



Importance of chloroplastic/mitochondrial interactions during elicitor- induced cell death in tobacco leaves: acceleration by light and low oxygen

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Importance of chloroplastic/mitochondrial interactions during elicitor- induced cell death in tobacco leaves: acceleration by light and low oxygen.

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INTRODUCTION

Light and oxygen are considered important in the execution of plant programmed cell death (PCD), principally because they are involved in the generation of reactive oxygen species (ROS).

We previously showed that the bacterial elicitor harpin N_{ea} from *Erwinia amylovora* induces an hypersensitive response (HR)-like PCD process in *N. sylvestris* leaves

In the light, harpin induces a respiratory burst involving alternative oxidase (AOX) engagement as determined by oxygen isotope discrimination (Vidal et al., 2007), changes in chloroplastic (Boccara et al. 2007), stomatal opening (Boccara et al. 20001) and changes in the expression of antioxidant genes (Garmier et al., 2002).

Here, we compare the HR-like responses of leaves elicited the

- in the light and in darkness

- in air and under very low oxygen (VL ox: < 0,1%)

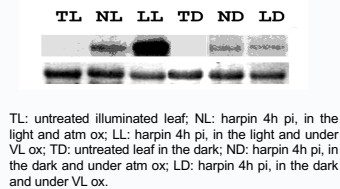
RESULTS

I.1 Symptoms induction

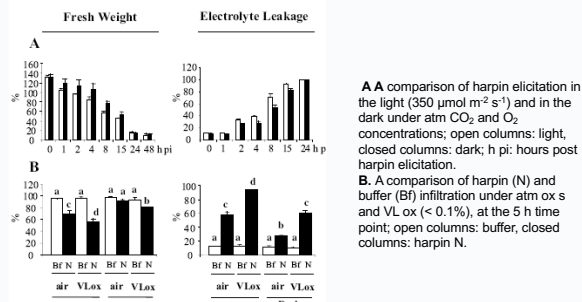
Harpin N-induced cell death was induced in the light and in darkness, in air and under anoxia.



I.2 Accumulation of transcripts of the tobacco Tnt1 transposon 4 hours post harpin N, indicates an active cell death process in all conditions



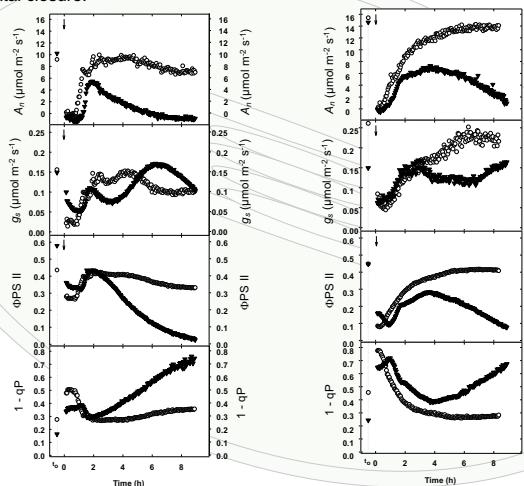
I.3 However, fresh weight and electrolyte leakage measurements indicate that cell death was delayed in the dark and accelerated under anoxia.



II Photosynthetic and Respiratory signatures of harpin-elicited leaves

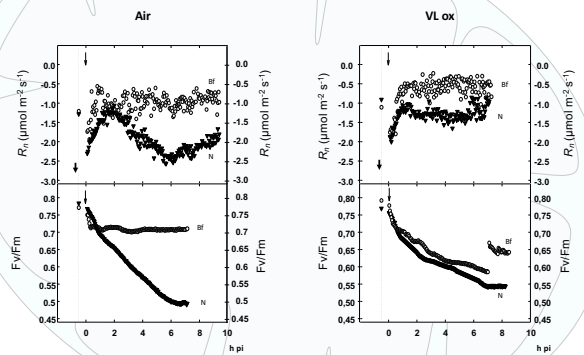
II.1 Photosynthetic and fluorescence parameters of light-elicited leaves

Collapse of photosynthesis from two hours post elicitation is caused by inactivation of PSII and not by stomatal closure.

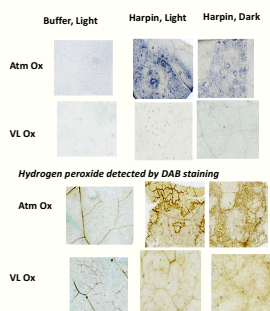


II.2 CO₂ release in leaves infiltrated in the dark

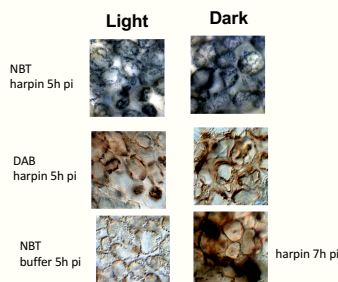
The respiratory burst induced in air was nearly abolished under VL ox, probably due to the inhibition of AOX which has lower affinity for oxygen than COX.



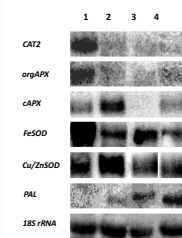
III.1 In situ detection of ROS 5 h post harpin elicitation.



III. 2 Microscopic examination indicate accumulation of reactive oxygen species inside chloroplasts in both the light and the dark.



III.3 Expression of antioxidant and defence genes 4 h post harpin elicitation (Northern).



Increased transcript levels of organellar antioxidant, CAT2, orgAPX and chlFeSOD, were observed in all conditions.

On the other hand, cytosolic Cu/Zn SOD and cAPX accumulated only in the light under atm conditions.

The PAL defence gene accumulated in all cases.

ROS accumulation peaked 4-6 h pi in both the light and the dark. In contrast it was strongly attenuated under VL ox.

A: harpin 3h pi, light; B: harpin 5h pi, light; C: harpin 5h pi, dark; D: harpin 3h pi, light; E: harpin 5h pi, light; F: harpin 5h pi, dark; G: harpin 7h pi, light; H: harpin 7h pi, dark; I: buffer 5h pi, light. Red arrows: chloroplasts; black arrows: collapsed cells. Bar: 1 cm = 100 µm