[Plant Signaling & Behavior 2:2, 131-132; March/April 2007]; ©2007 Landes Bioscience

Article Addendum

Localization of Superoxide in the Root Apex of *Arabidopsis*

Christophe Dunand
Claude Penel*

Laboratoire de Physiologie végétale; Université de Genève; Genève, Switzerland

*Correspondence to: Claude Penel; Laboratoire de Physiologie végétale; Université de Genève; 30, quai Ernest-Ansermet; CH-1211 Genève 4, Switzerland; Tel.: +41.22.379.30.16; Fax: +41.22.379.30.17; Email: Claude.Penel@bioveg.unige.ch

Original manuscript submitted: 03/07/07
Manuscript accepted: 03/07/07

Previously published online as a *Plant Signaling & Behavior* E-publication:
http://www.landesbioscience.com/journals/psb/abstract.php?id=4112

KEY WORDS

superoxide, hydrogen peroxide, cell elongation, transition zone, nitroblue tetrazolium

ACKNOWLEDGEMENTS

This work was supported by a grant of the Swiss National Science Foundation (3100A0-109325).

Addendum to:

Distribution of Superoxide and Hydrogen Peroxide in* Arabidopsis *Root and their Influence on Root Development: Possible Interaction with Peroxidases

Dunand C, Crèvecoeur M, Penel C

New Phytol 2007; 174:332-41
PMID: 17388896
DOI: 10.1111/j.1469-8137.2007.01995.x

ABSTRACT

Reactive oxygen species (ROS) fulfil many functions in plants. They have a signaling role in several physiological mechanisms, but they are also directly involved as substrates in important reactions, especially in the apoplast. Two ROS, superoxide and hydrogen peroxide, were shown to exhibit a typical accumulation pattern in the *Arabidopsis* root apex. While hydrogen peroxide is mainly present in the cell wall of fully elongated cells in the region of root hair formation, superoxide accumulation roughly coincides with the transition zone, between the meristem and the fast elongating zone. Developing lateral roots also exhibit a strong superoxide labeling with the same localization.

In a recent work,[1] we have shown that superoxide radical and hydrogen peroxide have different accumulation sites in *Arabidopsis* root tip. Hydrogen peroxide is mainly present in a region identified as "differentiation zone", according to the nomenclature used by Scheres et al.[2] This localization fits well with the role that was assigned to this ROS in the formation of root hairs.[3] This hypothesis was strengthened by the fact that umbelliferone, which promotes the in vitro and in vivo formation of hydrogen peroxide by peroxidases, induces the formation and the elongation of root hairs. In contrast, potassium iodide, a H_2O_2 scavenger, prevents the formation of root hairs, but does not completely abolished their initiation.

As for superoxide radical, it accumulates mainly in apoplast of cells ranging from the proximal part of root meristem to the point where cells initiate their fast elongation. This localization is in agreement with a role of superoxide in the cell elongation process.[1] This conclusion can be refined, taking into account the work of Baluška and coll.[4,5] Using various functional and structural criteria, these authors identified four distinct zones in the root apex of *Arabidopsis*. They introduced an additional zone, between the meristem and the fast elongating cells, named "transition zone". This region comprises cells which do not divide any more and are preparing their elongation. A reappraisal of the localization of superoxide accumulation in the light of this classification could suggest that this ROS is actually mainly associated with this transition zone, rather than with the beginning of the elongation zone. Figure 1 shows an *Arabidopsis* root stained for the presence of superoxide with nitroblue tetrazolium. It appears that the strong superoxide staining ranges from about 80 to 250 μm away from the root tip. The respective sizes of the various zones somewhat differ from the sizes reported (in ref. 5). It is difficult to precisely determine the border between the meristem and the transition zone, which should be around 120 μm. The fast elongation zone begins at about 240 μm. Fast elongating cells exhibit only a slight superoxide staining in their cell wall. Therefore, it appears that superoxide accumulates mainly in the wall of cells preparing their rapid elongation. It has been reported that cells in the transition zone undergo several modifications to prepare their growth. This includes reactions leading to cell wall loosening.[6,7] The presence of superoxide in the cell wall of those cells could participate in the onset of the loosening process, for example by interacting with peroxidases to produce hydroxyl radicals.[8]

When roots get older, the intensity of superoxide staining in the main root tip decreases, while the apex of the newly formed lateral roots exhibits a stronger reaction (Fig. 2). This could be related to the important growth potential of young lateral roots. The emerging root primordium is usually clearly positive (Fig. 2A) and in a fully formed lateral root, superoxide staining is concentrated in a zone between the meristem and elongated cells, most likely corresponding to the transition zone (Fig. 2B). In conclusion, superoxide radical seems to accumulate in the wall of cells preparing their elongation in the transition zone of *Arabidopsis* root apex.

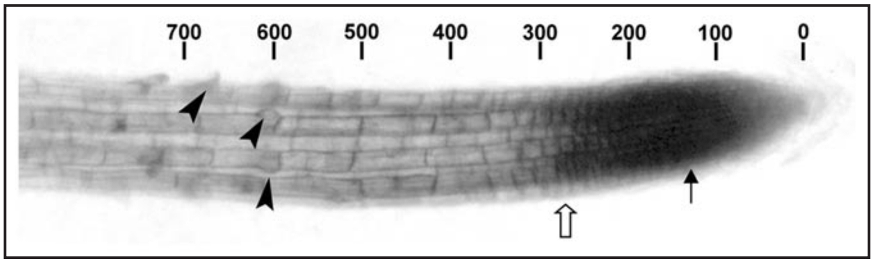

Figure 1. Distribution of superoxide radical in the root of a 7-day old *Arabidopsis* seedling stained with nitroblue tetrazolium. Growth conditions and staining procedure were as described (in ref. 1). The scale indicates µm, starting from the root cap junction. The picture was taken with a MZ 16 Leica stereomicroscope. Arrowheads point to root hairs in formation. Black arrow, basal limit of meristem. White arrow, onset of the fast elongation zone.

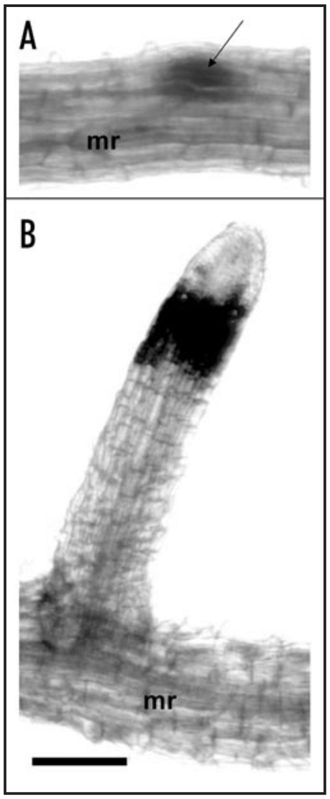

Figure 2. Detection of superoxide radical by nitroblue tetrazolium in a lateral root primordium marked by an arrow (A) and in a developing lateral root (B). mr, main root. Scale bar: 100 µm.

References

1. Dunand C, Crèvecoeur M, Penel C. Distribution of superoxide and hydrogen peroxide in *Arabidopsis* root and their influence on root development: Possible interaction with peroxidases. New Phytologist 2007; 174:332-41.
2. Scheres B, Benfey P, Dolan L. Root development. The *Arabidopsis* Book 2002; 1-18.
3. Foreman J, Demidchik V, Bothwell JHF, Mylona P, Mledema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan L. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 2003; 422:442-46.
4. Baluška F, Volkmann D, Barlow PV. Specialized zones of development in roots: View from the cellular level. Plant Physiol 1996; 112:3-4.
5. Verbelen JP, De Cnodder T, Le J, Vissenberg K, Baluška F. The root apex of *Arabidopsis thaliana* consists of four distinct zones of growth activities. Plant Signal Behav 2006; 1:e1-e9.
6. Vissenberg K, Martinez-Vilchez M, Verbelen JP, Miller JG, Fry SC. In vivo colocalization of xyloglucan endotransglycosylase activity and its donor substrate in the elongation zone of *Arabidopsis* roots. Plant Cell 2000; 12:1229-37.
7. Lee DK, Ahn JH, Song SK, Choi YD, Lee JS. Expression of an expansin gene is correlated with root elongation in soybean. Plant Physiol 2003; 131:985-97.
8. Chen SX, Schopfer P. Hydroxyl-radical production in physiological reactions. A novel function of peroxidase. Eur J Biochem 1999; 260:726-35.