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EFFECTS OF OZONE IN TWO DIFFERENTIALLY SENSITIVE POPLAR HYBRIDS: HISTOCYTOCHEMICAL AND MOLECULAR APPROACHES

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ozone stress, hypersensitive response (HR), programmed cell death (PCD), reactive oxygen species (ROS).

The leaf is considered the most sensitive organ to pollution and represents the first target for phyto-toxic action of ozone in sensitive plants. O_3 enters the leaf surface via the stomata and induces a hypersensitive-like response (HR) including an oxidative burst and both micro- and macro-scale cell-death. The reactive oxygen species (ROS), generated by O_3 dissociation, act non only as damaging agents but mainly as signal molecules for several signal transduction pathways, inducing a range of events interpreted as protective (defence responses).

In order to identify the basis of sensitivity to O_3 in arboreal species, we used two poplar hybrids characterized by a different susceptibility to O_3 (the ozone sensitive *Populus deltoides x maximowiczii*, Eridano clone and the more resistant *Populus x euoramericana*, I-214 clone) exposed to an acute ozone stress: within 24-48 h after the fumigation, only sensitive poplar clone leaves showed the typical dark-black necrosis. The differential sensitivity against acute O_3 stress of two poplar hybrids is firstly related to a different equipment of endogenous detoxifying antioxidant enzymes/metabolites and to a differential expression and regulation of specific genes after O_3 treatment. The anatomical characteristics of the sensitive poplar hybrid leaves (high stomatal density, low density of mesophyll cell packing) justify its increased sensitivity against ozone stress rather than the tolerant poplar hybrid. These anatomical characteristics can play an important role in O_3 uptake, favouring its entrance in mesophyll apoplast and its cyto-toxic activities on mesophyll cells (principally in palisade parenchyma) in the sensitive hybrids. Caryological investigations showed a clear differential response to O_3 stress in the two poplar clones: the leaves of the sensitive, sampled during the precocious phases of ozone fumigation, revealed a marked increase in the nuclear/nucleolar functional activity. In the sensitive poplar hybrid O_3 induced a rapid accumulation of callose localized in walls around the dying cells, as in response to wounding or pathogen infections. Besides, the rapid and well localized cell death, displayed in O_3 sensitive poplar leaves, exhibits some morphological and functional hallmarks of programmed cell death (PCD) processes, as: nuclear and cytoplasm shrinkage, DNA fragmentation, vacuoles lysis, incomplete cell wall breakage and collapse, plasmolysis, persistence of intact organelles until a late stage of death.