Acclimation potential to high irradiance of two cultivars of watermelon

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Abstract

The acclimation potential to high irradiance of two cultivars of watermelon, Reina de Corazones and Toro, calculated as the ratio of sun *vs.* shade activities of O_2^- and H_2O_2 scavenging enzymes and non-radiative energy dissipation, was similar. However, Reina de Corazones exhibited a higher capacity in absolute terms for photoprotection (harmless dissipation of absorbed light energy at PS 2 and ascorbate and O_2^- and H_2O_2 scavenging enzymes) suggesting a larger resistance of this cultivar to high irradiance. This could be seen as smaller decreases in fruit productivity and in lower oxidative injury as probed by malondialdehyde content in sun plants of Reina de Corazones than in Toro plants. Additionally, the results show that shading might be beneficial to both cultivars, presumably because it reduces the susceptibility of high irradiance-induced stress.

Additional key words: ascorbate, *Citrullus lanatus*, lipid peroxidation, non-photochemical quenching, productivity, scavenging enzymes.

Introduction

Mediterranean crops are often subjected to high irradiance stress during the summer which might initiate oxidative stress through an increase of production of activated oxygen species (Foyer *et al.* 1994). Acclimation of plants to high irradiance involves increased thermal energy dissipation associated with larger capacity for deepoxidation of their xanthophyll pools (Demmig-Adams 1998), higher rates of photorespiration (Heber *et al.* 1996), higher activities of the O_2^- and H_2O_2 scavenging enzymes (Cakmak and Marschner 1992) and higher ascorbate (AsA) pools (Logan *et al.* 1996).

At present, there is a paucity of knowledge regarding acclimation potential to high irradiance of Mediterranean crops and the potential influence of the irradiance on productivity. Therefore, the first objective of the present study was to determine the acclimation potential to high irradiance of two commonly used cultivars (Toro and Reina de Corazones) of watermelon (*Citrullus lanatus*). To achieve this goal, we have explored the capacity for non-radiative energy dissipation at PS2, antioxidant content, and the activities of O_2^- and H_2O_2 scavenging enzymes, oxidative strain and productivity in plants subjected either to a high and a low light regime. A second objective was to determine the influence of irradiance on productivity, owing to the great commercial value of Reina de Corazones, a watermelon without pips.

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Abbreviations: AP - ascorbate peroxidase; AsA - ascorbate; CAT - catalase; DHA - dehydroascorbate; Chl - chlorophyll; F_m - maximal fluorescence in dark adapted samples; F_o - minimal fluorescence in dark adapted samples; F_m' - maximal fluorescence in illuminated samples; GR - glutathione reductase; MDA - malondialdehyde; NPQ - non-photochemical quenching from Stern-Volmer equation; POD - peroxidase; PS - photosystem; SOD - superoxide dismutase; TAA - total ascorbate.

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Materials and methods

Plants: Seeds of watermelon [*Citrullus lanatus* (Thumb.) Matsum.] cvs. Toro and Reina de Corazones were germinated in vermiculite and maintained in a greenhouse for 3 weeks in the Estación Experimental Agraria de Carcaixent (Valencia, Spain). When the four first leaves were fully expanded, the plants were transferred to pots containing a comercial soil mixture (*Terraplant, BASF*, Uchte, Germany) and transferred outside. Plants were either exposed to full sunlight or artificially shaded using muslin. Irradiance measured at midday at the upper leaf level in the sun plots was around 1800 μmol m^{-2} s^{-1} whereas in the shade plots it was 800 μmol m^{-2} s^{-1}. The growth period extended from the begining of May to the beginning of September for both cultivars. Mean day/night temperature and relative humidy during the growth period were 27 ± 5/18.2 ± 3.5 °C and 47.4 ± 7 / 85.3 ± 6 %. Plants were watered daily.

Chlorophyll fluorescence: All measurements of modulated chlorophyll (Chl) fluorescence at ambient temperature were performed in the field, using a portable fluorometer (*PAM-2000 Walz*, Effeltrich, Germany). The maximum, F_m, and the minimum, F_o, fluorescence yield of dark-adapted leaves were determined prior to sunrise. F_o was determined on excitation of the upper surface of leaves with a weak measuring beam. The maximum Chl fluorescence yield (F_m) was determined with a 1.4 s saturating pulse of *ca.* 3000 μmol m^{-2} s^{-1}. The maximal fluorescence yield during exposure to daylight, F_m', was determined by means of saturating pulses. Quenching due to non-photochemical dissipation of absorbed light energy (NPQ) was determined as (F_m - F_m') / F_m' (Bilger and Björkman 1991).

Enzyme activities: Leaves (2 g) without the main midrib was homogenized in 10 cm^3 of 100 mM potassium phosphate buffer, pH 7.5, containing 2 mM EDTA and 2 % PVP. The slurry was centrifuged at 15 000 *g* for 20 min. The supernatant was filtered (*Millipore, Mitex* 0.5 μm) and utilized for enzyme analysis. All operations were carried out at 3 to 5 °C.

Peroxidase (POD) activity was analysed following the protocol described by Astorino *et al.* (1995) with slight modifications. The assay was performed in a 3 cm^3 cuvette containing 100 mM potassium phosphate buffer, 1 % guaiacol and 6 mM H_2O_2. Reaction assays were started by the addition of an appropiate volume of the tissue extract. Activity was determined by the increase in the absorbance at 470 nm (*8452A Hewlett Packard* spectrophotometer, Palo Alto, USA) due to the guaiacol oxidation.

Ascorbate peroxidase (AP) was determined by monitoring the decrease in A_{290} for 4 min in 3 cm^3 of reaction mixture contained 100 mM potassium phosphate buffer, 0.5 mM ascorbate, 0.4 mM H_2O_2 and the enzyme aliquot (Nakano and Asada 1981). Corrections were made for the oxidation of ascorbate in the absence of H_2O_2.

Catalase (CAT) activity was assayed in 3 cm^3 of reaction mixture containing 100 mM phosphate buffer, 6 mM H_2O_2 and the appropiate amount of enzyme. The decomposition of H_2O_2 was monitored at 240 nm (Cakmak and Marschner 1992) .

Glutathione reductase (GR) activity was determined by following the oxidation of NADPH at 340 nm as described by Rao (1992). Corrections were made for NADPH oxidation in the absence of GSSG.

Superoxide dismutase (SOD) activity was measured spectrophotometrically as described by Beyer and Fridovich (1987). In this assay, 1 unit of SOD is defined as the amount required to inhibit the photoreduction of nitroblue tetrazolium by 50 % .

Lipid peroxidation: was measured in terms of malondialdehyde (MDA) in leaves, according to the protocol proposed by Heath and Parker (1968) and taking into account the modifications made by (Dhindsa *et al.* 1981).

Ascorbate pools: The petiole and veins were removed from the excised leaves, which were immediately frozen with liquid nitrogen. Subsequently, 2 g were pulverized in liquid nitrogen and homogenized with 12 cm^3 of 2 % metaphosphoric acid . To pellet all debris the homogenate was centrifuged (4360 *g*, 4 °C, 10 min), the supernatant was filtered (*Millipore, Mitex* 0.5 μm). Ascorbate (AsA) and dehydroascorbate (DHA) were measured spectrophotometrically as described by Takahama and Oniki (1992) with a spectrophotometer (8452A *Hewlett-Packard*, Palo Alto, USA). Ascorbate concentration was determined by monitoring the absorbance decrease at 265 nm induced by the oxidation of AsA to DHA by ascorbate oxidase. To determine total ascorbate (TAA), DHA was reduced to AsA by adding dithiothreitol to a final concentration of 0.5 mM. The DHA concentration was calculated as the difference between TAA and AsA.

Results

Plant growth: Plant growth in the shade increased total fruit mass in both cultivars (Table 1), although the differences were non-significant. However, Toro plants experienced larger increases in total fruit mass than Reina de Corazones (120 % and 106 %, respectively).

Non-photochemical fluorescence quenching: Sun plants removed a higher percentage of absorbed light energy through thermal energy dissipation than shade plants (Table 1). Significant differences between sun and shade plants within each cultivar were found. NPQ was always higher in Reina de Corazones than in Toro, both in sun and shade plants.

Table 1. Total fruit mass [kg plant^{-1}], non-photochemical quenching calculated at midday and malondialdehyde content [nmol g^{-1}(f.w.)] in sun and shade leaves of cvs. Toro and Reina de Corazones. Means ± SD, n = 10 plants, except MDA where n = 5 plants. For comparison of means, analysis of variance (*ANOVA*) followed by the least significance difference (*LSD*) test, calculated at 95 % confidence level, was performed. Values followed by the same letter indicate no significant differences.

Cultivar	Treatment	Fruit mass	NPQ	MDA
Toro	Sun	5.71 ± 1.0a	2.041 ± 0.11a	56.4 ± 4.2a
Toro	Shade	6.90 ± 1.0a	1.335 ± 0.12b	30.1 ± 2.9b
R. de Corazones	Sun	27.55 ± 5.5b	2.793 ± 0.21c	38.5 ± 3.4c
R. de Corazones	Shade	29.35 ± 3.6b	2.025 ± 0.18a	23.5 ± 2.2d

Lipid peroxidation: Lipid peroxidation, measured as malondialdehyde (MDA) content (Table 1), was significantly higher in sun than in shade-grown plants. Additionally, significant differences in the degree of peroxidation induced by high irradiance were found between cultivars, with percentage increases of 187 % and 164 % in sun compared to shade plants in Toro and Reina de Corazones, respectively.

Activities of O_2^- and H_2O_2 scavenging enzymes: Sun plants of both cultivars exhibited enhanced activities of O_2^- and H_2O_2 scavenging enzymes (Table 2). Overall, enzyme activities were higher in Reina de Corazones than in Toro. Additionally, Reina de Corazones exhibited significant differences between sun and shade plants in all the enzyme activities assayed.

Table 2. Antioxidant enzyme activities in sun and shade leaves. Activities of peroxidase (POD), ascorbate peroxidase (AP), catalase (CAT) and glutathion reductase (GR) are expressed in [μmol g^{-1}(f.m.) s^{-1}], superoxide dismutase (SOD) activity as [U g^{-1} (f.m.)]. Means ± SD, n = 4. Values followed by the same letter indicate no significant differences.

Cultivar	Treatment	POD	AP	CAT	GR	SOD
Toro	Sun	0.0130 ± 0.0003a	0.196 ± 0.04ab	0.007 ± 0.0004a	0.040 ± 0.002ab	1586 ± 50a
Toro	Shade	0.0083 ± 0.0002b	0.133 ± 0.03a	0.005 ± 0.0003b	0.042 ± 0.004ab	658 ± 25b
R. de Corazones	Sun	0.0720 ± 0.0030c	0.447 ± 0.09c	0.009 ± 0.0004c	0.045 ± 0.011b	2117 ± 120c
R. de Corazones	Shade	0.0220 ± 0.0030d	0.255 ± 0.06b	0.007 ± 0.0003a	0.032 ± 0.004a	1012 ± 206d

Table 3. Ascorbate (AsA), dehydroascorbate (DHA), and total ascorbate (TAA = AsA + DHA) contents [mg g^{-1}(f.m.)] and the AsA/TAA ratio in sun and shade leaves of both cultivars. Means ± SD, n = 5. Values followed by the same letter indicate no significant differences.

Cultivar	Treatment	AsA	DHA	TAA	AsA/TAA
Toro	Sun	0.144 ± 0.02a	0.054 ± 0.01a	0.192 ± 0.03a	0.75 ± 0.03ab
Toro	Shade	0.112 ± 0.02a	0.078 ± 0.01b	0.190 ± 0.02a	0.59 ± 0.10a
R. de Corazones	Sun	0.189 ± 0.03b	0.036 ± 0.006c	0.203 ± 0.03a	0.84 ± 0.20b
R. de Corazones	Shade	0.141 ± 0.02a	0.062 ± 0.01a	0.203 ± 0.03a	0.69 ± 0.14ab

AsA and DHA concentrations: Of the total AsA, a higher proportion was in the reduced form (AsA) in sun plants of either cultivar than in their respective shade counterparts (Table 3). This yielded higher AsA/TAA ratio in sun than in shade plants. On the other hand, TAA contents did not differ significantly in sun and shade plants of both cultivars.

Discussion

The larger intrinsic capacity for photoprotective processes (harmless dissipation of absorbed light energy at PS2) and antioxidant quenching mechanisms (AsA pools and activity of O_2^- and H_2O_2 scavenging enzymes) exhibited by Reina de Corazones might confer this cultivar an increased resistance to high irradiance, in accordance to what has been demonstrated by other researchers (Cakmak and Marschner 1992, Streb *et al.* 1997). This could be seen as smaller decreases in fruit mass and in lower increases in oxidative injury, as probed by MDA content in shade *vs.* sun plants of Reina de Corazones than in Toro plants. Additionally, Reina de Corazones plants also exhibited a higher proportion of their AsA pool in the reduced form, suggesting a higher regeneration potential of the oxidized AsA.

Our results that sun leaves of both cultivars exhibited a much higher capacity for thermal energy dissipation was in accordance with current research (*e.g.*, Björkman and Demmig-Adams 1987, Demmig-Adams *et al.* 1995, Demmig-Adams 1998). Exposure to high irradiance predisposes the photosynthetic apparatus to overenergization and promotes the formation of toxic oxygen species (Hodgson and Raison 1991, Elstner *et al.* 1988). Therefore, the enhanced activities of antioxidant enzymes found in sun leaves of both cultivars might represent a physiological response for counteracting the adverse effects of exposure to high irradiance. Similar responses have been described in other species (*e.g.*, Gilham and Dodge 1987, Cakmak and Marschner 1992).

To summarise, the acclimation potential of Reina de Corazones and Toro plants measured as the relative increase of photoprotective and antioxidant quenching mechanisms was similar. However, their larger absolute values in Reina the Corazones might confer this cultivar a higher resistance to high irradiance stress.

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