

# Confocal imaging of Reactive Oxygen Species in *Arabidopsis* roots

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## INTRODUCTION

It is now generally accepted that cell extension during elongation growth is mediated by an irreversible extensibility of growth-limiting cell walls. This cell wall loosening has been regarded as a process mainly catalysed by expansins. Another alternative proposes a non enzymatic process involving reactive oxygen species (ROS) to perform this function *in vivo*. Elongating tissues usually produce ROS that are necessary for the cell growth process (Rodriguez et al., 2002; Schopfer et al., 2002). There are also experimental evidences showing that apoplastic peroxidases can produce ROS in presence of appropriate reducing molecules. On the other hand, these enzymes can induce cell wall loosening and growth by elongation as well as cross-linking of cell wall components (see Passardi et al., 2004).

Our first objective is to show that peroxidases are involved in ROS production in cell walls. We use hydroxyphenyl fluorescein (HPF) that is known to be converted to fluorescein by ROS. *Arabidopsis* roots were incubated in presence of HPF and fluorescence of fluorescein was detected *in vivo* using Confocal Laser Scanning Microscopy. The effect of different inhibitors on this fluorescence was examined.

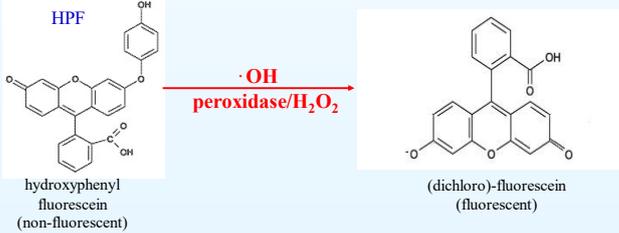
## PLANT MATERIAL

*Arabidopsis thaliana* (ecotype Columbia) seeds were germinated *in vitro* under conditions of 16 h light and 8 h darkness and were allowed to grow for 1 week.

## CONFOCAL LASER SCANNING MICROSCOPY

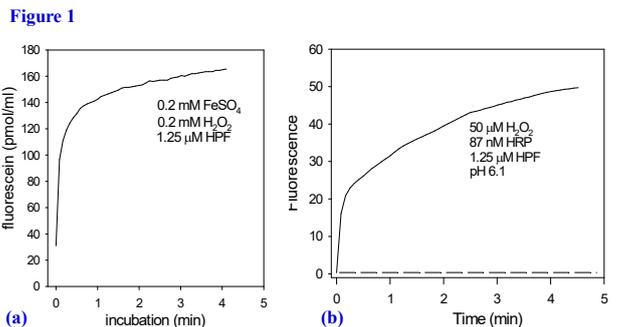
Plantlets were taken from agar and their roots were pre-incubated in phosphate buffer (pH 6.1) then in the same buffer containing HPF (10  $\mu$ M). Roots were also pre-incubated in presence of inhibitors before their incubation in HPF.

Root tips were then placed on a slide in a drop of buffer and covered with a coverslip. They were observed with a 20x water objective (NA: 0.71). Fluorescent images were collected with a Leica SP2 confocal laser-scanning microscope coupled to an inverted microscope. The 488 nm line of an argon ion laser was used for HPF excitation and the fluorescence detection was in the range of 506 à 586 nm.

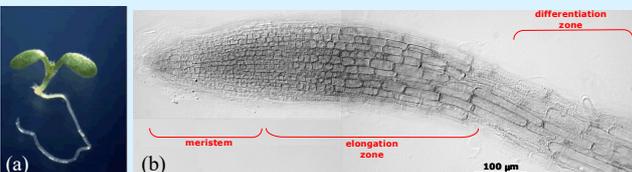


## RESULTS

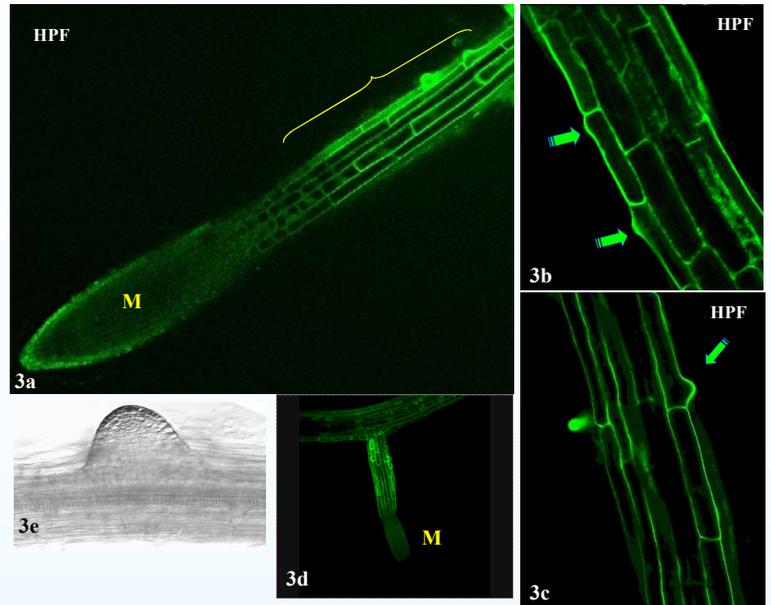
**Figure 1:** HPF is converted into fluorescein (a) by the hydroxyl radical (here formed by the reaction of  $\text{Fe}^{2+}$ ) and hydrogen peroxide but also (b) by hydrogen peroxide in the presence of peroxidase (here HRP).



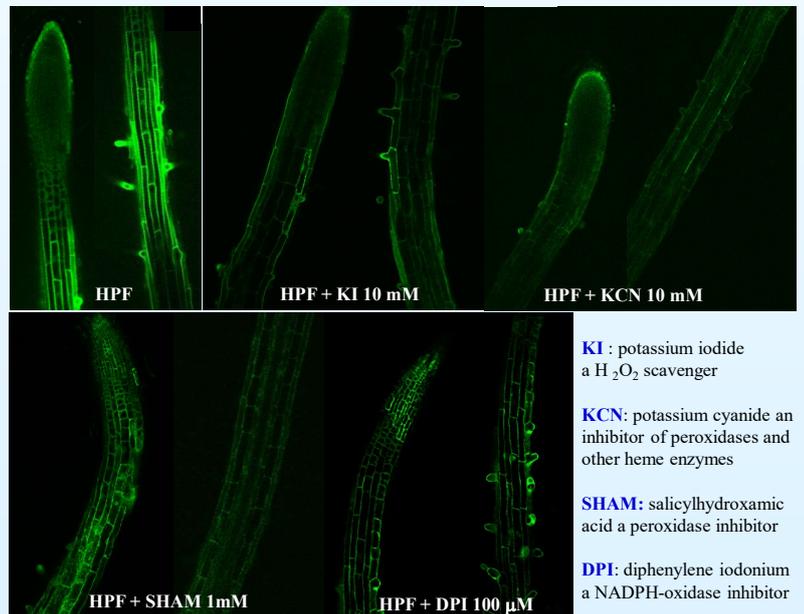
**Figure 2:** The confocal observations were focalised on the root tip of young seedlings (a) from the meristem to the differentiation zone (b)



**Figure 3:** in *Arabidopsis* roots incubated in HPF the fluorescence was detected mainly in the cell walls of a region comprised between the end of the elongation zone and the beginning of the differentiation zone (a) as well as in expanding root hairs (b, c) and the elongation part of lateral root (3d). No fluorescence was observed in the meristem (M) nor in very young roots in formation (see e, a transmission picture).



**Figure 4: effect of inhibitors.** The fluorescence detected in cell walls of roots incubated with HPF was strongly reduced by KI in both the elongating region and the root hairs. This reduction was still greater in roots treated with KCN. In roots treated with SHAM the cell wall fluorescence was strongly decreased in the elongating region and was almost undetectable in the root hairs zone. DPI also reduces the fluorescence in the elongating region and the root hairs (Left: meristem and elongating region; right: root hairs zone).



## DISCUSSION

HPF a fluorescent indicator of ROS is converted to fluorescein by hydroxyl radicals and by hydrogen peroxide in presence of peroxidases. *In vivo*, the fluorescence was detected in the cell walls preferentially in the elongating regions of roots. The strong reduction of this fluorescence by KI indicates that  $\text{H}_2\text{O}_2$  is involved in HPF oxidation. SHAM, which acts as a relatively specific peroxidase inhibitor and the less specific KCN also reduce the fluorescence, suggesting the involvement of peroxidases. Finally DPI has a clear inhibitory effect, indicating the involvement of the transplasmalemma NADPH oxidase, an enzyme known to produce  $\text{H}_2\text{O}_2$  in the cell walls.

These preliminary data suggest that HPF is a useful molecule to detect ROS *in vivo* and that the fluorescence seen in *Arabidopsis* roots cells is mainly due to the presence of hydrogen peroxide and peroxidases.

## References

Rodriguez et al., 2002, Plant physiol. 129, 1627-1632; Schopfer et al., 2002, Planta 214, 821-828; Liszskay et al., 2003, Planta 217, 658-667; Liszskay et al., 2004 Plant Physiol., 136, 1-10; Passardi et al., 2004, Trends in Plant Sci. 9, 534 -540