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CALCIUM INFLUXES AND PROTEIN KINASE ACTIVATION MEDIATE OZONE-INDUCED DEFENCE GENE EXPRESSION IN TOBACCO PLANTS

EDERLI L.*, CALDERINI O.**, PASQUALINI S.*

*) Department of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)
**) Institute of Plant Genetics - CNR, Via della Madonna Alta 130, 06128 Perugia (Italy)

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Ozone (O_3) can affect several processes in plants including the transcription of many defence associated genes, however, the mechanism by which O_3 brings about these changes remains largely unknown. We have shown that nitric oxide (NO) is a second messenger in the signalling cascade induced by O_3 leading to defence gene induction. The NO action is accomplished by both cGMP dependent and cGMP-independent pathways; in particular, the activation of *AOX1a* is independent from cGMP. By contrast, the early response of phenylalanine ammonia lyase (*PALa*) and the late response of pathogenesis related protein (*PR1a*) show critical dependence on cGMP. This research is aimed to unravel the role that calcium and protein kinases play in the transduction pathway leading to defence gene activation. O_3 induces rapid activation of a MAP kinase of approximately 48 kDa which by the immune-complex kinase activity assay was identified as salicylic acid induced protein kinase (SIPK). The SIPK activation was transient and the activity returned to basal level within 3 h of O_3-fumigation. When the O_3-induced NO accumulation was reverted by the NO quencher cPTIO no SIPK activation was detected in tobacco ozonated plants, suggesting that NO acts upstream of MAPK cascade. However, when we inhibited SIPK activity by the Ser/Thr kinase inhibitor staurosporine (Stau), we found a complete reversion of the O_3-induced *AOX1a* and *PALa* activation, and this suggests a central role of the protein kinase mediated phosphorylation in the O_3-induced up-regulation of these genes. For *PR1*a the picture is more complex. In tobacco plants challenged with O_3 *PR1a* was found to be up-regulated after 24 h; the application of Stau markedly induced *PR1*a mRNA accumulation in the absence of O_3, suggesting that a protein dephosphorylation mediates the *PR1a* expression in tobacco. An addictive effect on *PR1a* mRNA accumulation was found when both stimuli (Stau + O_3) were applied. We conclude that all the examined genes (*AOX1a*, *PALa* and *PR1a*) are dependent on protein kinases for their expression. Calcium has been demonstrated to be an important molecule that mediates signal transduction. To elucidate whether Ca^{2+} is involved in O_3-induction of target genes, cytosolic Ca^{2+} influx was inhibited by lanthanum chloride ($LaCl_3$) and ruthenium red (RR). Lanthanum chloride is known to compete externally with Ca^{2+} for channels located in the plasma membrane, while RR blocks the ryanodine ion channels that control Ca^{2+} mobilization from internal stores in both animals and plants. The *AOX1a* was transiently induced by O_3 with highest mRNA accumulation between 2 and 5 h of O_3 fumigation; the application of both RR and $LaCl_3$ did not change its expression profile, suggesting that *AOX1a* gene expression was not influenced by Ca^{2+}. A different picture was obtained for *PALa* expression which was induced during O_3-fumigation, but its activation was completely suppressed by RR, whereas was not influenced by $LaCl_3$. The pattern of *PR1a* expression under O_3, similarly to *AOX1a*, shows that the expression of this gene was independent

on Ca^{2+}, because neither RR and $LaCl_3$ did not suppress O_3-dependent induction. A working model showing how Ca^{2+} and protein kinases cross talk with NO-mediated transduction pathway in the induction of defence genes will be presented.