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Changes of paramagnetic species in cereal grains upon short-term ozone action as a marker of oxidative stress tolerance

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ABSTRACT

The increase of the concentration of ozone in the atmosphere, being the direct source of reactive oxygen species, results in the yield loss of agronomic crops. On the other hand, ozone is also used as a protector against microorganisms, living in plants and present in materials obtained from them, dangerous for human and animal health. In this work it has been studied if ozone in doses similar to those used for removal of microorganisms can have significant influence on the generation of stable organic radicals and changes in the character of transition metal ions and in the antioxidative biochemical parameters of cereal grains. The aim of this work was to find if the response of grains of three cereals (wheat, oat and barley) to ozone depended on their oxidative stress tolerance. The influence of direct short-term ozone application on grains of these cereals, each represented by two genotypes with different oxidative stress tolerance, was studied by biochemical analyses and by electron paramagnetic resonance (EPR) technique. Whole grains as well as their parts: embryo, endosperm and seed coat were subjected to ozone treatment for 30 min. Biochemical investigation of control samples showed that their antioxidant activity increased in order: wheat < oat < barley. EPR method revealed that character and the number of paramagnetic species (transition metal ions: Fe(III), Cu(II), Mn(II) and stable organic radicals) changed upon ozone exposure, depending on the kind of cereal, stress tolerance of particular genotype and the part of grain. The control samples of whole grains and their parts originating from sensitive genotypes contained higher amounts of stable organic radicals (semiquinone, phenoxyl and carbohydrate types) than those from tolerant ones. In embryos of grains from sensitive genotypes their amount increased upon ozone treatment stronger than in embryos from grains of tolerant cultivars. In seed coats and endosperms such relation was not found and the changes in the content of the radicals during ozone application were correlated with the amount of transition metal ions and were more intensive in parts of grains richer in easily oxidized iron species Fe(II), located in inorganic structures. On the contrary, Fe(II) ions situated in embryos were stabilized by organic matrix and did not undergo oxidation by ozone.

1. Introduction

Ozone has long been recognized as a stress factor that limits agricultural crops production worldwide (Fiscus et al., 2005). Various studies reported negative effects of ozone on plant yields, but the extent of the loss of plant productivity differed widely depending on the way of ozone applications and the kind of plants (Feng et al., 2008, 2010; Booker et al., 2009). The complex nature of metabolite changes in vegetative and reproductive organs and the dependence of plant development on the level of its sensitivity to stress action make it difficult to generalize the effects of ozone on plants (Black et al., 2000). Thus, the specific pathways and targets of ozone's attack within plant cells are still not fully known.

It was found that in leaves ozone treatment increased dark respiration (Tjoelker et al., 1995), decreased photosynthesis of plants and stimulated the antioxidant systems (Pell et al., 1997), especially by activation of enzymes (superoxide dismutases, catalase and/or peroxidases), as well as by synthesis of nonenzymatic antioxidants (Musselman and Massman, 1999). It is assumed that ozone enters the plants through their stomata and then rapidly reacts forming other reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radical (Sandermann, 2008).

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Besides this negative effects, ozone has numerous beneficial applications and is used in the food processing industry (Desvignes et al., 2008; Kim et al., 1999; Legeron, 1984; Maeba et al., 1988; Raila et al., 2006; Rakcejeva et al., 2014). The applications of ozone lead to reduction of the content of natural microflora, as well as bacterial, fungal and mould contaminations in cereal grains and their products. As a short-lived molecule (half-life of 20–50 min), ozone decomposes to diatomic oxygen, a natural component of the atmosphere, thus its short direct action is concentrated mainly on the cell membranes of bacteria or viruses attacking grain surface (Kharel and Amgain, 2010). On the other hand, such ozone treatment can lead also to disturbances of cellular grains activity. It was found that this process was connected with the changes in the content of important biochemical components in the grains, suggesting stressogenic effect of ozone (Rakcejeva et al., 2014).

One of the methods useful to quantitative and qualitative studies of such specific oxygen species is electron paramagnetic resonance (EPR) spectroscopy (Spasojević et al., 2011; Miller and Brudvig, 1991). In the case of leaves subjected to low levels of ozone, the direct EPR measurements revealed the appearance of $O_2^{-\bullet}$ signal (Runeckles and Vaartnou, 1997). On the basis of EPR studies it was showed that this radical was scavenged by chloroplastic SOD—enzyme systems and that this process was dependent on their scavenging capacity. Our earlier work performed on barley leaves subjected to oxidative stress (induced by drought) showed the dependence between the decrease of photosynthesis and the generation of stable organic radicals which was correlated with stress tolerance of cultivars (Filek et al., 2015). We postulated that these radicals were created during interaction of reactive oxygen species with chemical compounds of cell structures, such as proteins, lipids, carbohydrates and others (Łabanowska et al., 2013a, 2014). Such stable radical species may be produced by removal of hydrogen atom from carbon atom of carbohydrate molecule or from oxygen atom of OH group of other molecules (for example of amino acids or phenolic-like compounds) by reactive oxygen species (Łabanowska et al., 2014).

The increase of the number of stable radical species was observed not only in stressed plants but also in meal made from grains obtained from wheat subjected to ozone treatment (Reichenauer and Goodman, 2003). In turn, we noticed that exposure of meal to ozone resulted in the strong increase of the amount of stable radicals. Moreover, in non paramagnetic starch, which is the main component of grains, the ozone treatment caused the appearance of EPR signals of stable radicals (Łabanowska et al., 2013a, 2014). The presence of stable radicals was recorded also in not stressed whole wheat grains and in their inner parts (Łabanowska et al., 2012b). EPR studies showed that the number of these radicals was significantly higher in grains originating from plants of lower tolerance to stress. It was also found that the formation of the radicals was depended on the content and character of transition metal complexes present in grains (Łabanowska et al., 2012b, 2014). In the light of these results it seemed interesting to investigate if the changes in chemical character and content of paramagnetic species occurring upon oxidative stress, created by direct ozone application, in the doses typical for grain sterilization, were the characteristic feature of grains of various cereals.

The main objective of this research was to find indicators that allowed selection of cereals in terms of tolerance to environmental stresses at the level of grains. In this work we planned to differentiate grains of cereals (wheat, oat, barley) with various sensitivity to oxidative stress by studying the influence of ozone on the stable organic radicals generation and changes in antioxidant activities. We also checked if the responses of particular parts of grains to ozone treatment would be correlated with those of whole grains. The main parts: embryo – playing important role in plant development, endosperm – containing storage material for germinating plant and seed coat – preserving the inner parts of grain were chosen to investigation.

These studies could be also the source of information about the formation of stable radicals under direct oxygen species action on biomolecules localized within grains, without the effects of their diffusion through grain coats. The differences in activation of antioxidative system against oxidative stress were determined basing on generally accepted biochemical enzymatic (antioxidative enzymes) and nonenzymatic (glutathione and ascorbic acid) markers.

2. Material and methods

2.1. Plant material

Grains of spring genotypes of wheat (cv. Parabola – resistant and cv. Radunia – sensitive to oxidative stress) and oat (cv. Bingo – resistant and cv. Siwek – sensitive to oxidative stress) were obtained from the Institute of Plant Breeding (Radzików, Poland). The resistant genotype of barley (CAM/B1) was the breeding line from Syria (ICARDA International Centre for Agricultural Research in the Dry Areas, Aleppo), whereas the sensitive barley genotype (Maresi) was a German cultivar obtained from the Gene Bank in Praque (Czech Republic).

Embryos and endosperms, as well as seed coats (only for EPR studies) were isolated from about 500 grains of each crop cultivar, as it was described earlier (Kurdziel et al., 2015).

2.2. Ozone treatment

The ozone effect on investigated parts of grains was determined at the temperature of 293 K. Immediately after isolation, parts of grains were placed into 2 cm diameter × 4 cm high cylindrical PVC vials. Gas inlet and outlet was installed at the bottom and top lid of the cylinder, respectively. Ozone was generated using an FM-300 ozonator (Grecos, Poland) and was applied at the rate of 300 mg h^{-1} and a flow rate 3 dm^3 min^{-1} during 30 min. The exposure dose of ozone was close to that applied for removal of microbiological contamination from grains before their storage. Directly after ozone treatment samples were used for EPR measurements or stored at 193 K for biochemical analyses. As a control, samples not treated with ozone were used. EPR spectra of ozone treated grains were recorded also six days and one year after ozone exposure.

2.3. Biochemical analyses

Three methods of measurements of antioxidant activity were used to monitor and compare the total antioxidant activity of the grains. The oxygen radical absorption capacity (ORAC) assay, expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents, which measured inhibition of peroxyl radical-induced oxidation, was detected according to methods of Davalos et al. (2004) and Gillespie et al. (2007). A modified version of the Folin–Ciocalteu assay (Slinkard and Singleton, 1977) was used to determine the total phenolic contents (TPC). The absorbance was measured spectrophotometrically (Biochrom Ultrospec II, LKB, Sweden) at $\lambda = 765$ nm and gallic acid standard curve was prepared to express the phenolic content as mg GAE/100 g. The radical scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to modified method by Beta et al. (2005). Absorbance (A) was measured at $\lambda = 515$ nm. DPPH radical scavenging activity (%) and Trolox standards were calculated using the formula: (1-[A(sample)/A(control)] × 100). Results were reported in Trolox equivalent/g.