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Gene expression induced by chronic ozone in the Mediterranean shrub *Phillyrea latifolia*: analysis by cDNA-AFLP

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Summary Seedlings of *Phillyrea latifolia* L., a Mediterranean shrub, were exposed for 90 days to 110 nl l^{-1} ozone (O_3). Comparison of the cDNA-amplified fragment length polymorphism (cDNA-AFLP) patterns for treated and control plants allowed the identification and cloning of 88 differential sequences induced by O_3. The differential expression of 67 cloned sequences was further confirmed by RT-PCR. The functions of 36 cloned sequences, corresponding to seven of the twelve gene functional classes of *Arabidopsis*, were presumed on the basis of their homology with characterized gene sequences. Ozone induction of genes homologous to 24 of the clones has been reported in other plant species, whereas the induction of the 12 remaining sequences has not been observed before. Ozone activation of these newly identified genes could be a result of the chronic exposure to low O_3 concentration, because in most previous studies, acute treatments, involving high O_3 dosages, were applied. Possible roles of the cloned sequences in the response of *P. latifolia* to O_3 and other causes of oxidative stress are discussed.

Keywords: air pollution, gene differential expression, oxidative stress, RT-PCR.

Introduction

During the last century the tropospheric concentration of ozone (O_3) has significantly increased as a result of anthropogenic activities, causing extensive damage to wild and cultivated plants (Fowler et al. 1999). Chronic exposure of plants to low O_3 concentrations affects their photosynthetic capacity and vigor, causes premature leaf senescence, reduces crop yields and increases sensitivity to biotic and abiotic stresses (Ashmore 2005), whereas acute exposure of leaves to high O_3 concentrations produces symptoms similar to the hypersensitive response induced by pathogens (Kangasjärvi et al. 1994, 2005). Moreover, plant O_3 responses and the hypersensitive response share many physiological, biochemical and molecular features (Overmyer et al. 2003, Baier et al. 2005, Kangasjärvi et al. 2005). Comparison of sensitive and tolerant genotypes of several plant species has provided information on the molecular mechanism of lesion development caused by O_3. Hormone signaling and cross-talk determine the outcome of O_3 challenge at the cell level (Overmyer et al. 2003, Kangasjärvi et al. 2005). Reactive oxygen species (ROS), produced in the breakdown of O_3 after its penetration through the stomata, cause direct necrotic damage or trigger programmed cell death (PCD). The presence of O_3 or ROS in the apoplast activates several signal transduction pathways that control cell responses to increased oxidative load. The plant hormones salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) are involved in determining initiation, spread and containment of O_3-induced cell death (Overmyer et al. 2003, Kangasjärvi et al. 2005). The transmission of O_3-induced secondary signals is mediated by mitogen-activated protein kinase (MAPK) cascades (Ahlfors et al. 2004, Baier et al. 2005). Further insights into molecular signaling activated by oxidative stress and the specific induction of secondary messengers in response to O_3 have been provided by PCR-based suppression subtractive hybridization (SSH) and transcriptome analysis based on macro- and micro-arrays in *Arabidopsis* (Mahalingam et al. 2003, 2005, 2006, Tamaoki et al. 2003, Ludwikow et al. 2004, Miyazaki et al. 2004, Tosti et al. 2006).

Morphological and physiological effects of O_3 exposure have been studied in several forest species (Matyssek and Sandermann 2003), yet little is known of the underlying molecular bases (Langebartels et al. 2002). Molecular analysis of gene expression induced by O_3 would enable a better understanding of the effects of O_3 on forest trees (Sandermann and Matyssek 2004). So far, 14 O_3-induced genes from Norway spruce (*Picea abies* (L.) Karst), Scots pine (*Pinus sylvestris* L.), hybrid poplar (*Populus maximowizii* × *P. trichocarpa)*, birch (*Betula pendula* Roth.) and European beech (*Fagus sylvatica* L.) have been cloned and described (Langebartels et