hanada, A., Kuwahara, A., Endo, A., Okamoto, M., Yamauchi, Y., North, H., Marion-Poll, A., Sun, T.P., Koshiba, T., Kamiya, Y., Yamaguchi, S., Nambara, E.: Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. - *Plant J.* **48**: 354-366, 2006.
- Song, L., Zhou, X.-Y., Li, L., Xue, L.-J., Yang, X., Xue, H.-W.: Genome-wide analysis revealed the complex regulatory network of brassinosteroid effects in photomorphogenesis. -

- Mol. Plant **2**: 755-772, 2009.
- Tang, W., Deng, Z., Wang, Z.-Y.: Proteomics shed light on the brassinosteroid signaling mechanisms. - Curr. Opin. Plant Biol. **13**: 27-33, 2010.
- Tang, W., Wang, W., Chen, D., Ji, Q., Jing, Y., Wang, H., Lin, R.: Transposase-derived proteins FHY3/FAR1 interact with PHYTOCHROME-INTERACTING FACTOR1 to regulate chlorophyll biosynthesis by modulating HEMB1 during deetiolation in *Arabidopsis*. - Plant Cell **24**: 1984-2000, 2012.
- Vitória, A.P., Rodriguez, A.P.M., Cunha, M., Lea, P.J., Azevedo, R.A.: Structural changes in radish seedlings exposed to cadmium. - Biol. Plant. **47**: 561-568, 2003.
- Wann, E.V., Jourdain, E.L., Pressay, R., Lyon, B.G.: Effect of mutant genotypes *hp og^c* and *dg og^c* on tomato fruit quality. - J. amer. Soc. hort. Sci. **110**: 212-215, 1985.
- Weigel, D., Glazebrook, J.: Genetic Analysis of *Arabidopsis* Mutants. - CSH Protocols, Cold Spring Harbor 2008.
- Xia, X.-J., Zhou, Y.-H., Ding, J., Shi, K., Asami, T., Chen, Z., Yu, J.-Q.: Induction of systemic stress tolerance by brassinosteroid in *Cucumis sativus*. - New Phytol. **191**: 706-720, 2011.
- Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., Ma, Z.: Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. - Plant Physiol. **149**: 760-774, 2009.
- Xue, W., Li, X.Y., Zhu, J.T., Lin, L.S.: Effects of temperature and irradiance on photosystem activity during *Alhagi sparsifolia* leaf senescence. - Biol. Plant. **56**: 785-788, 2012.
- Zheng, B., Deng, Y., Mu, J., Ji, Z., Xiang, T., Niu, Q.-W., Chua, N.-H., Zuo, J.: Cytokinin affects circadian-clock oscillation in a phytochrome B- and *Arabidopsis* response regulator 4-dependent manner. - Physiol. Plant. **127**: 277-292, 2006.