

## ANALYSIS OF GENE EXPRESSION INDUCED BY OZONE IN DURUM WHEAT

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Tropospheric ozone (O<sub>3</sub>) is a harmful pollutant causing extensive damages to crops and communities of natural plants. The symptoms produced by O<sub>3</sub> in sensitive plants are similar to the leaf lesions caused by hypersensitive response (HR) induced by infection with incompatible pathogens. Moreover, recent researches have shown that O<sub>3</sub> and hypersensitive responses share also many physiological, biochemical and molecular features. New insights into the molecular responses to oxidative stress and in particular the signalling and the specificity of secondary messengers induced by ozone have been provided by PCR-based suppression subtractive hybridization and transcriptome analysis based on macro- and micro-arrays in *Arabidopsis*. Comparatively little is known on the molecular aspects of O<sub>3</sub> response in monocots and in particular in wheat, which does not develop specific and visible foliar symptoms in response to realistic O<sub>3</sub> doses. In wheat, however, O<sub>3</sub> may affect the productivity by promoting the leaf senescence, even though the extent of such effects may vary widely in relation to the exposure regimes and cultivar sensitivity. Durum wheat is one of the most important crops in the Mediterranean area, where ozone levels are considerably higher during the grain filling. In order to investigate the molecular responses induced by ozone in durum wheat, the O<sub>3</sub> tolerant cv Portorico was selected among 16 Italian cultivars on the basis of leaf damages and of some physiological parameters (gas exchanges, leaf chlorophyll fluorescence, membrane solute leakage and relative water content) detected after exposure to a single square wave of 200 nL L<sup>-1</sup> O<sub>3</sub> for 5 h. The expression profiles of the transcripts from two-week old ozonated and control plants were compared by a modified cDNA-AFLP technique. The analysis of about 2500 amplified transcripts from ozonated and control plants detected 165 differential products induced by O<sub>3</sub>. The nucleotide and deduced amino acid sequences of 122 out of 165 O<sub>3</sub>-induced clones, whose differential or preferential expression in ozonated plants was verified by RT-PCR analyses, were searched in the online DNA and protein databases. Significant homology was found for 88 differential products: 9 detected homologies only within the wheat ESTs, whereas 79 homologous sequences were found in all the searched databases. No significant homology was detected for the remaining 34 clones. On the basis of their homology with sequences in the databases with known functions, the 79 clones were assigned to eight functional groups: I) signal transduction (14 clones); II) cellular organization (9); III) protein synthesis/destination/degradation

(9); IV) metabolism (11); V) cell rescue/defence (11); VI) transcription (7); VII) energy (2); VIII) unknown (16). It is noteworthy that only 13 of them corresponded to wheat sequences reported in DNA and protein databases, whereas 44 were also found within the ESTs of wheat. Forty-five of the 63 isolated AFLP-tags, found to match genes with known functions, had previously been recognized as O<sub>3</sub>-responsive in *Arabidopsis*. Further characterization of the most interesting clones included their expression analysis in six durum wheat cultivars showing different sensitivity to O<sub>3</sub>. The differences and similarities of O<sub>3</sub> induced gene expression in sensitive and tolerant cultivars were discussed.