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Physiological and biochemical response to photooxidative stress of the fundamental citrus species

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ABSTRACT

Despite the economic importance of citrus, insights on the genetic response to stress are scarce. The aim of the present study was to compare fundamental citrus species for their response to photooxidative stress. The experiment was conducted under orchard conditions on three fundamental citrus species *C. medica* L., *C. reticulata* Blanco and *C. maxima* (Burm.) Merr., and on *Fortunella japonica* (Thunb.) Swing. We examined their respective net photosynthesis (*P*net), stomatal conductance (*Gs*) and chlorophyll fluorescence (*Fv*/*Fm*) on sun-acclimated leaves and shade-acclimated leaves returned under natural sunlight irradiance. To compare the respective response mechanism, we analyzed changes in oxidative status (hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)), reactive oxygen species (ROS)-scavenging enzymes (superoxide dismutase (SOD), catalase, ascorbate peroxidase), recycling enzymes (monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) and antioxidant metabolites (ascorbate and glutathione). Kumquat and pummelo exposed lower down-regulation and full recovery of photosynthetic parameters, lower accumulation of oxidized compounds associated with greater production of reduced glutathione (Gsh) and enhanced activity of the three ROS scavenging enzymes, especially SOD. Citron and mandarin showed a marked decrease and incomplete recovery in photosynthetic performance, mainly in *P*net and *Fv*/*Fm*, larger accumulation of oxidative parameters, slighter induction of antioxidant enzymes and down-regulation of reduced ascorbate (Asa) and Gsh synthesis. These results suggest that kumquat and pummelo have a greater tolerance to photooxidative stress than citron and mandarin.

1. Introduction

Citrus is the world's most economically important fruit crop. Strictly, true citrus plants comprise six genera: *Clymenia*, *Eremocitrus*, *Microcitrus*, *Poncirus*, *Fortunella* and *Citrus*. Scora (1975) and Barrett and Rhodes (1976) considered *Citrus medica* L. (citron), *C. maxima* (Burm.) Merr. (pummelo) and *C. reticulata* Blanco (mandarin) to be the three fundamental species of *Citrus*, the other species resulting from hybridization of these true species. This view has recently gained support from various biochemical and molecular studies (Federici et al., 1998; Barkley et al., 2006; Fanciullino et al., 2006). Allopatric evolution has resulted in strong genetic and also phenotypic differentiation between these *Citrus* taxa (Garcia-Lor et al., 2012).

Citrus trees are continuously exposed to changes in light and temperature in their natural environment. Global climatic warming may cause these changes to become increasingly pronounced in both frequency and magnitude, particularly in the north Mediterranean area. In this region, the summer season is characterized by high temperatures and dryness, whereas in winter, day temperature is generally moderate and night temperatures often dip below 5 °C. At these two periods, the radiation loads can reach high levels. Sunlight contains high-energy ultraviolet radiation (UV, 280–400 nm) and photosynthesis is one of the processes most sensitive to high irradiance (Demmig-Adams and Adams, 1992). Under such conditions, trees are liable to suffer photoinhibition, defined as the slow, reversible decline in photochemical efficiency that occurs under photooxidative stress (Krause et al., 2001). This process is

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Table 1
Genotypes used for physiological and biochemical analysis and their corresponding rootstocks.

Genotype				Corresponding rootstock		
Abbreviation	Common name	Tanaka system	ICVN[a] No.	Common name	Tanaka system	ICVN[a] No.
CC	Corsican citron	*Citrus medica* L.	0100613	Volkamer lemon	*Citrus limonia* Osbeck	0100729
WLM	Willowleaf mandarin	*Citrus deliciosa* Ten.	0100133	Volkamer lemon	*Citrus limonia* Osbeck	0100729
MK	Marumi kumquat	*Fortunella japonica* (Thunb.) Swingle	0100482	Volkamer lemon	*Citrus limonia* Osbeck	0100729
PP	Pink pummelo	*Citrus maxima* (Burm.) Merr.	0100322	Trifoliate orange	*Poncirus trifoliata* (L.) Raf.	0110480

[a] International citrus variety numbering.

frequent in trees of warm regions, where the light intensity can reach levels over 1800 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (Favaretto et al., 2011). The ability to cope with photoinhibition ranges greatly among plant species (Kitao et al., 2006). Numerous studies have shown that photosystem II (PSII) is the primary target of photoinhibitory damage (Aro et al., 1993). Photoinhibition of PSII can be easily detected in vivo by a decrease in the dark-adapted ratio of variable to maximum chlorophyll a fluorescence (Fv/Fm) (Krause and Weis, 1991). A decrease in this ratio indicates a stressful condition, and a reduction in the maximum quantum efficiency of PSII, which thereby compromises the plant's photosynthetic potential (Maxwell and Johnson, 2000).

A common effect of most environmental factors is an increased production of reactive oxygen species (ROS) in green plant cells, a situation called photooxidative stress, driven by the light energy absorbed in excess of assimilatory requirements (Foyer et al., 1994). These harmful ROS such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) are involved in the mechanism of photoinhibition (Asada, 1999). The production of ROS in plant cells is enhanced by conditions that limit CO_2 fixation, such as drought, salt, heat and cold stresses, and by the combination of these conditions with strong light (Foyer and Noctor, 2003). Because aerobic organisms, such as plants, live in a highly oxidative environment, they have evolved efficient antioxidant systems protecting them from the damaging effects of ROS (Asada, 1999) such as decreased protein synthesis, damage to DNA and membrane lipids (Mackerness et al., 2001; Frohnmeyer and Staiger, 2003). These antioxidant mechanisms employ (i) ROS-scavenging enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, 1.11.1.11), (ii) recycling enzymes of the ascorbate-glutathione cycle, such as monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2), and (iii) low molecular weight antioxidants, such as reduced ascorbic acid (Asa) and reduced glutathione (Gsh). Some authors have reported that antioxidative systems play a major role in protecting plants from the harmful effects of excess light energy (Foyer et al., 1994; Favaretto et al., 2011). Thus, antioxidative systems have been found to be of paramount importance in the response and tolerance of trees to environmental stress (Polle and Rennenberg, 1993).

Some authors have shown that citrus physiology is adversely affected by abiotic stresses, such as drought (Avila et al., 2012), waterlogging (Hossain et al., 2009) and salinity (Balal et al., 2012; Brumos et al., 2009). Currently, experiments have been mainly performed on the most common rootstocks under the superimposition of a specific stress with strong light. For instance, it was found that the Cleopatra mandarin was very sensitive to flooding stress and tolerant to salt stress, whereas Carrizo citrange showed the opposite behavior (Arbona et al., 2008; Brumos et al., 2009). Thus, a heterogeneous response to oxidative stress between rootstocks exists under homogeneous cultural conditions. To date, no study has focused on the possible differences of stress response that could exist between citrus species and, especially, for the species at the origin of the broad genetic diversity of cultivated citrus.

The main objective of this work was to compare fundamental citrus species for their response to oxidative stress. Thus, individual trees grown under orchard conditions were submitted to photooxidative stress by controlling light conditions of the leaves. We measured the main photosynthetic traits (net photosynthesis, stomatal conductance and chlorophyll a fluorescence), the oxidative status (H_2O_2 and malondialdehyde (MDA) contents), the activities of the main antioxidant enzymes (SOD, CAT, APX, MDHAR, DHAR, GR) and the level of the main hydrophilic antioxidant molecules (ascorbate and glutathione) of the four fundamental citrus species. These measurements were performed on sun-acclimated leaves and on one-week shade-acclimated leaves returned under natural sunlight irradiance. The results allow discussing the responses of the citrus species to photooxidative stress.

2. Materials and methods

2.1. Plant material and growth conditions

Experiments were carried out on leaves from 8-year-old trees with genotypes belonging to the *Citrus* and *Fortunella* genera (Table 1) growing in the experimental orchards of the Station de Recherches Agronomiques INRA-CIRAD of San Giuliano, Corsica, France (42° 18′ 55″ N, 9° 29′ 29″ E; 51 m a.s.l., under a Mediterranean climate and on soil derived from alluvial deposits and classified as fersiallitic, pH range 6.0–6.6). The trees were about 2.0 m high, spaced 6 m × 4 m, and subjected to homogeneous growing conditions to reduce environmental effects. Water was supplied every day on the basis of 100% replacement of actual evapotranspiration estimated from the equation of Monteith (1965). Fertilizers were supplied, and insects and diseases were controlled according to the recommendations of the local Department of Agriculture.

The experiment was conducted from September 23, 2010 to October 10, 2010 on clear days. For each of the basic true species of the *Citrus* genus and of the *Fortunella* genus (Table 1), three trees were analyzed. We isolated two independent sections on each of the three trees. The first section did not undergo any special treatment, and served as control. On this section, the leaves were kept uncovered throughout the experimental period to receive 100% sunlight irradiance. The leaves of the second section were shaded using a 90% shade cloth. This shade cloth allowed the actual transmission of 9.2% sunlight irradiance (90% shade cloth). We checked that spectra were not modified by shading, using a Li-Cor Li-1800 spectrometer. After one week of adaptation, the photooxidative stress was applied. The leaves were completely uncovered and received full light. Each treatment was allocated among the three selected trees of each genotype of the trial at three different periods (Fig. 1). At each period, one tree of each genotype was studied. In this way, the variability associated with different days of measurement was included in the intraspecific variability. On each section, physiological measurements and samplings were performed 0, 3, 6, 24 and 48 h after the end of the shading period. The same leaves were used for physiological parameter measurements. On each tree, three fully expanded leaves from spring of the current year's