Plant Physiology and Biochemistry 112 (2017) 261–269

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

The ozone-like syndrome in durum wheat (*Triticum durum* Desf.): Mechanisms underlying the different symptomatic responses of two sensitive cultivars

Valentina Picchi [a], Robert Monga [b], Riccardo Marzuoli [c], Giacomo Gerosa [c], Franco Faoro [b, *]

[a] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Unità di ricerca per i processi dell'industria agroalimentare, Via G. Venezian 26, 20133 Milan, Italy
[b] Department of Agricultural and Environmental Sciences - Production, Land, Agroenergy, University of Milan, Via Celoria 2, Milano, Italy
[c] Department of Mathematics and Physics, Catholic University of Brescia, Via dei Musei 41, Brescia, Italy

ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
9 January 2017
Accepted 11 January 2017
Available online 12 January 2017

Keywords:
Ozone
Wheat
Stomata
Oxidative stress
Cell death
Chloroplasts

ABSTRACT

Colombo and Sculptur are two modern durum wheat cultivars that, in previous studies, proved to be very sensitive to ozone injury in terms of eco-physiological parameters and significant grain yield loss. Nevertheless, their response regarding leaf visible symptoms was very different; Sculptur showed almost no symptoms, even after several weeks of ozone exposure, whereas Colombo showed in a few weeks typical ozone-like symptoms (chlorotic/necrotic spots). The mechanisms underlying this different response has been studied with a biochemical and microscopical approach. Plants were grown in Open-Top Chambers (OTCs) and exposed to charcoal filtered and ozone enriched air. Flag leaves were analyzed at two phenological stages (pre- and post-anthesis). At pre-anthesis the ascorbate pool was significantly lower in Colombo, which also underwent an increase in the oxidized glutathione content and abundant H_2O_2 deposition in mesophyll cells around the substomatal chamber. No or scarce H_2O_2 was found at both phenological stages in ozone exposed leaf tissues of Sculptur, where stomata appeared often closed. In this cultivar, transmission electron microscopy showed that chloroplasts in apparently undamaged mesophyll cells were slightly swollen and presented numerous plastoglobuli, as a result of a mild oxidative stress. These results suggest that Sculptur leaves remains symptomless as a consequence of the higher content of constitutive ascorbate pool and the synergistic effect of stomata closure. Instead, Colombo shows chlorotic/necrotic symptoms because of the lower ROS (Reactive Oxygen Species) scavenging capacity and the less efficient stomata closure that lead to severe damages of groups of the mesophyll cells, however leaving the surrounding photosynthetic tissue functional.

1. Introduction

The detrimental effect of tropospheric ozone (O_3) on the vegetation in general, and on the agricultural crops in particular, has been demonstrated in a large number of studies (reviewed in Ainsworth et al., 2012; in Rai and Agrawal, 2012). This pollutant, that enters the plant leaves exclusively through open stomata, produces in leaf tissues several reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl ($OH^•$) and hydroperoxyl ($HO_2^•$) radicals (Overmyer et al., 2009; Vahisalu et al., 2010). ROS cause peroxidation of lipids, chlorophyll bleaching, protein oxidation, damage to nucleic acids and destruction of cell membranes, particularly those of the chloroplasts (Karberg et al., 2005; Vaultier and Jolivet, 2015). In response to ROS production, plants trigger a number of antioxidative stress-related defense mechanisms (Kangasjärvi et al., 1994), among them: ascorbate, phenolics, α-tocopherol, glutathione, carotenoids and scavenging enzymes, i.e. superoxide dismutases, catalases and several peroxidases (Temmerman et al., 2002; Paoletti et al., 2008). When ROS production overwhelms antioxidant defenses, cells undergo severe damages and, ultimately, cell death. These damages may result in a number of different leaf symptoms on sensitive plants depending on the species and the exposure type. Chronic exposure, as a

* Corresponding author.
E-mail address: franco.faoro@unimi.it (F. Faoro).

http://dx.doi.org/10.1016/j.plaphy.2017.01.011
0981-9428/

consequence of low ozone concentrations (<40 ppb) for the entire life of a plant, with some episodes of high concentration (>80 ppb), either periodically or accidentally, cause chlorosis (yellowing due to the chlorophyll breakdown, often distributed in spots over the leaf) and bronzing (red-brown pigmentation caused by phenylpropanoid accumulation) (Krupa et al., 2001). When ozone concentrations rise over 80 ppb for some days or weeks, chlorosis and chlorotic spots may degenerate in necrotic lesions of different tonalities depending on the species (Krupa et al., 2001; Chaudhary and Agrawal, 2015). However, photosynthesis and growth can be inhibited even in absence of leaf visible symptoms when damages are restricted to the inactivation of important enzymes such as ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) or to the induction of stomata closure (reviewed in Iriti and Faoro, 2008). At the individual level, ozone exposure can induce significant losses of crop yields and quality (Sarkar and Agrawal, 2010; Gerosa et al., 2009). For example, wheat yield losses due to ambient ozone concentrations were estimated at about 20–27% in the Mediterranean region (Fumagalli et al., 2001) and at about 6–17% in the central Italy (Fagnano et al., 2009), while even more severe losses, up to 29%, were reported in China (Feng et al., 2008).

Among wheat species, *Triticum durum* is quite sensitive to ozone damages, though this sensitivity may vary between different cultivars (Gerosa et al., 2014; Monga, 2015). In previous studies we showed that two highly productive cultivars, Colombo and Sculptur, were significantly damaged by the pollutant considering ecophysiological parameters and grain yield (Monga et al., 2015; Monga, 2015). Nevertheless, their symptomatic response in terms of leaf visible symptoms was very different. Sculptur was almost symptomless, even after several weeks of ozone exposure, while Colombo showed in a few weeks typical ozone-like symptoms, consisting in chlorotic spots, often degenerating into necrotic lesions. The observed reduction in stomatal conductance (g_s) induced by ozone in both cultivars could partially explain the grain yield losses but not their different symptomatic responses (Monga et al., 2015; Monga, 2015).

To unravel the mechanisms underlying this different responses, we have undertaken a biochemical and microscopical study aimed at verifying whether the lack of visible symptoms in Sculptur is linked to a better capacity of ROS scavenging or to other mechanisms of avoidance of ozone effect, such as stomata closure, that would also explain the loss of productivity observed in this symptomless cultivar.

2. Materials and methods

2.1. Plant material and ozone treatments

The experiment was performed between March and July 2014 in the Open-Top Chamber (OTC) experimental site of Curno, Bergamo (45° 41′17″ N, 9°36′40″ E, elev. 242 m a.s.l.), in Northern Italy. Seeds of two cultivars (Colombo and Sculptur) of durum wheat (*Triticum durum* Desf.) were sown on 3 March in pots (25-cm diameter, 23-cm height, V = 11 L) filled with 10 kg of standard commercial soil (Koro Excell). Each pot contained 3 plants, and a total of 6 pots of each cultivar were placed in each OTCs 46 days after sowing (DAS). A detailed description of OTCs was reported in Monga et al. (2015). Plants entered into anthesis phase 76 and 78 DAS for Sculptur and Colombo, respectively. Both cultivars were harvested at 118 DAS. Plants were fertilized twice during the experiment with urea 46%, 39 (1 g per pot) and 70 (0.5 g per pot) DAS. They also were automatically irrigated to maintain the soil water content of the pots close to field capacity in order to avoid water stress.

The OTCs were arranged in 3 blocks of 4 OTCs corresponding to 4 different levels of ozone treatments: i) Charcoal-Filtered OTCs (CF-OTCs), where the air filtration system assured an abatement of approximately 50% of the ambient air O_3 concentration; ii) non-filtered OTCs (NF-OTCs), where the ambient air was not filtered; (iii) non-filtered OTCs with moderate O_3-enriched air (OZ+-OTCs), and iv) non-filtered OTCs with high O_3-enriched air (OZ++-OTCs), in which the O_3 concentration inside was, respectively, 30% and 60% higher than the ambient air. The average hourly concentration of the latter was around 30 ppb.h from March to May and around 40 ppb.h in June. Ozone fumigation was performed daily from 9 a.m. to 5 p.m., from 28th March to 18th June 2014, and ozone concentration inside the chambers was continuously monitored. Further details are given in Monga et al. (2015).

Plants exposure to ozone was expressed as AOT40 (Accumulated Ozone exposure over a Threshold of 40 ppb). AOT40 was calculated as the sum of the exceedances of ozone concentrations over 40 ppb during daylight hours (Fuhrer et al., 1997).

2.2. Leaf visible symptoms assessment

Ozone visible symptoms were monitored daily from the starting of ozone fumigation looking for the onset of chlorotic and necrotic spots that are typical of ozone injury on durum wheat (Picchi et al., 2010). Once the first visible symptoms appeared, the diffusion of the symptoms was evaluated by imaging leaves with a common PC scanner. Four samples of flag leaves (2 leaves for cv. Colombo and 2 leaves for cv. Sculptur) were randomly collected from each OTC, twice during the growing season (14th and 27th of May). The extent of foliar injury was assessed by estimating the injured adaxial leaf area on digitalized images at 300 dpi with an image analyzer (Global Lab©, Data Translation, USA) and the percent of chlorotic/necrotic area per cm^2 was determined.

2.3. Biochemical analysis

Samples for biochemical analysis and microscopic inspection were collected in the morning from 10 to 11 a.m. on the 14th and 27th of May 2014, in pre- and post-anthesis phenological stage, respectively. Five fully expanded flag leaves of Colombo and Sculptur were chosen from each OTC. Leaves were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis. Three replicates were maintained for all measurements.

2.3.1. Ascorbic and dehydroascorbic acid determination

Frozen foliar tissue (300 mg) was ground with liquid nitrogen in a pre-cooled pestle. Five ml of metaphosphoric acid at 6% was immediately added to the powder. The homogenate was vortexed for 30 s and then centrifuged at 12,000 rpm for 15 min at 4 °C.

L-ascorbic acid (AsA) was quantified by analyzing diluted aliquots of the prepared extracts by HPLC, as previously described (Picchi et al., 2012). The oxidized form (dehydroascorbic acid, DHA) was determined by the "subtractive" method after measurement of the total ascorbate (AsA + DHA) content following reduction with dithiothreitol (DTT). The reduction was carried out according to Davey et al. (2003). Briefly, 100 μl of plant extract was added to 50 μl of a solution of 200 mM DTT in 400 mM Tris base. This generated a final pH of 6–6.8. The reaction was stopped after 15 min at room temperature by acidification with a further 50 μl of 8.5% orthophosphoric acid. Reduced extracts were then diluted with 0.02 M orthophosphoric acid and immediately analyzed by HPLC. The analytical column was a 250 × 6 mm i.d., Intersil ODS-3, maintained at 40 °C. The isocratic elution was performed using 0.02 M mobile phase orthophosphoric acid at a flow rate of 0.7 ml/min. Samples of 20 μl were injected and monitored at 254 nm. The identity of the AsA peak was confirmed by coelution with authentic standards and the concentration of AsA was calculated from the experimental