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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).

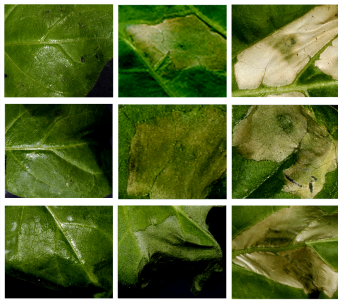
Here, we compare the HR-like responses of leaves elicited the

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

RESULTS

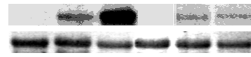
I.1 Symptoms induction

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

TL NL LL TD ND LD



TL: untreated illuminated leaf; NL: harpin 4h pi, in the light and atm ox; LL: harpin 4h pi, in the light and under VL ox; TD: untreated leaf in the dark; ND: harpin 4h pi, in the dark and under atm ox; LD: harpin 4h pi, in the dark and under VL ox.

SUMMARY

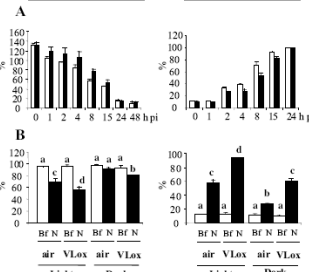
The present work shows that, in contrast with other plant PCD systems, neither light nor oxygen are necessary to harpin N-induced cell death. In all conditions, the HR-like response is an active process, involving contrasting responses of defence and antioxidant genes. Moreover, examination of response signatures in mitochondria (respiration) and in chloroplasts (photosynthesis), together with the intracellular localisation of ROS accumulation, provides compelling evidence for strong interactions between respiration and photosynthetic metabolism in the modulation of the cell death. While extra-mitochondrial ROS are not key signals in the execution of the regulated cell suicide programme, mitochondrial signals could be of major importance.

REFERENCES

Boccara et al. (2001) IR thermography revealed a role for mitochondria in presymptomatic cooling during harpin-induced hypersensitive response. *Plant J.* 28, 663-670.
Boccara et al. (2007) Early chloroplastic alterations analysed by optical coherence tomography (OCT) during harpin-induced hypersensitive response. *Plant J.*, in press.
Garmier et al. (2002) Changes in antioxidant expression and harpin induced hypersensitive response in a *Nicotiana sylvestris* mitochondrial mutant. *Plant Physiol. Biochem.* 40, 561-566
Vidal et al. (2007) Lack of respiratory chain Complex I impairs AOX engagement and modulates redox signaling during elicitor-induced cell death in tobacco. *Plant Cell* 19, 640-655.

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

Fresh Weight Electrolyte Leakage



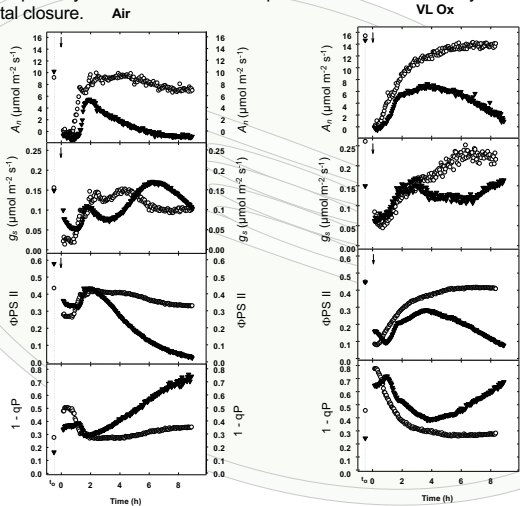
A A comparison of harpin elicitation in the light (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and in the dark under atm CO_2 and O_2 concentrations; open columns: light, closed columns: dark; h pi: hours post harpin elicitation.

B A comparison of harpin (N) and buffer (Bf) infiltration under atm ox and VL ox (< 0.1%), at the 5 h time point; open columns: buffer, closed columns: harpin N.

II Photosynthetic and Respiratory signatures of harpin-elicited leaves

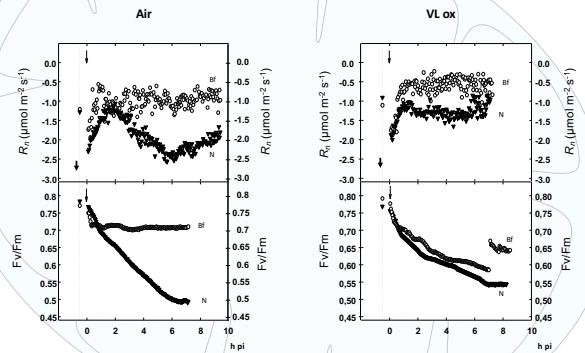
II.1 Photosynthetic and fluorescence parameters of light-elicited leaves

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

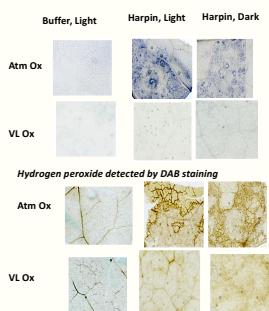


II.2 CO_2 release in leaves infiltrated in the dark

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

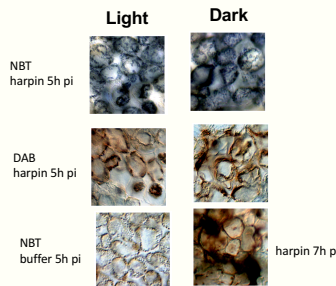


III.1 *In situ* detection of ROS 5 h post harpin elicitation.



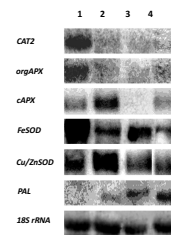
ROS accumulation peaked 4-6 h pi in both the light and the dark. In contrast it was strongly attenuated under VL ox.

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



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

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



1: untreated leaf in the light under atm ox; 2: harpin-infiltrated leaf in the light under atm ox; 3: harpin-infiltrated leaf in the dark under atm ox; 4: harpin-infiltrated leaf in the light under VL ox.

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.