OXIDATIVE STRESS



Phenol metabolism of two cultivars of durum wheat (Triticum durum Desf.) as affected by ozone and flooding stress

Cristina Nali^{3,4} | Giacomo Lorenzini^{3,4} | Limin Ma^{1,2}

Chong Chen^{1,2} Songsong Chen^{1,2} Rohit Kumar Jha^{1,2} Lorenzo Cotrozzi³

¹State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai, China

²Shanghai Institute of Pollution Control and Ecological Security, Shanghai, China

³Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

⁴CIRSEC, Centre for Climate Change Impact, University of Pisa, Pisa, Italy

Correspondence

Limin Ma, State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering. Tongji University, Shanghai, 200092, China. Email: Imma@tongji.edu.cn

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Abstract

To compare the phenolic responses under oxidative stressors, plants of two Italian cultivars of durum wheat (Claudio and Mongibello) were (a) exposed to ozone (O_2) (80 ppb, 5 hr/day for 70 consecutive days), with the aim to investigate the changes of phenolic compound contents in their leaves, or (b) flooded (seven consecutive days). Plants showed O₃-induced visible injury, but their photosynthetic performance was not affected by the pollutant. Specifically, Claudio showed a higher O₃ tolerance than Mongibello. The major value of the present study is undoubtedly the pioneering investigation of phenolic metabolism of durum wheat under O₃. We identified 12 foliar phenolic compounds in all leaf samples (i.e. controls, exposed to O₃ and flooded): ten phenolic acids, a flavanol (catechin hydrate) and a phenolic aldehyde (syringaldehyde). Overall, O₃ exposure resulted in accumulations of phenolic compounds, especially in Claudio. These responses can be likely considered a fine-regulated repair process that equipped Claudio stressed plants with an antioxidant system capable of scavenging oxidative stress. Different phenolic variations were found in flooded plants, suggesting that phenolic response to environmental constraints is stress specific. Our study confirms that investigations and characterization of specific phenolic profiles of crop cultivars under oxidative stress may be helpful in breeding programmes.

KEYWORDS

air pollution, catechin hydrate, oxidative stress, phenolic acids, plant secondary metabolism, syringaldehyde

1 | INTRODUCTION

Ozone (O_3) in the near-surface atmosphere (i.e. the troposphere) is an important and widespread air pollutant that can influence global environmental change and many biological activities (The Royal Society, 2008). Tropospheric O₃ is produced through a series of complex photochemical reactions by a variety of precursors, such as nitrogen oxides (NO_v) and volatile organic compounds (VOCs), under light conditions (Krupa et al., 2001). Ozone can harm humans and animals (Bhuiyan, Khan, Zaman, & Hishan, 2018; Nuvolone, Petri, & Voller, 2018), as well as plants. It reduces crop and forest productivity, inducing huge economic loss, as well as compromises ecosystem performance and biodiversity. Since the 20th century, Europe, America, Japan and many other geographical areas have frequently experienced serious O₃ pollution scenarios, making this problem become an increasingly concern in the environmental science (Krupa et al., 2001; Lefohn et al., 2018). More recently, strong evidence has been provided that ambient O3 pollution may be substantially reducing yields of important crops across China and other Asian regions (Gaude et al., 2014; Wang, Manning, Feng, & Zhu, 2007).

Flooding caused by heavy or continuous rainfall in areas with poorly drained soil is another serious environmental stress 2 WILEY - Journal & Agronomy & Crop Science

worldwide and is increasing in frequency due to changes in global climate (Garssen, Baattrup-Pedersen, Voesenek, Verhoeven, & Soons, 2015). Flooding with freshwater, although less harmful than waterlogging with saltwater, poses a multitude of constraints on growth. survival and reproduction of many plants (Armstrong, Brändle, & Jackson, 1994; Jackson & Colmer, 2005), since under hypoxic conditions due to flooding, ATP production and energy transformation through oxidative phosphorylation are impaired (Bailey-Serres, Lee, & Brinton, 2012). Similarly to O₃, flooding significantly reduces the growth and yield of several crops (Komatsu, Hiraga, & Yanagawa, 2012; Li et al., 2018).

Ozone is a strong oxidant that enters the leaves through the open stomata and in the apoplastic substomatal cavity reacts with the water-soluble matrix of the cell wall to form a large amount of reactive oxygen species (ROS), such as singlet oxygen (¹O₂), superoxide anion (O_2^{-}) , hydroxyl radical (OH') and hydrogen peroxide (H₂O₂) (Sandermann, 1996). Excessive generation of ROS, especially H₂O₂, has been also shown under hypoxia in flooded plants, a phenomenon mainly related to mitochondria functioning (Mustroph, 2018; Shalygo, Domanskaya, Radyuk, Shcherbakov, & Dremuk, 2012; Yordanova & Popova, 2007). ROS are highly reactive molecules because of unpaired electrons in their structure and react with several biological macromolecules in cell, such as carbohydrates, nucleic acids, lipids and proteins, altering their functions. Regulation of redox state is critical for cell viability, activation, proliferation and organ function (Heath, 1987; Sandermann, 1996; Sharma, Jha, Dubey, & Pessarakli, 2012). Protection mechanisms against ROS involving non-enzymatic and enzymatic antioxidant systems are thus essential traits of both O3- and flooding-tolerant plants helping to protect lipids and other macromolecules from oxidative damage (Chernikova, Robinson, Lee, & Mulchi, 2000; Sandermann, Ernst, Heller, & Langebartels, 1998; Shalygo et al., 2012).

Among non-enzymatic antioxidant systems, it has been reported that phenolic compounds have a great efficacy (Cheynier, Comte, Davies, Lattanzio, & Martens, 2013; Cotrozzi et al., 2018; Pellegrini et al., 2018a, 2018b, 2019). Phenolic compounds are usually arranged in two groups-simple phenols (such as the cinnamic, p-coumaric, syringic and salicylic acids) and more complex molecules, often containing aromatic rings linked together (Kulbat, 2016). The aromatic 6-C ring of phenolic compounds is synthesized in the shikimic acid pathway chiefly from the precursor amino acid phenylalanine, thanks to the action of phenylalanine ammonia-lyase (PAL), an enzyme that since long has been demonstrated to play a crucial role in differential response of plants to O₃ (Biagioni, Nali, Heimler, & Lorenzini, 1998), although our knowledge on this topic is still scarce and fragmentary. Differently, phenolic variations in flooded plants have not been largely investigated.

Triticum durum Desf. ("durum wheat") is a key member of the genus Triticum; its gluten content is relatively high, so it is suitable for macaroni, solid vermicelli and couscous (Šramková, Gregová, & Šturdík, 2009). Durum wheat is mainly cropped in the Mediterranean basin, the Northern Plains between United States of America and Canada, and within the desert areas of southwest of United States

and Northern Mexico, but is also widely cultivated in North Africa, Turkey, Syria and other Asian countries (Kadkol & Sissons, 2016; Li et al., 2018). In most of these areas, durum wheat have commonly to cope with major climatic constraints, mainly elevated temperatures, drought and high O3 levels, but also flooding (this is expected to increase in the near future). Most of available literature concerns O₃ effects on common wheat (T. aestivum L.), although durum wheat is also known to be both O₃ (Fangmeier, Brockerhoff, Grüters, & Jäger, 1994; Ollerenshaw & Lyons, 1999; Picchi, Francini, Nali, & Lorenzini, 2006) and flooding sensitive (Burgos, Stamp, & Schmid, 2001; Pampana, Masoni, & Arduini, 2016), even if somewhat contrasting results have been reported based on genotypic differences in tolerance and on intensity and duration of the stress exposure (Gerosa et al., 2014; Mustroph, 2018; Pampana et al., 2016).

Due to the long history of wheat breeding all over the world, a huge number of cultivars have become available. These breeding processes focused on high yield and food quality, often concomitant with a loss of genetic diversity and stress resistance. However, plant material capable of maintaining acceptable yield performances even under severe conditions is needed to ensure the food security of millions of worldwide wheat consumers. Strategies to achieve this general breeding goal include (a) screening of a wide range of cultivars under a specific stress condition; (b) selection of tolerant cultivars; and (c) improving our knowledge on the intimate mechanisms which module the stress-cell interactions, with special regard to the set-up of defence/detoxification reactions (Mustroph, 2018).

In the present work, two Italian cultivars of durum wheat were cultured under controlled conditions and (a) exposed to O₃, with the aims to investigate the changes of phenolic compound monomers content in their leaves, so to reveal their response and acclimation/ adaptation mechanism(s) under O₃ pollution; or (b) flooded, to better understand the role of phenolic metabolism in the oxidative stressplant interaction, and to compare the phenolic responses under two different oxidative stressors.

MATERIALS AND METHODS 2

2.1 | Plant material and treatments

Seeds of durum wheat (T. durum Desf.) commercial cultivars Claudio and Mongibello were sown in plastic pots (3.5-L, 1 plant pot⁻¹) containing a growing mix of mature manure:organic soil:peat (1:1:1 in volume) and a slow-release N-P-K (15-11-13) fertilizer, at a dose suggested by the supplier (ANPEL). Pots were then kept under charcoal-filtered air in a greenhouse at the field-station of San Piero a Grado of the Department of Agriculture, Food and Environment, University of Pisa (Italy, UTMWGS84: E 608510 - N 4837241, 3 m a.s.l.), until the stage of "first leaf unfolded/second leaves unfolded" (BBCH-Code [BBCH-C] 11-12; 20-day-old seedlings), according to Lancashire et al. (1991).

Two independent experiments were then performed to investigate the effects of O₃ and flooding on durum wheat, using a completely randomized design. Uniform-sized seedlings were distributed among four had hoc built perspex fumigation chambers (ca. 1 m³ in volume; two chambers per treatment) and (a) exposed to charcoal-filtered air (controls) or a target concentration of 80(±10) ppb of O_3 (ca. 160 μ g/m³ at 20°C and 101.3 kPa, 5 hr/day, in the form of a square wave between 10:00 and 15:00 hr) for 70 consecutive days; or (b) kept under charcoal-filtered air and flooded by adding water (2-L pot^{-1}) above the soil surface to immerse seedlings for seven consecutive days. Ozone was generated by a Fisher 500 air-cooled generator (Fisher America Inc.), supplied with pure oxygen, and mixed with the inlet air entering the fumigation chambers; its concentration in the fumigation chambers was continuously monitored with a Serinus 10 analyser (Ecotech Acoem Group).

Throughout the whole experiment, plants were kept in a greenhouse under 21 ± 3°C of temperature, 65 ± 7% of relative humidity (RH), natural light/dark cycle, and well-watered to field capacity (except for flooded plants). To avoid a "chamber" effect, chambers used for charcoal-filtered air and O₃ exposure were switched every 3-4 days (also moving the plants they contained). For further details of the fumigation facilities, see Cotrozzi et al. (2017).

Ecophysiological investigations and leaf (the third fully expanded trifoliate leaves of stolons) collections for biochemical analyses of control and O₃-treated plants were performed at seven (BBCH-C 13), 28 (BBCH-C 23-32), 50 (BBCH-C 59) and 70 (BBCH-C 65-69) days from the beginning of the exposure (FBE), when the Accumulated exposure Over a Threshold of 40 ppb (AOT40, http:// www.emep.int/mscw/definitions.pdf) was 1,400, 5,600, 10,000 and 14,000 ppb h, respectively. Leaf sampling of flooded plants was performed at 7 days FBE (BBCH-C 13). For each combination of cultivar, treatment and time, completely expanded leaves were collected, mixed, divided into aliquots, instantly frozen in liquid nitrogen and stored at -80°C until biochemical analyses.

2.2 | Foliar visible symptoms

The onset of O₃-induced foliar visible symptoms was daily checked. Mature foliar symptoms were also quantified at the end of the exposure as the percentage of necrotic area on the adaxial surface by manually overlaying a transparent plastic grid (4 mm) and counting the percentage of intersections covering injured areas with respect to healthy ones (Pellegrini, Francini, Lorenzini, & Nali, 2011).

2.3 **Ecophysiological investigations**

Ecophysiological investigations were performed between 11:00 and 13:00 (solar time; i.e. when maximum gas exchanges usually occur) on one completely expanded leaf per plant. CO₂ assimilation rate (A) was measured under light saturated conditions (photosynthetic active radiation of about 800 μ mol photons/m² s⁻¹), ambient CO₂ concentration (ca. 390 µmol/mol) and 60% of RH, using an Infrared Gas Analyzer (CIRAS-1; PP-Systems). Parameters of modulated Journal & Agronomy ... Crop Science WILEY

chlorophyll a fluorescence were measured with a PAM-2000 fluorometer (Walz) after 40 min of dark adaptation. The maximal PSII photochemical efficiency in the dark was calculated as $F_v/F_m = (F_m - F_m)$ F_0)/ F_m , where F_0 and F_m are minimum (recorded with a weak measuring beam of 0.1 μ mol photons/m² s⁻¹) and maximum (determined with a saturating pulse of 8,000 μ mol photons/m² s⁻¹ for 1 s) fluorescence, respectively (Genty, Briantais, & Baker, 1989).

2.4 | Phenolic compounds

The extraction of phenolic compounds was performed according to Sgarbi, Baroni Fornasiero, Lins, and Medeghini Bonatti (2003) with some minor modifications. Samples (50 mg, fresh weight) were homogenized and extracted with 10 ml of 85% (v/v) aqueous methanol for 24 hr at room temperature. After centrifugation at 10,000 g for 10 min, the supernatant was evaporated to dryness under reduced pressure. Residues were again dissolved in 2 ml of 85% aqueous methanol and then applied to a Sep-Pack RP-18 column (Merck KGaA), pre-conditioned with the extraction solvent. The loaded cartridge was washed with 2 ml of the extraction solvent and the residue used for the analysis by reverse-phase highperformance liquid chromatography (HPLC; Varian HPLC System [Varian Inc.], equipped with a UV-Vis variable wavelength detector [Varian Inc., Star 9050, USA], two-pump system [Varian Inc., Star 9010, USA], valve with a 25- μ l sample loop and a 150 mm × 4.6 mm Alltima C18 column [Grace]) kept at 22°C. Elution was performed with gradients of eluent A (water with 1% acetic acid, v/v) and eluent B (methanol:acetonitrile:acetic acid, 95:5:1 in volume), at a flow rate of 1.0 ml/min. Initial eluent conditions were 5% B and 95% A and ran as isocratic for 2 min, followed by four linear gradients: (a) increase to 25% B within 8 min, (b) increase to 40% B within 10 min, (c) increase to 50% B within other 10 min, and (d) up to 100% B in 10 min once again, and then holding 5 min. After that, initial conditions were reached within 5 min. The injected sample volume was 25 µl. Compounds were detected by UV absorbance at 280 nm. Peak identifications and quantifications were performed with authentic compounds used as external standards: benzoic acid (CAS: 65-85-0), caffeic acid (CAS: 331-39-5), chlorogenic acid (CAS: 327-97-9), p-coumaric acid (CAS: 4501-31-9), 3,4-dihydroxybenzoic acid (CAS: 99-50-3), ferulic acid (CAS: 537-98-4), gallic acid (CAS: 149-91-7), sinapic acid (CAS: 530-59-6), syringic acid (CAS: 530-57-4), vanillic acid (CAS: 121-34-6), catechin hydrate (CAS: 154-23-4), syringaldehyde (CAS: 134-96-3). All standards were from Sigma-Aldrich.

2.5 | Statistics

Normal distribution of data was preliminary analysed following the Shapiro-Wilk test. The effects of cultivar (Cv), growth stage (Gs), O₃ treatment (O₂) and their interactions on ecophysiological traits and phenolic compounds were tested by using a three-way analysis of variance (ANOVA). The Tukey HSD test was used as the post hoc 4 WILEY Journal & Agronomy & Crop Science

test. Relations among phenolic compounds from O3 experiment were evaluated using Pearsons's correlations. Only strong correlations (-.6 \geq r \geq .6) are reported below. A discriminant analysis was applied to the full set of phenolic data to select the compounds that best discriminated among cultivars (Claudio and Mongibello), growth stages (BBCH-C 13, BBCH-C 23-32, BBCH-C 59 and BBCH-C 65-69) and O₃ treatments (0 and 80 ppb, 5 hr/day, 70 days). The effects of Cv, flooding (F) and their interaction on phenolic compounds were instead determined by a two-way ANOVA, followed by the post hoc Tukey's HSD test. Effects with $p \le .05$ were considered statistically significant. Statistical analyses were performed in JMP 13.2 (SAS Institute Inc., Cary, NC, USA).

3 RESULTS

3.1 | Ozone

3.1.1 | Visible foliar injury and ecophysiological traits

At the end of the fumigation, plants of both cultivars showed visible foliar injury in form of a widespread chlorosis which developed in bifacial minute (1-2 mm Ø) ivory necrotic lesions scattered among the leaf veins of completely expanded leaves. In Mongibello, the onset of visible injury was at 5 days FBE (AOT40: 1,000 ppb h), whereas in Claudio, it was at 12 days FBE (AOT40: 2,400 ppb h). The final percentage of leaf necrotic area was higher in Mongibello than Claudio ($p \le .05$, data not shown). No visible injury was detected in charcoalfiltered controls.

Significant $Cv \times Gs \times O_3$ interactions were found for both A and F_v/F_m (although $Cv \times Gs$ was not significant for both traits, as well as Cv for A and Cv × O_3 for F_v/F_m ; Table 1): O_3 only induced a concomitant decrease of A and F_{v}/F_{m} at 28 days FBE in Mongibello (-61 and -5% in comparison with controls, respectively), which then recovered at the following times of analysis (Figure 1).

3.1.2 Phenolic variations induced by ozone

A total of 12 phenolic compounds were detected in the leaves of both cultivars under all the environmental conditions (i.e. controls, exposed to O_3 , and flooded; Tables 2 and 5). The interaction $Cv \times Gs \times O_3$ was significant for all of them, except for sinapic acid. The effects of the singular factors and their binary interactions were also mostly significant, except for Cv, O_3 and $Cv \times Gs$ on 3,4-dihydroxybenzoic acid, O_3 and $Cv \times O_3$ on sinapic acid and syringaldehyde, only $Cv \times Gs$ on ferulic acid, and only $Cv \times O_3$ on p-coumaric and syringic acids, as well as on catechin hydrate (Table 2).

Variations of phenolic compounds induced by O₃ are shown in Figures 2-5. In Claudio, O₃ increased the levels of benzoic, chlorogenic, p-coumaric and syringic acids only at 28 days FBE (ca. 3-, 4-, 2.5-, and 1-fold higher than controls, respectively; Figures 2a, **TABLE 1** *p*-values of three-way ANOVA for the effects of cultivar (Cv; Claudio and Mongibello), growth stage (Gs; 7, 28, 50 and 70 days), ozone treatment (O_3 ; 0 and 80 ppb, 5 hr/day) and their interactions on CO2 assimilation rate (A) and maximal PSII photochemical efficiency in the dark (F_v/F_m) of wheat

	df	А	F_v/F_m
Cv	1	0.222	0.018
Gs	3	<0.001	<0.001
O ₃	1	<0.001	0.0231
Cv × Gs	3	0.118	0.127
$Cv \times O_3$	1	0.003	0.801
$Gs \times O_3$	3	0.002	<0.001
$Cv \times Gs \times O_3$	3	0.045	0.002

Note: Significant values ($p \le .05$) are shown in bold. Abbreviation: df, degrees of freedom.

e, 3a, 4c), of caffeic and ferulic acids at both 28 and 50 days FBE (caffeic: +53% and ca. 4-fold, respectively, Figure 1c; ferulic: ca. 2and 5-fold, Figure 3e), and of 3,4-dihydrobenzoic and gallic acids, as well as of catechin hydrate and syringaldehyde, only at 50 days FBE (ca. 3-, 3.5-, 4-, and 3-fold, respectively; Figures 3c, 4a, 5a, c). At 7 days FBE, noteworthy, benzoic acid and syringaldehyde showed higher values in controls of Claudio than in those of Mongibello (as well as in their related O₃-treated plants). No significant differences between treatments were shown at the end of the exposure for all these compounds. In Mongibello, O3 only increased p-coumaric acid at 28 days FBE (+28%, Figure 3b), ferulic acid at 28 and 70 days FBE (+37% and more than 150-fold, respectively; actually, ferulic acid in controls of Mongibello was severely higher than in those of Claudio at 7 days FBE, to then constantly decrease throughout the experiment; Figure 3f), and of catechin hydrate at 50 days FBE (ca. 12-fold; Figure 5b). Vanillic acid increased through time in control plants of both cultivars, especially in Claudio, whereas O₃ limited these raises keeping the levels lower than controls at 50 and 70 days FBE in Claudio (-90 and -47%, respectively; Figure 3d), while only at 70 days FBE in Mongibello (-51%, Figure 4e). Sinapic acid increased through time and significant differences between cultivars were observed only at 70 days FBE when Claudio showed higher values than Mongibello (treatments averaged); whereas significant differences between treatments were observed only at 50 days FBE when O₃treated plants showed higher values than controls (cultivars averaged, data not shown).

3.1.3 | Correlations among phenolic compounds

Correlations among phenolic compounds are reported in Table 3. In both Claudio and Mongibello, positive correlations were found for p-coumaric acid with chlorogenic acid, ferulic acid with benzoic and caffeic acids, gallic acid with 3,4-dihydroxybenzoic acid and syringaldehyde, and between vanillic and sinapic acids. Negative correlations were found between vanillic and syringic acids. In Claudio,

FIGURE 1 CO₂ assimilation rate (A) (a, b) and maximal PSII photochemical efficiency in the dark (F_v/F_m) (c, d) in wheat cultivars Claudio (circle, *left*) and Mongibello (square, *right*) exposed to 0 (open) or 80 (closed) ppb of ozone (5 hr/day) at different growth stages (7, 28, 50, 70 days). Data are shown as mean ± standard deviation (n = 3). According to the three-way ANOVA and the Tukey's HSD *post hoc* test, different letters indicate significant differences ($p \le .05$). Vertical dashed line separates the cultivars



TABLE 2 *p*-values of three-way ANOVA for the effects of cultivar (Cv; Claudio and Mongibello), growth stage (Gs; 7, 28, 50 and 70 days), ozone treatment (O_3 ; 0 and 80 ppb, 5 hr/day) and their interactions on phenolic compounds of wheat

	Cv	Gs	0 ₃	Cv × Gs	$Cv \times O_3$	$Gs \times O_3$	$Cv \times Gs \times O_3$
df	1	3	1	3	1	3	3
Benzoic acid	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	<0.001
Caffeic acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Chlorogenic acid	0.004	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
p-coumaric acid	<0.001	<0.001	<0.001	<0.001	0.211	<0.001	0.010
3,4-dihydrobenzoic acid	0.352	<0.001	0.259	0.918	<0.001	0.025	0.005
Ferulic acid	<0.001	<0.001	<0.001	0.128	<0.001	<0.001	<0.001
Gallic acid	<0.001	<0.001	<0.001	<0.001	<0.001	0.043	<0.001
Sinapic acid	<0.001	<0.001	0.250	<0.001	0.726	<0.001	0.200
Syringic acid	<0.001	<0.001	0.021	<0.001	0.537	0.020	<0.001
Vanillic acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Catechin hydrate	<0.001	<0.001	<0.001	<0.001	0.222	<0.001	<0.001
Syringaldehyde	<0.001	<0.001	0.403	<0.001	0.151	<0.001	<0.001

Note: Significant values ($p \le .05$) are shown in bold.

Abbreviation: df, degrees of freedom.

p-coumaric acid was also positively related with caffeic and ferulic acids. Ferulic acid was also positively related with chlorogenic acid, as well as syringic acid with benzoic acid, catechin hydrate with 3,4-di-hydroxybenzoic and gallic acids, and syringaldehyde with 3,4-di-hydroxybenzoic acid. Negative correlations were found between syringic and sinapic acids; and for syringaldehyde with chlorogenic and vanillic acids. In Mongibello, chlorogenic acid was also positively related with caffeic and 3,4-dihydroxybenzoic acids. 3,4-dihydroxybenzoic acid was also positively related with *p*-coumaric acid. More negative correlations were also reported in Mongibello for sinapic and vanillic acids: sinapic acid was negatively related with benzoic,

caffeic, chlorogenic and ferulic acids; vanillic acid was negatively related with caffeic, chlorogenic, *p*-coumaric, 3–4-dihydroxybenzoic, ferulic and gallic acids.

3.1.4 | Canonical discriminant analysis

The canonical discriminant analysis gave 11 significant new canonical variables (CAN1-10: $p \le .001$; CAN11: $p \le .01$). Among these CANs, the first four accounted for 90.0% of the total variability (Table 4), thus indicating that the multivariate structure of the



FIGURE 2 Contents of benzoic (a, b), caffeic (c, d) and chlorogenic (e, f) acids in wheat cultivars Claudio (circle, *left*) and Mongibello (square, *right*) exposed to 0 (open) or 80 (closed) ppb of ozone (5 hr/day) at different growth stages (7, 28, 50, 70 days). Data are shown as mean \pm standard deviation (n = 3). According to the three-way ANOVA and the Tukey HSD *post hoc* test, different letters indicate significant differences ($p \le .05$). Vertical dashed line separates the cultivars

original variables (i.e. the 12 phenolic compounds detected) can be well represented by CAN1-4. CAN1 and CAN2 together accounted for the 65.1% of the total variability, and markedly separated the two varieties. Across growth stages and O₃ treatments, Claudio showed higher scores (only plants exposed for 70 days to O₃ showed negative scores for both CAN1 and CAN2), while Mongibello showed lower scores (Figure 6a). The phenolic compounds which mostly affected this discrimination were benzoic and syringic acids (positively related with CAN1), as well as p-coumaric and gallic acids (negatively and positively related with CAN2, respectively), and catechin hydrate and syringaldehyde (positively related with CAN2; Table 4). CAN3 and CAN4 together accounted for the 24.9% of the total variability, mostly separating plants sampled at the last growth stages from those previously sampled (only Claudio plants sampled after 28 days and exposed to O₃ were separated from the others, by CAN4). Among plants sampled after 50 days, CAN3 discriminated Claudio plants kept under filtered air from all the others, while CAN4 separated plants exposed to different O₃ levels. Among plants sampled after 70 days, CAN3 separated the cultivars as well as the O₃ treatments, while CAN4 separated only the varieties (Figure 6b). CAN3 was negatively related with 3,4-dihydroxybenzoic acid, and

positively related with sinapic and vanillic acids. CAN4 was positively related with caffeic and chlorogenic acids (Table 4).

3.2 | Flooding

3.2.1 | Phenolic variations induced by flooding

Variations of phenolic compounds induced by flooding are shown in Table 5. The interaction $Cv \times F$ was significant for caffeic, *p*coumaric, 3,4-dihydroxybenzoic, gallic, sinapic and syringic acids. Caffeic and syringic acids were higher in controls of Claudio than in those of Mongibello (+29% and *ca*. 5-fold, respectively), but flooding increased caffeic acid only in cultivar Claudio (+32%, in comparison with controls) and syringic acid only in cultivar Mongibello (7-fold). Oppositely, *p*-coumaric acid was higher in controls of Mongibello than those of Claudio (*ca*. 2.5-fold) and was reduced by flooding only in cultivar Mongibello (-60%, reaching Claudio's levels). 3,4-dihydroxybenzoic, gallic and sinapic acids did not show significant differences between controls of the two cultivars and always increased under flooding. However, 3,4-dihydroxybenzoic

FIGURE 3 Contents of *p*-coumaric (a, b), 3,4-dihydroxybenzoic (c, d) and ferulic (e, f) acids in wheat cultivars Claudio (circle, *left*) and Mongibello (square, *right*) exposed to 0 (open) or 80 (closed) ppb of ozone (5 hr/day) at different growth stages (7, 28, 50, 70 days). Data are shown as mean \pm standard deviation (*n* = 3). According to the three-way ANOVA and the Tukey HSD *post hoc* test, different letters indicate significant differences (*p* ≤ .05). Vertical dashed line separates the cultivars



and gallic acids increased more in Claudio (*ca.* 3- and 2.5-fold, respectively vs. +55 and + 36%), while sinapic acid increased more in Mongibello (*ca.* 23-fold vs. ca. 5.5-fold). Chlorogenic and ferulic acids, as well as catechin hydrate, showed significant effects for both the singular factors, *Cv* and *F.* Overall, chlorogenic and ferulic acids were higher in Mongibello, and flooding increased chlorogenic acid while decreased ferulic acid; catechin hydrate was higher in cultivar Claudio and decreased under flooding. Only a significant effect of *Cv* was observed for benzoic and syringic acids, with higher values of both acids found in Claudio. No significant effects were reported for vanillic acid.

4 | DISCUSSION

The global demands for various grains, including durum wheat, are expected to increase substantially in the coming years, due to the ever-growing human population's needs for food, feed and fuel. Thus, providing consistent or increased durum grain to the world market is one of the priorities for policymakers, researchers and farmers (Li et al., 2018). Abiotic stresses are one of the major constraints to crop production and food security worldwide and the significant role that O_3 is expected to play in deteriorating the global crop production makes O_3 a hidden threat to global food security (Ashmore, 2005; Tai, Val Martin, & Heald, 2014). Therefore, emphasis should be given for a better understanding of crop responses to O_3 for pragmatic implication for remedies and management.

The degree to which plants develop visible injury is usually used in many intra- and inter-species comparisons as an indicator of their O3 sensitivity (Cotrozzi et al., 2018). In agreement with previous studies performed in controlled, semi-controlled and field conditions (Gerosa et al., 2014; Monga et al., 2015; Picchi et al., 2010; Picchi, Monga, Marzuoli, Gerosa, & Faoro, 2017; Reichenauer, Goodman, Kostecki, & Soja, 1998), our results confirmed the O₃ sensitivity of *T. durum* in terms of visible injury, given the rapid and extensive development of necrotic areas observed. Furthermore, based on the onset and diffusion of these manifestations, Claudio seemed to be less sensitive than Mongibello. Visible cell death symptoms were likely associated with the presence of extensive H2O2 deposits, localized mainly on the guard cells and epidermal cell walls of the peristomatal cells, and indicative of an oxidative burst induced by O3, according to previous histo-cytochemical symptom validations by Picchi et al. (2010) and Monga



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FIGURE 4 Contents of gallic (a, b), syringic (c, d) and vanillic (e, f) acids in wheat cultivars Claudio (circle, *left*) and Mongibello (square, *right*) exposed to 0 (open) or 80 (closed) ppb of ozone (5 hr/day) at different growth stages (7, 28, 50, 70 days). Data are shown as mean \pm standard deviation (n = 3). According to the three-way ANOVA and the Tukey HSD *post hoc* test, different letters indicate significant differences ($p \le .05$). Vertical dashed line separates the cultivars

FIGURE 5 Contents of catechin hydrate (a, b) and syringaldehyde (c, d) in wheat cultivars Claudio (circle, *left*) and Mongibello (square, *right*) exposed to 0 (open) or 80 (closed) ppb of ozone (5 hr/day) at different growth stages (7, 28, 50, 70 days). Data are shown as mean \pm standard deviation (*n* = 3). According to the three-way ANOVA and the Tukey HSD *post hoc* test, different letters indicate significant differences (*p* ≤ .05). Vertical dashed line separates the cultivars

TABLE 3 Pearson's correlation matrix describing relationships among phenolic compounds in wheat cultivars Claudio (lower triangular part) and Mongibello (upper triangular part)

	Ben	Caf	Chl	p-cou	3,4-dih	Fer	Gal	Sin	Syr	Van	Cat hyd	Syri
Ben	-	0.57	0.33	0.07	0.00	0.84	0.29	-0.69	0.37	-0.51	-0.08	0.17
Caf	0.57	-	0.61	0.36	0.36	0.67	0.54	-0.77	0.56	-0.74	0.06	0.52
Chl	0.37	0.54	-	0.69	0.61	0.44	0.46	-0.60	0.53	-0.75	-0.16	0.51
p-cou	0.47	0.68	0.91	-	0.71	0.13	0.19	-0.30	0.45	-0.64	0.34	0.14
3,4-dih	0.23	0.48	-0.24	-0.03	-	0.19	0.71	-0.40	0.38	-0.63	0.35	0.57
Fer	0.80	0.80	0.75	0.77	0.15	-	0.52	-0.90	0.27	-0.66	-0.22	0.47
Gal	-0.02	0.32	-0.37	-0.21	0.87	-0.05	-	-0.58	0.40	-0.60	0.24	0.89
Sin	-0.48	-0.11	0.48	0.28	-0.48	-0.03	-0.39	-	-0.31	0.76	-0.28	-0.53
Syr	0.84	0.52	0.21	0.40	0.35	0.59	0.08	-0.64	-	-0.67	0.58	0.23
Van	-0.59	-0.33	0.34	0.10	-0.56	-0.25	-0.41	0.88	-0.66	-	-0.33	-0.52
Cat hyd	-0.17	0.33	-0.32	-0.17	0.77	-0.10	0.96	-0.25	-0.10	-0.27	-	0.18
Syri	0.22	0.14	-0.61	-0.47	0.73	-0.06	0.72	-0.58	0.23	-0.62	0.57	-

Note: Significant correlations ($p \le .05$) are italicized. Strong correlations ($-0.6 \ge r \ge 0.6$) are bolded.

Abbreviations: 3,4-dih, 3,4-dihydroxybenzoic acid; Ben, benzoic acid; Caf, caffeic acid; Cat hyd, catechin hydrate; Chl, chlorogenic acid; Fer, ferulic acid; Gal, gallic acid; p-cou, p-coumaric acid; Sin, sinapic acid; Syr, syringic acid; Syri, syringaldehyde; Van, vanillic acid.

TABLE 4 Correlations between the first four canonicals (CAN) and the original variables

	CAN1	CAN2	CAN3	CAN4
Benzoic acid	0.74	-0.17	-0.37	0.29
Caffeic acid	0.40	0.17	-0.29	0.77
Chlorogenic acid	0.07	-0.46	0.05	0.75
p-coumaric acid	-0.47	-0.65	-0.47	0.15
3,4-dihydroxybenzoic acid	-0.04	0.39	-0.64	0.30
Ferulic acid	0.52	-0.53	-0.28	0.46
Gallic acid	0.11	0.74	-0.41	0.30
Sinapic acid	-0.36	-0.01	0.80	0.43
Syringic acid	0.60	0.26	-0.27	0.27
Vanillic acid	-0.19	0.09	0.92	0.20
Catechin hydrate	-0.25	0.74	-0.38	0.35
Syringaldehyde	0.43	0.70	-0.28	0.10
Eigenvalue	180.8	114.4	76.4	36.7
Variance explained (%)	39.9	25.2	16.8	8.1
Cumulative variance (%)	39.9	65.1	81.9	90.0

Note: Strong correlations $(-.6 \ge r \ge .6)$ are bolded. Discriminant analysis was applied to the full set of phenolic data to select the compounds that best discriminated among cultivars, growth stages and O₃ treatments.

et al. (2015) on several durum wheat cultivars exposed to elevated O3. The accumulation of ROS as the common actual cause of cellular and foliar damage induced by O₃ is largely known (Faoro & Iriti, 2009).

Reactive oxygen species -induced alterations of clorenchyma, usually expressed by chlorosis, yellowish spots, and necrosis, are often associated to alterations of the photosynthetic performance (Cotrozzi et al., 2018; Pellegrini et al., 2011). The lower O_3 tolerance of Mongibello than Claudio deduced on the basis of visible symptoms was confirmed by ecophysiological investigations, since reductions of both A and F_v/F_m ratio, the most widely used photo-oxidative stress markers (Fenollosa & Munné-Bosch, 2018), were only observed in Mongibello, at 28 days FBE. However, our study shows a relative O₃ tolerance of durum wheat in terms of photosynthetic performance, since also Mongibello later recovered optimal A and F_v/F_m values (i.e. similar to controls). This outcome indicates that T. durum can be more O₃ tolerant than common wheat, according with previous investigations. Reichenauer et al. (1998) found that A and F_v/F_m values were negatively affected by chronic O₃ exposure in two O₃ sensitive cultivars of *T. aestivum*, but not in the O₃ tolerant cv. Extradur of T. durum. Specifically, similarly to Mongibello in the present study, Extradur was able to recover an optimal F_v/F_m ratio after an initial decrease. Herbinger et al. (2002) also did not report any negative effects of O_3 on stomatal conductance and $F_v/$ F_m of Extradur. Similarly, Gerosa et al. (2014) did not find any O_3 induced stomatal closure in other two T. durum cultivars, Virgilio (O₃ sensitive) and Neodur (O3 tolerant), and Monga et al. (2015) only reported slight reductions of stomatal conductance in both O₃ sensitive (Sculptur and Colombo) and O3 tolerant cultivars (Pharaon, Gallareta and Vitrón), and no F_v/F_m ratio decreases.

Overall, the works mentioned above show that (a) there is not a regular correlation between O₃ symptoms severity and yield reduction, suggesting that these parameters cannot be always indicative of O₃ sensitivity for wheat; and (b) the O₃ tolerance is not caused by reduced O₃ uptake via the stomata and higher photosynthetic performance, but due to a better ability of wheat cells to cope with the O₃-induced oxidative stress. The study by Feng, Wang, Pleijel, Zhu, and Kobayashi (2016) on the effects of O₃ on photosynthesis of five winter wheat cultivars with different sensitivity shows that the differential response is linked to the level of antioxidant enzymes,



FIGURE 6 Discrimination of cultivar (Claudio, black; Mongibello, red), growth stage (7 days, circle; 28 days, square; 50 days, triangle; 70 days, diamond) and ozone treatment (0 ppb, open; 80 ppb, 5 hr/day, closed) on the basis of canonical discriminant analysis. The first four canonicals (CAN) are shown (CAN1-CAN2, a; CAN3-CAN4, b). Discriminant analysis was applied to the full set of phenolic data to select the compounds that best discriminated among cultivars, growth stages and O₃ treatments

TABLE 5 Contents of phenolic compounds in wheat cvs Claudio and Mongibello submerged (flooding) or not-submerged (control)

	Claudio		Mongibello		p	p		
	Control	Flooding	Control	Flooding	Cv (df: 1)	F (df: 1)	Cv × F (df: 1)	
Ben	19.06 ± 0.09	19.55 ± 3.55	9.12 ± 0.15	6.41 ± 0.94	<0.001	0.326	0.170	
Caf	$0.31 \pm 0.04 \text{ b}$	0.41 ± 0.01 c	0.22 ± 0.01 a	0.17 ± 0.04 a	<0.001	0.260	0.003	
Chl	1.13 ± 0.10	5.08 ± 0.94	4.16 ± 1.06	7.32 ± 1.11	0.001	<0.001	0.466	
p-cou	0.31 ± 0.01 a	0.38 ± 0.04 a	0.78 ± 0.03 b	0.31 ± 0.06 a	<0.001	<0.001	<0.001	
3,4-dih	$0.10\pm0.00~\text{a}$	0.32 ± 0.03 c	0.11 ± 0.00 a	0.17 ± 0.01 b	<0.001	<0.001	<0.001	
Fer	2.21 ± 0.32	0.73 ± 0.24	3.94 ± 0.20	2.25 ± 0.87	<0.001	<0.001	0.708	
Gal	6.39 ± 0.80 a	15.57 ± 0.31 c	6.17 ± 0.24 a	8.40 ± 0.45 b	<0.001	<0.001	<0.001	
Sin	0.61 ± 0.13 a	3.30 ± 1.40 b	0.30 ± 0.13 a	6.82 ± 1.39 c	0.023	<0.001	0.010	
Syr	0.39 ± 0.08 bc	$0.13 \pm 0.01 \text{ ab}$	0.08 ± 0.03 a	0.56 ± 0.19 c	0.319	0.107	<0.001	
Van	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.519	0.347	0.892	
Cat hyd	3.07 ± 0.88	1.34 ± 0.15	2.02 ± 0.64	0.43 ± 0.05	0.015	<0.001	0.843	
Syri	2.62 ± 0.26	2.55 ± 0.38	0.95 ± 0.08	1.16 ± 0.24	<0.001	0.674	0.396	

Note: Data are shown as mean \pm standard deviation. *P*-values of two-way ANOVA for the effects of cultivar (Cv), flooding (F) and their interactions on phenolic compounds of wheat are shown. Significant values ($p \le .05$) are shown in bold. According to the Tukey HSD *post hoc* test, different letters indicate significant differences ($p \le .05$).

Abbreviation: df, degrees of freedom. For abbreviations, see Table 3.

rather than stomatal conductance, and highlights the need for breeding plant varieties higher in such enzymes. A similar conclusion was reached by Picchi et al. (2017) who showed that the differential O_3 sensitivity of the *T. durum* cultivars Colombo and Sculptur was related to their regulations of ascorbate and glutathione, among the main non-enzymatic antioxidants (Blokhina, Virolainen, & Fagerstedt, 2003), these being a mechanism also reported for the response to other abiotic stressors (Paradiso et al., 2008).

Phenolic compounds are plant secondary metabolites derived from the phenylpropanoid pathway with flavonoids and phenolic acids being the major groups found in cereals (Atanasova-Penichon, Barreau, & Forget, 2016; Balmer, Flors, Glauser, & Mauch-Mani, 2013). Phenolic acids and flavonoids account for a substantial proportion of total antioxidant activity in cereal leaves and grains, and dietary intake of plant phenolics have been linked to a range of positive health impacts (Del Rio et al., 2013; Rempelos et al., 2018). Few O_3 experiments have been conducted on the mechanisms of the secondary metabolism of wheat cultivars, and little is known of the role played by phenolic compounds in the ROS scavenging and antioxidant mechanism (Fatima, Singh, Mukherjee, Agrawal, & Agrawal, 2018; Wang, Zhu, Zheng, & Liu, 2014; Yadav et al., 2019). More generally, also few studies have investigated the phenolic metabolism of durum wheat, only focusing on grain contents (Branković et al., 2015; Di Loreto et al., 2018; Pasqualone et al., 2014; Shamloo et al.,

2017), except for the work of Stagnari et al. (2017) where sprouts and wheatgrass (about 5 and 12 cm shoot length, respectively) of *T. durum* cultivar Creso were also assessed. Thus, the major value of the present study is undoubtedly the pioneering investigation of phenolic metabolism of durum wheat under O_3 .

We identified 12 phenolic compounds in the leaves of *T. durum* cvs Claudio and Mongibello. Ten of these compounds were simple phenolic acids. Phenolic acids are non-flavonoid polyphenolic compounds, which can be further divided into two main types, benzoic acid and cinnamic acid derivatives based on C1-C6 and C3-C6 backbones (Cheynier et al., 2013). We identified five compounds for each type (benzoic acids: benzoic, 3–4-dihydroxybenzoic, gallic, syringic and vanillic acids; cinnamic acids: caffeic, chlorogenic, *p*-coumaric, ferulic and sinapic acids). The two remaining compounds belonged to flavanols (catechin hydrate), a specific class of flavonoids, and to phenolic aldehydes (syringaldehyde). Most of these compounds were already identified in previous durum wheat investigations in both grains and shoot tissues (Branković et al., 2015; Di Loreto et al., 2018; Pasqualone et al., 2014; Shamloo et al., 2017; Stagnari et al., 2017).

Overall, O₃ exposure resulted in accumulations of phenolic compounds, according with previous reports on common wheat (Fatima et al., 2018; Wang et al., 2014; Yadav et al., 2019). This response occurred at 28 and/or 50 days FBE but not at the last time of analysis (i.e. 70 days FBE), likely because plants at senescence (as confirmed by drops of the photosynthetic process of controls) were not more able to activate this mechanism and/or promoted the allocation of resources to other processes. Only vanillic acid, which similarly increased in senescing plants of both cultivars, was reduced by O₂ (with a negative correlation with syringic acid). A substantial induction of the phenolic metabolism under O3 was observed in Claudio, while it was less activated in Mongibello. Thus, the increase of phenolic concentration can be likely considered a repair process that equipped Claudio stressed plants with an antioxidant system capable of scavenging ROS, limiting cell death and avoiding photosynthetic impairment (Cheynier et al., 2013).

Given the number of ways phenolics are able to act as antioxidants, as well as their multiple functions in plants, the observed inductions of specific compounds at specific times can be interpreted as an orchestrated fine regulation adopted by plants to cope with the chronic O₃-induced oxidative stress (Cheynier et al., 2013). In Claudio, most of the phenolic acids were triggered by O₃ already at 28 days FBE, with caffeic and ferulic acids remaining at higher levels also at the following time of analysis. Only 3,4-dihydroxybenzoic and gallic acids were later induced at 50 days FBE, similarly to catechin hydrate and syringaldehyde. Interestingly, the flavanol syringaldehyde was the only compound showing very similar raises between cultivars, since it increased at 50 days also in Mongibello (where only slight increases of p-coumaric and ferulic acids were also observed). This outcome may confirm the key role of flavonoids in ROS scavenging under chronic O₃-induced oxidative stress (Cheynier et al., 2013; Cotrozzi et al., 2018). These cultivar-specific fine regulations of the phenolic metabolism adopted by plants to cope with O₃ stress trough time was confirmed by the several significant correlations among compounds.

Phenolic profiles can be valuable chemical markers of specific species and cultivars under variable environments (Cotrozzi et al., 2018; Medina-Medrano et al., 2015). Interestingly, the canonical discriminant analysis showed that benzoic, syringic, *p*-coumaric and gallic acids, together with catechin hydrate and syringaldehyde had a higher power in discriminating the cultivars, whereas 3,4-dihydroxy-benzoic, sinapic and vanillic acids were the compounds that mostly discriminated both the cultivars and the treatments in older plants, at 50 and 70 days FBE. This outcome further suggests that investigations and characterizations of specific phenolic profiles of crops under oxidative stress may be helpful in breeding programmes.

Finally, the investigation of phenolic metabolism in flooded plants is another innovative aspect of the present study. However, we did not find similar responses among plants exposed to different oxidative stresses (i.e. O₃ and flooding), as we instead expected. Flooding only induced an accumulation of chlorogenic, 3,4-dihydroxybenzoic, gallic and sinapic acids in both the cultivars, with the former increasing similarly in both the cultivars, the second and the third increasing more in Claudio than in Mongibello, and the latter increasing more in Mongibello. Flooding-induced reductions of some phenolic compounds were also observed: p-coumaric acid only in Mongibello, while ferulic acid and catechin hydrate in both the species. These responses were likely due to the short duration of flooding more than to the activation of other adaptive systems (e.g. aerenchyma, Dennis et al., 2000), as supported by the findings of Pampana et al. (2016) who reported a reduction of grain yield of two varieties (Claudio and Svevo) only when waterlogging was prolonged for more than 20 days. Although O₃ and flooding are similarly able to induce oxidative stress in plants (Mustroph, 2018; Sandermann, 1996), our data show that the phenolic response to these environmental constraints is stress specific, with a higher activation induced under O₃ than under flooding, another aspect to take into account when breeding future cultivars.

5 | CONCLUSIONS

The most sustainable long-term strategy to alleviate the effects of tropospheric O_3 on agricultural crops (and human health) would certainly be to reduce air pollution, but various emission scenarios suggest that O_3 precursor levels may continue to rise in many parts of the world in the coming decades, leading to regional hotspots of O_3 pollution. Increased flooding frequency is also expected for the near future due to changes in global climate. Therefore, adaptation of crop production to rising levels of O_3 and flooding is a solution as a mid-term strategy to avoid yield losses, and to ensure food security in those highly populated parts of the world (Biswas et al., 2009; Tai et al., 2014). According to Frei (2015), this could be performed by shifting crop calendars to avoid major episodes of high O_3 levels, but a modelling study suggested that this measure had little effect on a global scale (Teixeira et al., 2011). A second option would be to breed crops, which are better WILEY-

adapted to environmental constraints. The genotype by environment interaction highlighted by the present study suggests that genotype selection is of crucial importance (especially if exacerbated stress conditions are forecasted). Our study also shows that investigations and characterization of specific phenolic profiles of crop cultivars under oxidative stress may be helpful in breeding programmes. Given the different phenolic responses observed under O_3 and flooding, these investigations should be performed for specific stressors, testing variable intensities and durations of stress exposure.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

SUBMISSION DECLARATION

The authors declare that the work here described has not been published previously, and that it is not under consideration for publication elsewhere, and its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

ORCID

Chong Chen D https://orcid.org/0000-0002-1743-7511 Lorenzo Cotrozzi D https://orcid.org/0000-0002-4401-3896 Limin Ma D https://orcid.org/0000-0001-9459-963X

REFERENCES

- Armstrong, W., Brändle, R., & Jackson, M. B. (1994). Mechanisms of flood tolerance in plants. Acta Botanica Neerlandica, 43, 307–358. https:// doi.org/10.1111/j.1438-8677.1994.tb00756.x
- Ashmore, M. R. (2005). Assessing the future global impacts of ozone on vegetation. *Plant, Cell and Environment*, 28, 949–964. https://doi. org/10.1111/j.1365-3040.2005.01341.x
- Atanasova-Penichon, V., Barreau, C., & Forget, F. R. (2016). Antioxidant secondary metabolites in cereals: Potential involvement in resistance to *fusarium* and mycotoxin accumulation. *Frontiers in Microbiology*, 7, 566. https://doi.org/10.3389/fmicb.2016.00566
- Bailey-Serres, J., Lee, S. C., & Brinton, E. (2012). Waterproofing crops: Effective flooding survival strategies. *Plant Physiology*, 160, 1689– 1709. https://doi.org/10.1104/pp.112.208173
- Balmer, D., Flors, V., Glauser, G., & Mauch-Mani, B. (2013). Metabolomics of cereals under biotic stress: Current knowledge and techniques. Frontiers in Plant Science, 4, 1–12. https://doi.org/10.3389/ fpls.2013.00082
- Bhuiyan, M. A., Khan, H. U. R., Zaman, K., & Hishan, S. S. (2018). Measuring the impact of global tropospheric ozone, carbon

dioxide and sulfur dioxide concentrations on biodiversity loss. *Environmental Research*, 160, 398–411. https://doi.org/10.1016/j. envres.2017.10.013

- Biagioni, M., Nali, C., Heimler, D., & Lorenzini, G. (1998). PAL activity and differential ozone sensitivity in tobacco, bean and poplar. *Journal of Phytopathology*, 145, 533–539. https://doi. org/10.1111/j.1439-0434.1997.tb00362.x
- Biswas, D. K., Xua, H., Yang, J. C., Li, Y. G., Chen, S. B., Jiang, C. D., ... Jiang, G. M. (2009). Impacts of methods and sites of plant breeding on ozone sensitivity in winter wheat cultivars. *Agriculture, Ecosystems* and Environment, 134, 168–177. https://doi.org/10.1016/j. agee.2009.06.009
- Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. Annals of Botany, 91, 179–194. https://doi.org/10.1093/aob/mcf118
- Branković, G., Dragičević, V., Dodig, D., Knežević, D., Kandić, V., Šurlan-Momirović, G., & Sečanski, M. (2015). Phytic acid, inorganic phosphorus, antioxidants in bread and durum wheat and their associations with agronomic traits. Agricultural and Food Science, 24, 183–194. https://doi.org/10.23986/afsci.49729
- Burgos, S. T., Stamp, P., & Schmid, J. E. (2001). Agronomic and physiological study of cold and flooding tolerance of spelt (*Triticum spelta* L.) and wheat (*Triticum aestivum* L.). Journal of Agronomy and Crop Science, 187, 195–201. https://doi. org/10.1046/j.1439-037x.2001.00516.x
- Chernikova, T., Robinson, M. J., Lee, E. H., & Mulchi, C. L. (2000). Ozone tolerance and antioxidant enzyme activity in soybean cultivars. *Photosynthesis Research*, *64*, 15–26. https://doi.org/10.1023/A:10265 00911237
- Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry*, 72, 1–20. https:// doi.org/10.1016/j.plaphy.2013.05.009
- Cotrozzi, L., Campanella, A., Pellegrini, E., Lorenzini, G., Nali, C., & Paoletti, E. (2018). Phenylpropanoids are key players in the antioxidant defense to ozone of European ash, *Fraxinus excelsior*. *Environmental Science and Pollution Research*, 25, 8137–8147. https:// doi.org/10.1007/s11356-016-8194-8
- Cotrozzi, L., Pellegrini, E., Guidi, L., Landi, M., Lorenzini, G., Massai, R., ... Nali, C. (2017). Losing the warning signal: Drought compromises the cross-talk of signalling molecules in *Quercus ilex* exposed to ozone. *Frontiers in Plant Science*, 8, 1020. https://doi.org/10.3389/ fpls.2017.01020
- Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxidants & Redox Signaling, 18, 1818–1892. https://doi.org/10.1089/ars.2012.4581
- Dennis, E. S., Dolferus, R., Ellis, M., Rahman, M., Wu, Y., Hoeren, F. H., ... Peacock, W. J. (2000). Molecular strategies for improving waterlogging tolerance in plants. *Journal of Experimental Botany*, 51, 89–97. https://doi.org/10.1093/jexbot/51.342.89
- Di Loreto, A., Bosi, S., Montero, L., Bregola, V., Marotti, I., Sferrazza, R. E., ... Cifuentes, A. (2018). Determination of phenolic compounds in ancient and modern durum wheat genotypes. *Electrophoresis*, *39*, 2001–2010. https://doi.org/10.1002/elps.201700439
- Fangmeier, A., Brockerhoff, U., Grüters, U., & Jäger, H.-J. (1994). Growth and yield responses of spring wheat (*Triticum aestivum* L. cv. Turbo) grown in open-top chambers to ozone and water stress. *Environmental Pollution*, 83, 317–325. https://doi. org/10.1016/0269-7491(94)90153-8
- Faoro, F., & Iriti, M. (2009). Plant cell death and cellular alterations induced by ozone: Key studies in Mediterranean conditions. *Environmental Pollution*, 157, 1470–1477. https://doi.org/10.1016/j. envpol.2008.09.026

Journal & Agronomy and Crop Science

- Fatima, A., Singh, A. A., Mukherjee, A., Agrawal, M., & Agrawal, S. B. (2018). Variability in defence mechanism operating in three wheat cultivars having different levels of sensitivity against elevated ozone.
- org/10.1016/j.envexpbot.2018.06.015 Feng, Z. Z., Wang, L., Pleijel, H., Zhu, J., & Kobayashi, K. (2016). Differential effects of ozone on photosynthesis of winter wheat among cultivars depend on antioxidative enzymes rather than stomatal conductance. *Science of the Total Environment*, 572, 404-411. https://doi.org/10.1016/j.scitotenv.2016.08.083

Environmental and Experimental Botany, 155, 66-78. https://doi.

- Fenollosa, E., & Munné-Bosch, S. (2018). Photoprotection and photo-oxidative stress markers as useful tools to unravel plant invasion success. In A. Sánchez-Moreiras, & M. Reigosa (Eds.), Advances in plant ecophysiology techniques (pp. 153–175). Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-319-93233-0_9
- Frei, M. (2015). Breeding of ozone resistant rice: Relevance, approaches and challenges. *Environmental Pollution*, 197, 144–155. https://doi. org/10.1016/j.envpol.2014.12.011
- Garssen, A. G., Baattrup-Pedersen, A., Voesenek, L. A. C. J., Verhoeven, J. T. A., & Soons, M. B. (2015). Riparian plant community responses to increased flooding: A meta-analysis. *Global Change Biology*, 21, 2881–2890. https://doi.org/10.1111/gcb.12921
- Gaude, S., Jena, C., Chate, D. M., Beig, G., Pfister, G. G., Kumar, R., & Ramanathan, V. (2014). Reductions in India's crop yield due to ozone. *Geophysical Research Letters*, 41, 5685–5691. https://doi. org/10.1002/2014GL060930
- Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica Et Biophysica Acta* (BBA) - *General Subjects*, 990, 87–92. https://doi.org/10.1016/ S0304-4165(89)80016-9
- Gerosa, G., Marzuoli, R., Finco, A., Monga, R., Fusaro, I., & Faoro, F. (2014). Contrasting effects of water salinity and ozone concentration on two cultivars of durum wheat (*Triticum durum* Desf.) in Mediterranean conditions. *Environmental Pollution*, 193, 13–21. https ://doi.org/10.1016/j.envpol.2014.05.027
- Heath, R. L. (1987). The biochemistry of ozone attack on the plasma membrane of plant cells. In J. A. Saunders, L. Kosak-Channing, & E. E. Conn (Eds.), *Phytochemical effects of environmental compounds. Recent Advances in Phytochemistry* (vol. 21) (pp. 29–54). Boston, MA: Springer. https://doi.org/10.1007/978-1-4613-1931-3_2
- Herbinger, K., Tausz, M., Wonisch, A., Soja, G., Sorger, A., & Grill, D. (2002). Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiology and Biochemistry*, 40, 691–696. https://doi.org/10.1016/ S0981-9428(02)01410-9
- Jackson, M. B., & Colmer, T. D. (2005). Response and adaptation by plants to flooding stress. Annals of Botany, 96, 501–505. https://doi. org/10.1093/aob/mci205
- Kadkol, G. P., & Sissons, M. (2016). Durum wheat: Overview. Encyclopedia of Food Grains, 1, 117–124. https://doi.org/10.1016/ B978-0-12-394437-5.00024-3
- Komatsu, S., Hiraga, S., & Yanagawa, Y. (2012). Proteomics techniques for the development of flood tolerant crops. *Journal of Proteome Research*, 11, 68–78. https://doi.org/10.1021/pr2008863
- Krupa, S., McGrath, M. T., Andersen, C. P., Booker, F. L., Burkey, K. O., Chappelka, A. H., ... Zilinskas, B. A. (2001). Ambient ozone and plant health. *Plant Disease*, *85*, 4–17. https://doi.org/10.1094/ PDIS.2001.85.1.4
- Kulbat, K. (2016). The role of phenolic compounds in plant resistance. Biotechnology and Food Science, 80, 97–108.
- Lancashire, P. D., Bleiholder, H., van den Boom, T., Langelüddeke, P., Stauss, R., Weber, E., & WitzenBerger, A. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, 119, 561–601. https://doi.org/10.1111/j.1744-7348.1991.tb04895.x

- Lefohn, A. S., Malley, C. S., Smith, L., Wells, B., Hazucha, M., Simon, H., ... Gerosa, G. (2018). Tropospheric ozone assessment report: Global ozone metrics for climate change, human health, and crop/ecosystem research. *Elementa*, *6*, 28. https://doi.org/10.1525/elementa.279
- Li, L., Niu, Y., Ruan, Y., DePauw, R. M., Singh, A. K., & Gan, Y. (2018). Agronomic advancement in tillage, crop rotation, soil health, and genetic gain in durum wheat cultivation: A 17-year Canadian story. Agronomy, 8, 193. https://doi.org/10.3390/agronomy8090193
- Medina-Medrano, J. R., Almaraz-Abarca, N., González-Elizondo, M. S., Uribe-Soto, J. N., González-Valdez, L. S., & Herrera-Arrieta, Y. (2015).
 Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae). *Botanical Studies*, *56*, 24. https://doi. org/10.1186/s40529-015-0101-y
- Monga, R., Marzuoli, R., Alonso, R., Bermejo, V., González-Fernández, I., Faoro, F., & Gerosa, G. (2015). Varietal screening of ozone sensitivity in Mediterranean durum wheat (*Triticum durum*, Desf.). *Atmospheric Environment*, 110, 18–26. https://doi.org/10.1016/j. atmosenv.2015.03.040
- Mustroph, A. (2018). Improving flooding tolerance of crop plants. Agronomy, 8, 160. https://doi.org/10.3390/agronomy8090160
- Nuvolone, D., Petri, D., & Voller, F. (2018). The effects of ozone on human health. *Environmental Science and Pollution Research*, *25*, 8074–8088. https://doi.org/10.1007/s11356-017-9239-3
- Ollerenshaw, J. H., & Lyons, T. (1999). Impacts of ozone on the growth and yield of field-grown winter wheat. *Environmental Pollution*, 106, 67–72. https://doi.org/10.1016/S0269-7491(99)00060-3
- Pampana, S., Masoni, A., & Arduini, I. (2016). Grain yield of durum wheat as affected by waterlogging at tillering. *Cereal Research Communications*, 44, 706–716. https://doi.org/10.1556/0806.44.2016.026
- Paradiso, A., Berardino, R., de Pinto, M. C., Sanità di Toppi, L., Storelli, M. M., Tommasi, F., & De Gara, L. (2008). Increase in ascorbate-glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants. *Plant and Cell Physiology*, 49, 362–374. https://doi.org/10.1093/pcp/pcn013
- Pasqualone, A., DelVecchio, L. N., Lacolla, G., Piarulli, L., Simeone, R., & Cucci, G. (2014). Effect of composted sewage sludge on durum wheat: Productivity, phenolic compounds, antioxidant activity, and technological quality. *Journal of Food, Agriculture & Environment*, 12, 276–280.
- Pellegrini, E., Campanella, A., Cotrozzi, L., Tonelli, M., Nali, C., & Lorenzini, G. (2018a). Ozone primes changes in phytochemical parameters in the medicinal herb *Hypericum perforatum* (St. John's wort). *Industrial Crops and Products*, 126, 119–128. https://doi.org/10.1016/j.indcr op.2018.10.002
- Pellegrini, E., Campanella, A., Cotrozzi, L., Tonelli, M., Nali, C., & Lorenzini, G. (2018b). What about the detoxification mechanism underlying ozone sensitivity in *Liriodendron tulipifera? Environmental Science and Pollution Research*, 25, 8148–8160. https://doi.org/10.1016/10.1007/ s11356-017-8818-7)
- Pellegrini, E., Francini, A., Lorenzini, G., & Nali, C. (2011). PSII photochemistry and carboxylation efficiency in *Liriodendron tulipifera* under ozone exposure. *Environmental and Experimental Botany*, 70, 217–226. https://doi.org/10.1016/j.envexpbot.2010.09.012
- Pellegrini, E., Hoshika, Y., Dusart, N., Cotrozzi, L., Gérard, J., Nali, C., ... Paoletti, E. (2019). Antioxidative responses of three oak species under ozone and water stress conditions. *Science of the Total Environment*, 647, 390–399. https://doi.org/10.1016/j.scitotenv.2018.07.413
- Picchi, V., Francini, A., Nali, C., & Lorenzini, G. (2006). Photosynthetic responses of two *Triticum durum* varieties exposed to chronic ozone fumigation. *Journal of Plant Pathology*, 88, S23.
- Picchi, V., Iriti, M., Quaroni, S., Saracchi, M., Viola, P., & Faoro, F. (2010). Climate variations and phenological stages modulate ozone damages in field-grown wheat. A three-year study with eight modern cultivars in Po Valley (Northern Italy). Agriculture, Ecosystems and Environment, 135, 310–317. https://doi.org/10.1016/j.agee.2009.10.012

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- Picchi, V., Monga, R., Marzuoli, R., Gerosa, G., & Faoro, F. (2017). The ozone-like syndrome in durum wheat (*Triticum durum* Desf.): Mechanisms underlying the different symptomatic responses of two sensitive cultivars. *Plant Physiology and Biochemistry*, 112, 261–269. https://doi.org/10.1016/j.plaphy.2017.01.011
- Reichenauer, T. G., Goodman, B. A., Kostecki, P., & Soja, G. (1998). Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury, photosynthetic activity and free radical content. *Physiologia Plantarum*, 104, 681-686. https://doi.org/10.103 4/j.1399-3054.1998.1040423.x
- Rempelos, L., Almuayrifi, A. M., Baranski, M., Tetard-Jones, C., Eyre, M., Shotton, P., ... Bilsborrow, P. (2018). Effects of agronomic management and climate on leaf phenolic profiles, diseases severity, and grain yield in organic and conventional wheat production systems. *Journal of Agricultural and Food Chemistry*, 66, 10369–10379. https:// doi.org/10.1021/acs.jafc.8b02626
- Sandermann, H. Jr (1996). Ozone and plant health. Annual Review of Phytopathology, 34, 347–366. https://doi.org/10.1146/annur ev.phyto.34.1.347
- Sandermann, H. Jr, Ernst, D., Heller, W., & Langebartels, C. (1998). Ozone: An abiotic elicitor of plant defense reactions. *Trends in Plant Science*, 3, 47–50. https://doi.org/10.1016/S1360-1385(97)01162-X
- Sgarbi, E., Baroni Fornasiero, R., Lins, A. P., & Medeghini Bonatti, P. (2003). Phenol metabolism is differentially affected by ozone in two cell lines from grape (Vitis vinifera L.) leaf. Plant Science, 165, 951–957. https://doi.org/10.1016/S0168-9452(03)00219-X
- Shalygo, N. V., Domanskaya, I. N., Radyuk, M. S., Shcherbakov, R. A., & Dremuk, I. A. (2012). Accumulation of hydrogen peroxide and functioning of defense system in overwatered barley seedlings. *Russian Journal of Plant Pathology*, *59*, 748–756. https://doi.org/10.1134/ S1021443712050147
- Shamloo, M., Babawale, E. A., Furtado, A., Henry, R. J., Eck, P. K., & Jones, P. J. H. (2017). Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. *Scientific Reports*, 7, 9133. https://doi.org/10.1038/s41598-017-09681-5
- Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012, 1–26. https://doi.org/10.1155/2012/217037
- Šramková, Z., Gregová, E., & Šturdík, E. (2009). Chemical composition and nutritional quality of wheat grain. Acta Chimica Slovaca, 2, 115–138.

- Stagnari, F., Galieni, A., D'Egidio, S., Falcinelli, B., Pagnani, G., Pace, R., ... Benincasa, P. (2017). Effects of sprouting and salt stress on polyphenol composition and antiradical activity of einkorn, emmer and durum wheat. *Italian Journal of Agronomy*, 12, 848. https://doi. org/10.4081/ija.2017.848
- Tai, A. P. K., Val Martin, M., & Heald, C. L. (2014). Threat to future global food security from climate change and ozone air pollution. *Nature Climate Change*, 4, 817–821. https://doi.org/10.1038/nclimate2317
- Teixeira, E., Fischer, G., van Velthuizen, H., van Dingenen, R., Dentener, F., Mills, G., ... Ewert, F. (2011). Limited potential of crop management for mitigating surface ozone impacts on global food supply. *Atmospheric Environment*, 45, 2569–2576. https://doi.org/10.1016/j. atmosenv.2011.02.002
- The Royal Society (2008). Ground-level ozone in the 21st century: future trends, impacts and policy implications. Science Policy Report 15/08. London, UK: The Royal Society.
- Wang, J., Zhu, J., Zeng, Q., & Liu, G. (2014). Phenolic compounds and antioxidant ability responses to experimental free-air ozone exposure in two wheat cultivars. Bulletin of Environmental Contamination and Toxicology, 93, 625–631. https://doi.org/10.1007/ s00128-014-1375-6
- Wang, X., Manning, W., Feng, Z., & Zhu, Y. (2007). Ground-level ozone in China: Distribution and effects on crop yields. *Environmental Pollution*, 147, 394–400. https://doi.org/10.1016/j.envpol.2006.05.006)
- Yadav, D. S., Rai, R., Mishra, A. K., Chaudhary, N., Mukherjee, A., Agrawal, S. B., & Agrawal, M. (2019). ROS production and its detoxification in early and late sown cultivars of wheat under future O₃ concentration. *Science of the Total Environment*, 659, 200–210. https://doi. org/10.1016/j.scitotenv.2018.12.352
- Yordanova, Y. R., & Popova, P. L. (2007). Flooding-induced changes in photosynthesis and oxidative status in maize plants. Acta Physiologiae Plantarum, 29, 535–541. https://doi.org/10.1007/ s11738-007-0064-z

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