Ozone sensing and early signaling in plants: An outline from the cloud

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ABSTRACT

All along their life, plants and trees are exposed to various stresses, and particularly to abiotic ones. Ozone (O_3) is one of the most important air pollutants, whose ground levels keep increasing as a result of climate change. High O_3 concentrations deeply affect plants and cells, and impact worldwide crop and forest production. In plant leaves, O_3 directly interferes with surface tissues or reaches mesophyll cells through stomata. In this case, O_3 is almost immediately degraded into reactive oxygen species (ROS) in the apoplastic space of plant cells. For plants to acclimate to O_3, the O_3 stress signal has to be perceived at the cellular level and relayed to the nucleus to lead to cell reprogramming. The aim of this review is to focus on different O_3-sensing localizations, *i.e.*, epicuticular waxes, the cell wall and the plasma membrane, and to detail the different early signaling components related to these sites – in particular lipids, membrane proteins (G proteins, NADPH oxidases and ion channels) and MAP kinases. Finally, some interesting putative membrane-related O_3 signaling components are presented as clues to be validated in future investigations.

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1. Introduction

Changes in the environment such as light intensity, temperature, water content or air composition affect plant metabolism and development. Plants have to cope with these environmental constraints. As soon as variations occur, they have to detect the stress and turn on a whole battery of mechanisms leading to acclimation. Nowadays, due to anthropic activities, ozone (O_3) is a more and more widespread pollutant with concentration peaks usually occurring in spring and summer, and a constant increase of its ground levels since the pre-industrial period (Stevenson et al., 2006). O_3 affects plants in different manners, depending on its concentration (Renaut et al., 2009). The main visible symptoms of injury are chlorosis and necrosis on leaves (Karnosky, 1976; Karnosky et al., 2007) notably due to cell death. Senescence is accelerated (Karnosky et al., 1996). Severe damage and productivity losses in crops and forests are observed. For example, economic losses on winter wheat or rice in India for the 2002–2007 period are estimated to be between 100 and 1000 million US dollars annually (Avnery et al., 2011a,b; Debaje, 2014). Avnery et al. (2011a, b); Avnery et al. (2011a,b) estimated the global risk of O_3 surface pollution to three major crops (soybean, maize and wheat) by 2030: they predict total global agricultural losses ranging between 12 and 35 billion US dollars annually, *i.e.*, 1–17 billion US dollars more than economic crop production losses in the year 2000.

Two distinct types of O_3 stresses can be differentiated depending on O_3 concentrations: chronic and acute stress. In ambient air conditions, O_3 concentration is roughly about 40–50 nL L^{-1}. O_3 peaks may occur up to around 120 nL L^{-1} in some polluted areas (Royal Society, 2008). Acute or high O_3 concentrations, above 150–200 nL L^{-1} and up to 1000 nL L^{-1}, are not so widespread in natural conditions, but these high concentrations are used in growth chambers in order to switch on O_3 signaling components and bring them to light.

Increased O_3 concentrations during chronic or acute O_3 exposure induce several plant responses at the biochemical and molecular levels (Agrawal et al., 2002b Tamaoki et al., 2003; Ludwikow et al., 2004; Mahalingam et al., 2006; Ludwikow and Sadowski, 2008; Castagna and Ranieri, 2009; Dizengremel et al., 2009; Renaut et al., 2009; Cho et al., 2013). Hence, for plants to acclimate to O_3 stress, high O_3 concentrations have to be perceived at the cellular level and relayed to the nucleus to lead to cell reprogramming. Stomatal closure is a good example of an O_3-induced response (Ainsworth et al., 2012) leading to O_3 acclimation (Guidi et al., 2001; Hoshika et al., 2013). Without an acclimation program, oxidative stress will ultimately lead to cell death. These signal amplification and transduction events are O_3-signaling events.

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O_3 toxicity has long been believed to be mostly due to the formation of oxidative products resulting from O_3 degradation (Heath, 1994). In plant leaves, O_3 directly interferes with surface tissues, or reaches mesophyll cells through stomata. In this case, the O_3 molecule is almost immediately degraded, into reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radicals ($HO^\bullet$) or superoxide $O_2^{\bullet-}$ in the apoplastic space of plant cells. Then apoplastic ROS can translocate inside cells. But O_3 also induces active ROS production from several sources. For example, in birch, O_3 induces H_2O_2 accumulation first at the plasma membrane *via* NADPH-oxidases and at the cell wall *via* peroxidases, and then H_2O_2 production continues in the cytoplasm, mitochondria and peroxisomes (Pellinen et al., 1999; Vahisalu et al., 2010). In *Arabidopsis*, guard cell chloroplasts are the first detectable ROS source (Joo et al., 2005). ROS production is a very common response to stress, and the dual role of these molecules is now well established (Mittler, 2002). ROS act as deleterious molecules that have toxic effects on DNA, proteins, lipids and carbohydrates but they also have a role in initiating signaling in response to stress (Jaspers and Kangasjärvi, 2010). Therefore cells permanently have to fine-tune the balance between these antagonistic roles of ROS throughout their life-time.

ROS production is an early event shared among different biotic and abiotic stresses. Consequently, oxidative stress and ROS signaling have been broadly reviewed (Neill et al., 2002; Baier et al., 2005; Kangasjärvi et al., 2005; Shapiguzov et al., 2012; Baxter et al., 2014; Wrzaczek et al., 2013). The aim of this review is to stress the importance of the onset of O_3-induced stress signaling pathways in response to high O_3 concentrations. So the present paper focuses on the different O_3-sensing localizations, *i.e.*, epicuticular waxes, the cell wall and the plasma membrane. These early targets are crucial to O_3 signaling. They are not only the places where O_3 molecules are degraded into ROS, but also the places where specific O_3 signals are initiated, as highlighted in this review. Then either new ROS are produced as part of the signaling pathways, or new signaling components (*e.g.*, lipids, proteins) are triggered. The different early signaling components in relation to these sites, in particular lipids, membrane proteins (G proteins, NADPH oxidases and ion channels) and MAP kinases are detailed below. Finally, some interesting putative membrane-related O_3 signaling components are presented as clues to be validated by future investigations.

The role of phytohormone (ethylene, salicylic acid, jasmonate, ABA, nitric oxide) signaling during O_3 stress is not detailed in this paper. For review papers about this aspect, readers can refer to Kangasjärvi et al. (2005),Tamaoki (2008) and Vainonen and Kangasjärvi (2014).

2. Epicuticular waxes

The epidermis as well as epicuticular waxes (EW) are directly in contact with atmospheric O_3 so that they are *de facto* putative sites of primary perception. Waxes are composed of a heterogeneous mixture of lipids. They protect the underlying epidermal cells from the early influence of climate and possibly of air pollution. If air pollutants can alter the complex composition of waxes, a detrimental effect on their structure should be visible, possibly followed by enhanced sensitivity to air pollution (Lütz et al., 1990). Alterations in EW structure and chemical composition were recently shown to affect plants' responses to abiotic and biotic stresses (Riikonen et al., 2010). Yet oxidative transformation alone cannot account for the *in vivo* degradation of wax tubules on conifer needles, even at O_3 concentrations well above ambient conditions (Jetter et al., 1996). Surface characteristics of leaves from different tree species – *Betula papyrifera* (Riikonen et al., 2010), *Populus tremuloides* (Mankovska et al., 2005), *Picea abies* (Lütz et al., 1990), or *Picea pungens* (Jetter et al., 1996) – exposed to O_3 treatment were analyzed, and O_3 seemed to have no significant impact on EW biosynthesis, composition or crystal habit. However, O_3 had a significant negative impact on aspen wax quality and this impact was more severe on O_3-sensitive clones as rust colonization on leaf surfaces increased under O_3 exposure (Mankovska et al., 2005). Moreover, the important primary role of leaf surface in O_3 responses was suggested by Kontunen-Soppela et al. (2007) in a study on two birch genotypes with different O_3 sensitivity levels. Long-term O_3 exposure (7 years) increased the levels of compounds related to leaf cuticular wax formation such as triterpenoids, squalene, dammaran-3-ol, 1-dotriacontanol, dotriacontane and 1-hexacosanol (Kontunen-Soppela et al., 2007). Comparative metabolite profiling among genotypes showed that some of these compounds could provide growth-related tolerance to O_3 (Kontunen-Soppela et al., 2007). In addition, waxes could be more affected by combined environmental factors: high $CO_2 + O_3$ exposure significantly altered the fatty acid and primary alcohol proportions of wax, whereas O_3 alone did not (Riikonen et al., 2010).

3. The cell wall

The plant cell wall is a dynamic composite structure. Its molecular and mechanical properties change in response to biotic or abiotic stresses (Lindner et al., 2012). Cell walls and the apoplast are directly in contact with O_3 and its oxidant derivatives. O_3 is immediately degraded into ROS in the apoplastic space. Relative long-term exposure to O_3 leads to increased cell wall lignification (Cabané et al., 2012). Within 2 h after the beginning of O_3 exposure, O_3 induced H_2O_2 accumulation at the cell wall and plasma membrane levels from cell wall peroxidase activity (Pellinen et al., 1999; Ranieri et al., 2003). Changes induced by O_3 in cell wall components or by cell wall-produced H_2O_2 are possible initial steps of stress signal initiation. Therefore, apoplastic receptors, *e.g.*, plasma membrane-localized kinase proteins with extracellular domains at the interface between the cell wall and the inner cell appear as primary candidates for signal perception at the cell surface. Interestingly, extracellular ROS produced by O_3 transcriptionally regulated genes coding for Cysteine-rich Receptor-like Kinases (CRKs), which have a transmembrane domain (Wrzaczek et al., 2010). More than half of the 44 *CRKs, i.e.*, 25 of them, displayed more than two-fold higher mRNA abundance after 1-h exposure to 250 ppb O_3 (Wrzaczek et al., 2010). As CRKs possess a DUF26 domain with four conserved cysteines and as cysteines are sensitive to redox modifications, they could act as ROS sensors in the apoplast (Wrzaczek et al., 2010). In the same line, ROS production in different cellular compartments produced different transcriptional profiles on the *CRK* gene subfamily. Apoplastic ROS triggered transcriptional induction of *CRKs*, whereas chloroplastic ROS mainly decreased their expression (Wrzaczek et al., 2010).

4. Plasma membrane lipids

As a cell barrier connecting external stimuli and internal transduction, the plasma membrane is a critical player in stress signaling. Different alterations of the plasma membrane in response to O_3 were documented as early as several decades ago, *e.g.*, higher membrane permeability through rapid loss of K^+ in *Chlorella* (Heath and Frederick, 1979). This increased leakiness could be biophysically explained by the fact that O_3 exposure induces the formation of gel-phase lipids, and the co-existence of liquid-crystalline and gel-phase lipids greatly increases the permeability of lipid bilayers (Pauls and Thompson, 1980). More recent studies show that important conformational switches in the structure of many phospholipids occur following membrane oxidation (review in Farmer and Mueller, 2013).