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SHORT COMMUNICATION

Superoxide anion generation response to wound in Arabidopsis hypocotyl cutting

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ABSTRACT

Cutting is a frequently used model to study the process of adventitious root formation, and excision of cuttings leads to rapid wound response signaling. We recently showed that as a wound signal, reactive oxygen species (ROS, mainly hydrogen peroxide) participate in adventitious root induction of hypocotyl cuttings through regulation of auxin biosynthesis and transport. Here, superoxide anion ($O_2^{-\bullet}$), an early type of ROS, exhibited rapid burst at the cutting site immediately in response to wounding in Arabidopsis hypocotyl cuttings. Diphenylene iodonium chloride (DPI, inhibitor of NADPH oxidase) overwhelmingly suppressed $O_2^{-\bullet}$ propagation through the hypocotyl. Compared to wild type, $O_2^{-\bullet}$ burst only occur in cut base, and upward transduction were inhibited completely in NADPH oxidase mutant *AtRbohD*. These results indicate $O_2^{-\bullet}$ generation and propagation in response to wound and via NADPH oxidase in adventitious root induction of hypocotyl cuttings.

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The formation of adventitious roots is a multifactorial process in cuttings, which is very important for asexual propagation of plant, but the understanding of its regulation mechanism is not entirely clear. The origin of adventitious root rooting in stem cuttings is the removal of primary root system, which is mainly caused by two primary stimuli: the isolation of the cut part from the whole plant and wounding at the cutting site.[1,2] Jasmonates, ethylene, and reactive oxygen species (ROS) are widely produced in response to wound.[3–5]

ROS are reactive forms of molecular oxygen, and mainly include superoxide anion ($O_2^{-\bullet}$), singlet oxygen, hydrogen peroxide (H_2O_2), and hydroxyl radical.[6] In recent years, beyond the traditional belief of ROS as toxic by-products of metabolic processes, more and more evidences show that ROS can be an important signal molecule in plant growth, development, and stress response.[7,8] Our recent study indicates that wound-induced ROS participate in AR induction through regulation of auxin biosynthesis and transport.[9]

$O_2^{-\bullet}$ is a free radical formed by molecular oxygen metabolism in organism, and it can be further converted into H_2O_2.[10] NADPH oxidase (NOX) is an oxidase located in the plasma membrane. It can obtain electrons from NADPH on the inner side of the plasma membrane and transfer them to molecular oxygen, so that O_2 can be excited to form $O_2^{-\bullet}$ at the outer side of plasma membrane through single-electron reduction reaction. Subsequently, $O_2^{-\bullet}$ is converted to H_2O_2 by SOD catalysis or natural disproportionation. Studies have shown that NOX widely exists in plants, and it can respond to growth, development, and abiotic stress.[11,12]

Here, we detected the changes of intracellular $O_2^{-\cdot}$level in hypocotyl after cuttings by using $O_2^{-\cdot}$–sensitive fluorophore DHE.[13] It was observed that intracellular $O_2^{-\cdot}$ are rapidly generated at the cutting site as a wound-induced signal, then propagated upward along the hypocotyl and mainly distributed in the stele of hypocotyl (Figure 1). To further investigate the spatial–temporal variation of wound-induced $O_2^{-\cdot}$ in whole hypocotyl cuttings, 6-mm-long hypocotyls were conceptually divided into three sections (base, middle, and upper) (Figure 2). DHE fluorescence intensity of basal part increased quickly and reached strongest at 8 min after cutting, and the fluorescence in the middle part was the strongest at 16 min and that in upper at 22 min (Figure 2). The result indicates that the more to the upper part of hypocotyl, the less $O_2^{-\cdot}$ level increased.

Diphenylene iodonium chloride (DPI), an inhibitor of NADPH oxidase, completely inhibited upward $O_2^{-\cdot}$ propagation, although it had no obvious effect on the $O_2^{-\cdot}$ generation at the cutting base (Figure 1), suggesting that NADPH oxidase is involved in $O_2^{-\cdot}$ upward propagation, but not in the basal $O_2^{-\cdot}$ burst. We further examined the changes of $O_2^{-\cdot}$ in NADPH oxidase mutant *AtRbohD*. Compared to wild-type plants (Figure 2), the $O_2^{-\cdot}$ burst occurred only at the cutting base and $O_2^{-\cdot}$ upward propagation was completely inhibited in *AtRbohD* (Figure 3). These results unequivocally indicate that *RBOHD* mediates $O_2^{-\cdot}$ upward propagation. Combined with our previous results,[9] the consistency and similarity of $O_2^{-\cdot}$ and H_2O_2 signal changes confirms that the wound-induced ROS is NADPH oxidase-dependent. We conclude that $O_2^{-\cdot}$ is generated and propagated through the hypocotyl of the cuttings, similarly to H_2O_2, consistent with the role of these ROS in long distance signaling of adventitious root induction.

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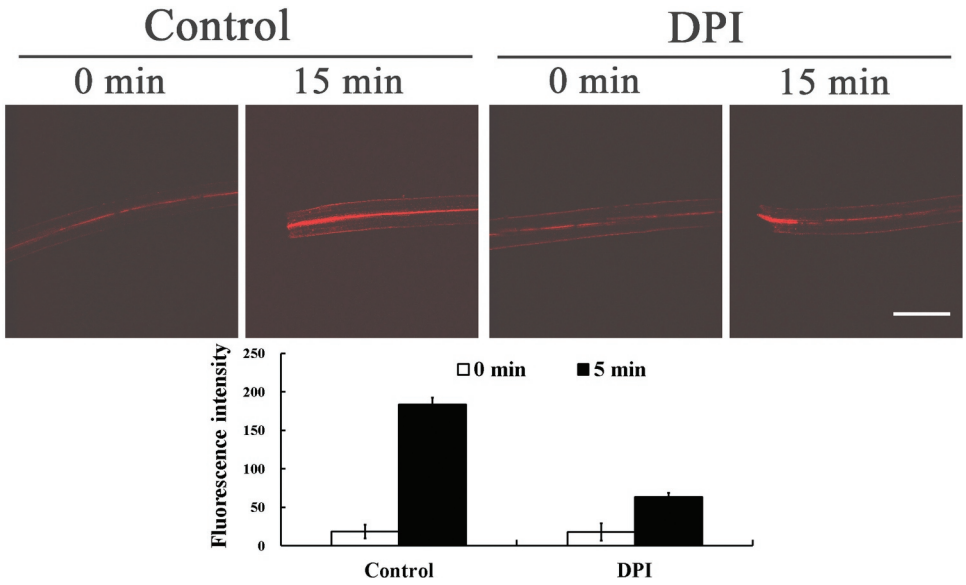

Figure 1. Detection of intracellular $O_2^{\cdot-}$ generation response to excision of primary roots in Arabidopsis wild type (Col-0) hypocotyl. Wild-type seedlings were preloaded with 250 μM DHE or combined with 3 μM DPI, then fluorescence images were detected at the base of the hypocotyl at the indicated time after excision. A Leica TCS-SP5 confocal laser scanning microscope was used for visualization and imaging (excitation at 543 nm and emission at 580–600 nm). Data of fluorescence pixel intensities are displayed as means ±SE of three replicates, each replicate with 3 seedlings. Bar = 250 μm.

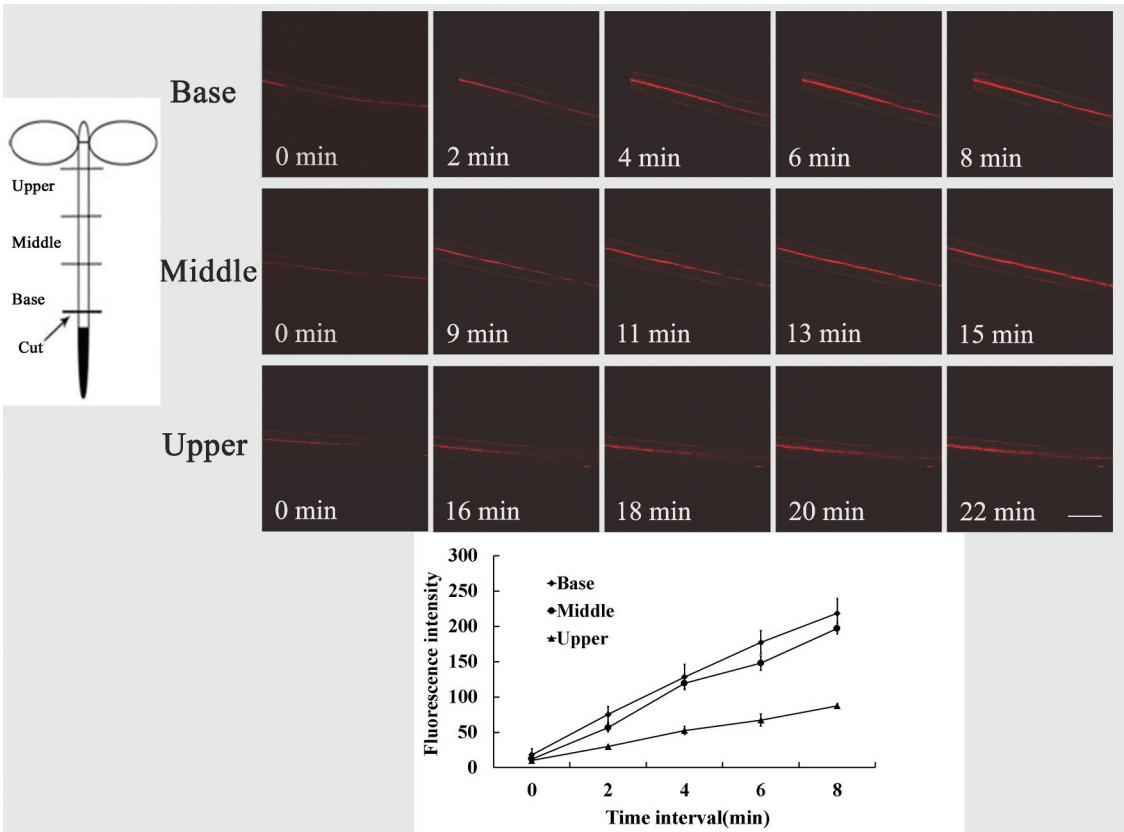

Figure 2. Spatial–temporal variation of intracellular $O_2^{\cdot-}$ fluorescence in different parts of Arabidopsis wild-type (Col-0) hypocotyls cuttings. Wild-type seedlings were preloaded with DHE, then fluorescence images of intracellular $O_2^{\cdot-}$ were detected at the base, middle, and upper hypocotyl at different times after excision of primary roots. Data of fluorescence pixel intensities are displayed as means ±SE of three replicates, each replicate with 3 seedlings. Bar = 250 μm.

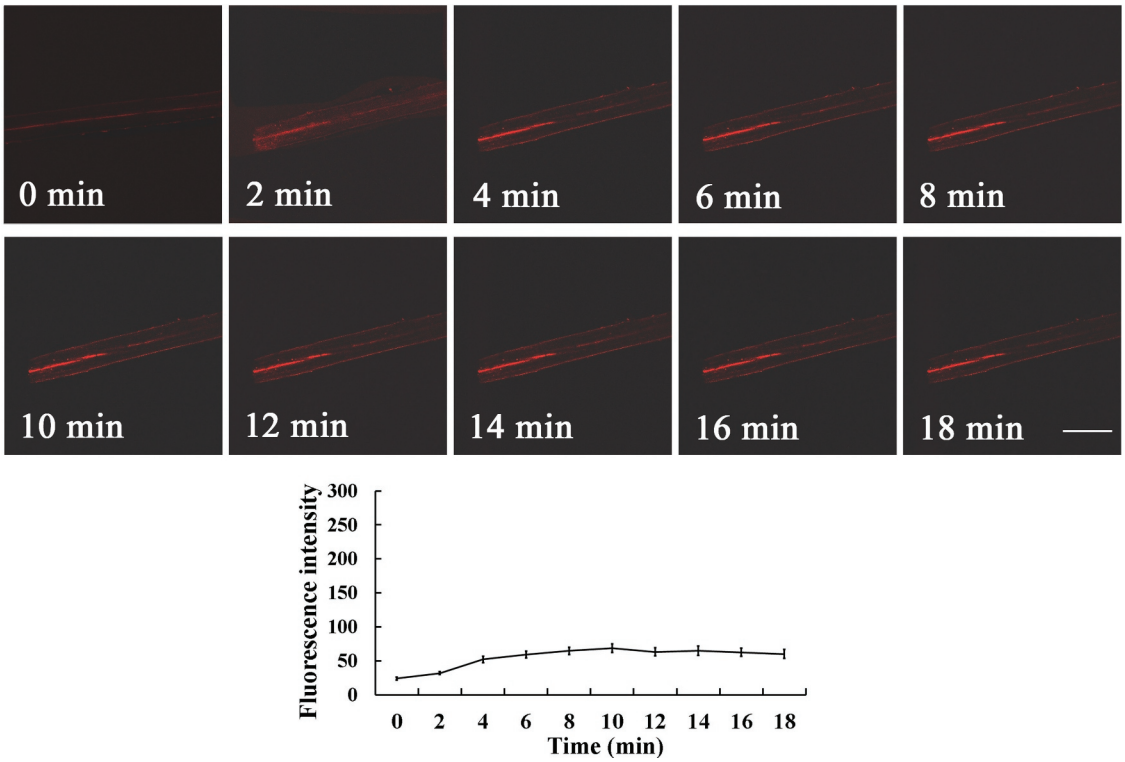

Figure 3. Changes in $O_2^{\cdot-}$ fluorescence in hypocotyls of Arabidopsis NADPH oxidase mutants *AtRbohD*. Seedlings of mutant *AtRbohD* were preloaded with DHE, and then fluorescence images were obtained at the hypocotyl base at different times after excision. Data of fluorescence pixel intensities are displayed as means ±SE of three replicates, each replicate with 3 seedlings. Bar = 250 μm.

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Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the author.

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