SHORT COMMUNICATION

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# Hydrogen peroxide is involved in cGMP modulating the lateral root development of *Arabidopsis thaliana*

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3',5'-cyclic guanosine monophosphate (cGMP) and hydrogen peroxide ($H_2O_2$) function as the important signaling molecule which promote the lateral root development of *Arabidopsis thaliana*. In this study, interestingly, application of 8-Br-cGMP (the membrane permeable cGMP analog) promoted the endogenous $H_2O_2$ production. In addition, the decrease of endogenous $H_2O_2$ also inhibited the effect of cGMP on the lateral root development. Thus, $H_2O_2$ maybe act as a downstream signaling of cGMP molecule which is involved in the lateral root development of *Arabidopsis*. We further found that $H_2O_2$ affected cGMP modulating polar auxin transport. When the endogenous $H_2O_2$ level was inhibited, the effect of cGMP on the acropetal auxin transport and the basipetal auxin transport was removed. Moreover, *pin2* was insensitive for cGMP and $H_2O_2$ suggesting that PIN2 protein plays an important role in cGMP and $H_2O_2$ modulating the lateral root development of *Arabidopsis*.

The main determinant of root systems structure is the lateral root formation.[1] Therefore, researching the mechanism of lateral root formation and growth is very important in agricultural production. *Arabidopsis thaliana* as an important model plant, the molecular mechanism of *Arabidopsis* lateral root development has been extensively studied. In *Arabidopsis*, lateral root development can be divided into three stages. The lateral root initiates the pericycle cells of the xylem.[1] Division and expansion of pericycle cells form the lateral root primordium.[2] At the stage of emergence, the primordium forms an active meristem and becomes a new lateral root.[2]

Auxin plays a key role in lateral root development. It is proposed that indole acetic acid (IAA) is necessary at lateral root primordia formation and lateral root development.[3] Laskowski et al. have suggested that IAA is required for isolated lateral root primordia which promote the rapidly division of the pericycle cells.[4] However, developing lateral root primordia obtain IAA via polar auxin transport.[5] In the root, IAA is polarly transported, from shoot to the root apex (acropetal, rootward) and toward the root-shoot junction (basipetal, shootward).[5]

In the past many years, hydrogen peroxide ($H_2O_2$) is considered to be a toxic cellular metabolite in the environmental stress. Recently, many reports suggest that $H_2O_2$ functions is an important signaling molecule in plants.[6] The production of $H_2O_2$ is regulated by PM-bound NADPH oxidase complex in plants and the inhibitor of the NADPH oxidase can decrease endogenous $H_2O_2$ level in both mammals and plants.[7] In plants, a lot of physiological and biochemical processes are regulates by $H_2O_2$ signal, including programmed cell death, stomatal closure, hypersensitive response, the development of cell wall and pollen development.[8-10] Recently, there are many reports demonstrate that $H_2O_2$ is involved in mung bean adventitious rooting development.[11] Our work found that the density of *Arabidopsis* lateral root markedly increased after 10 μM $H_2O_2$ treatment for 5 d (**Fig. 1A**). In addition, application of diphenylene iodonium (DPI, a PM NADPH oxidase inhibitor) inhabited the density of lateral root in *Arabidopsis* (**Fig. 1A**). These data suggest that $H_2O_2$ maybe act as an essential signaling molecule which is involved in the lateral root development of *Arabidopsis*.

3', 5'-cyclic guanosine monophosphate (cGMP) is an important secondary messenger, it is involved in various developmental processes in plants, such as chloroplast development, seed germination, pollen germination and stomatal closure.[12-14] Recently, our study demonstrates that cGMP can regulate polar auxin transport in the root of *Arabidopsis* and promotes *Arabidopsis* lateral root development.[15] However, when the endogenous $H_2O_2$ level was inhibited by DPI, the effect of cGMP on the lateral root was removed (**Fig. 1A**). Interestingly, in this study, we also found that application of 8-Br-cGMP (the membrane permeable cGMP analog) induced the endogenous $H_2O_2$ accumulation (**Fig. 1B**).

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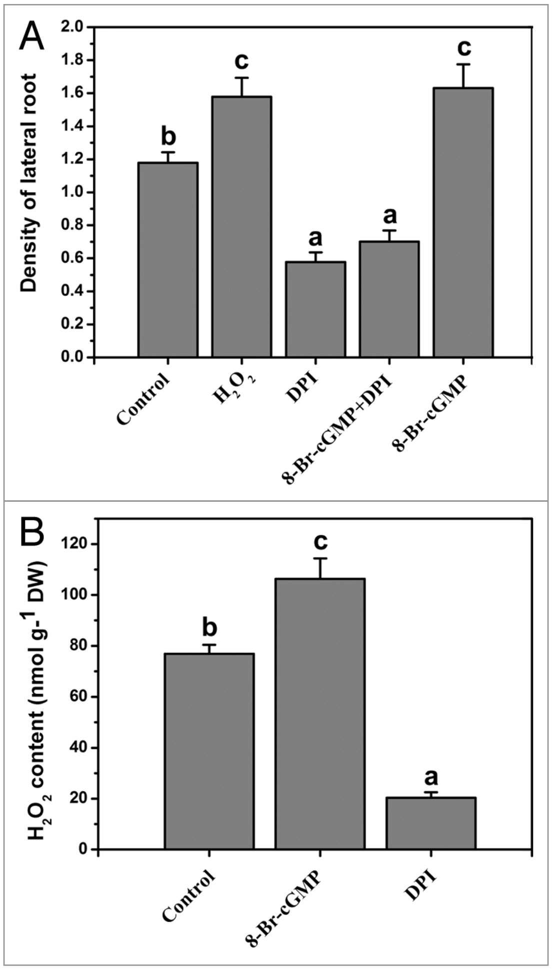

**Figure 1.** (**A**) Effects of 8-Br-cGMP and $H_2O_2$ on the density of lateral root in wild-type plant. Five days old seedlings to various treatments for 5 d. (**B**) Effects of 8-Br-cGMP on the endogenous $H_2O_2$ level in wild-type *Arabidopsis* roots. Seven-day-old seedlings to various treatments for 6 h. Ten μM $H_2O_2$, 40 μM 8-Br-cGMP and 5 μM DPI were used for various treatments. Mean values and SE were calculated from three replicates. Within each set of experiments, bars with different letters were significantly different at the $p < 0.01$ level.

Application of exogenous $H_2O_2$ also could promote the density of lateral root in *Arabidopsis* (**Fig. 1A**). Thus, the function of $H_2O_2$ seen to be involved in cGMP modulating the lateral root development of *Arabidopsis*. We further understand the interaction between cGMP and $H_2O_2$ on polar auxin transport in *Arabidopsis* roots. We has demonstrated that the activity and expression of *DR5::GUS* can be increased in root tips by 8-Br-cGMP. However, in this work we found that when the endogenous $H_2O_2$ level was inhibited by DPI, the effect of cGMP on the activity and expression of *DR5::GUS* was removed (**Fig. 2A and B**). cGMP could enhance the acropetal auxin transport and the basipetal auxin transport in *Arabidopsis* roots. However cGMP modulating polar auxin transport was also inhibited by DPI (**Fig. 2C and D**). Therefore, we suggest that $H_2O_2$ and cGMP are necessary signaling molecule which respond for the lateral root development of *Arabidopsis* and $H_2O_2$ maybe act as a downstream signaling molecule involved in cGMP modulating polar auxin transport.

In plants, a substantial amount of data demonstrate that PIN family proteins act as the auxin efflux carriers which are the essential facilitators of polar auxin transport.[16,17] PIN proteins bind at the plasma membrane and show asymmetric subcellular localization. PIN proteins regulate polar auxin transport in roots and thus the local auxin gradients build up that promote the lateral root formation. Previous studies have shown that mutation of PINs cause the phenotypic changes of root in *Arabidopsis*.[16,18] In *pin1,2* double mutant, the root cannot elongation and the root meristem is inhibited.[19] In different developmental processes, PIN proteins can be regulated by various signaling molecule, such as cGMP.[3,15] cGMP functions as a signaling molecule to regulate the PIN family gene expression (*PIN1*, *PIN2*, *PIN3* and *PIN7*) and affects polar distribution of PIN protein.[11] $H_2O_2$ is involved in cGMP modulating polar auxin transport in *Arabidopsis* roots. We have also analyzed the effect of cGMP and $H_2O_2$ on *pin2* mutant plant.[20] The obtained results showed that *pin2* was insensitive for cGMP and $H_2O_2$ (**Fig. 2E and F**). These data suggest that PIN2 protein plays an important role in cGMP and $H_2O_2$ modulating the lateral root development of *Arabidopsis*.

In conclusion, both cGMP and $H_2O_2$ are necessary signaling molecule which respond for the lateral root development. The relationship between cGMP and $H_2O_2$ shed a new light on the complex signaling mechanisms during the lateral root development in *Arabidopsis*. $H_2O_2$ functions as a downstream signaling molecule involved in cGMP modulating the lateral root development of *Arabidopsis*.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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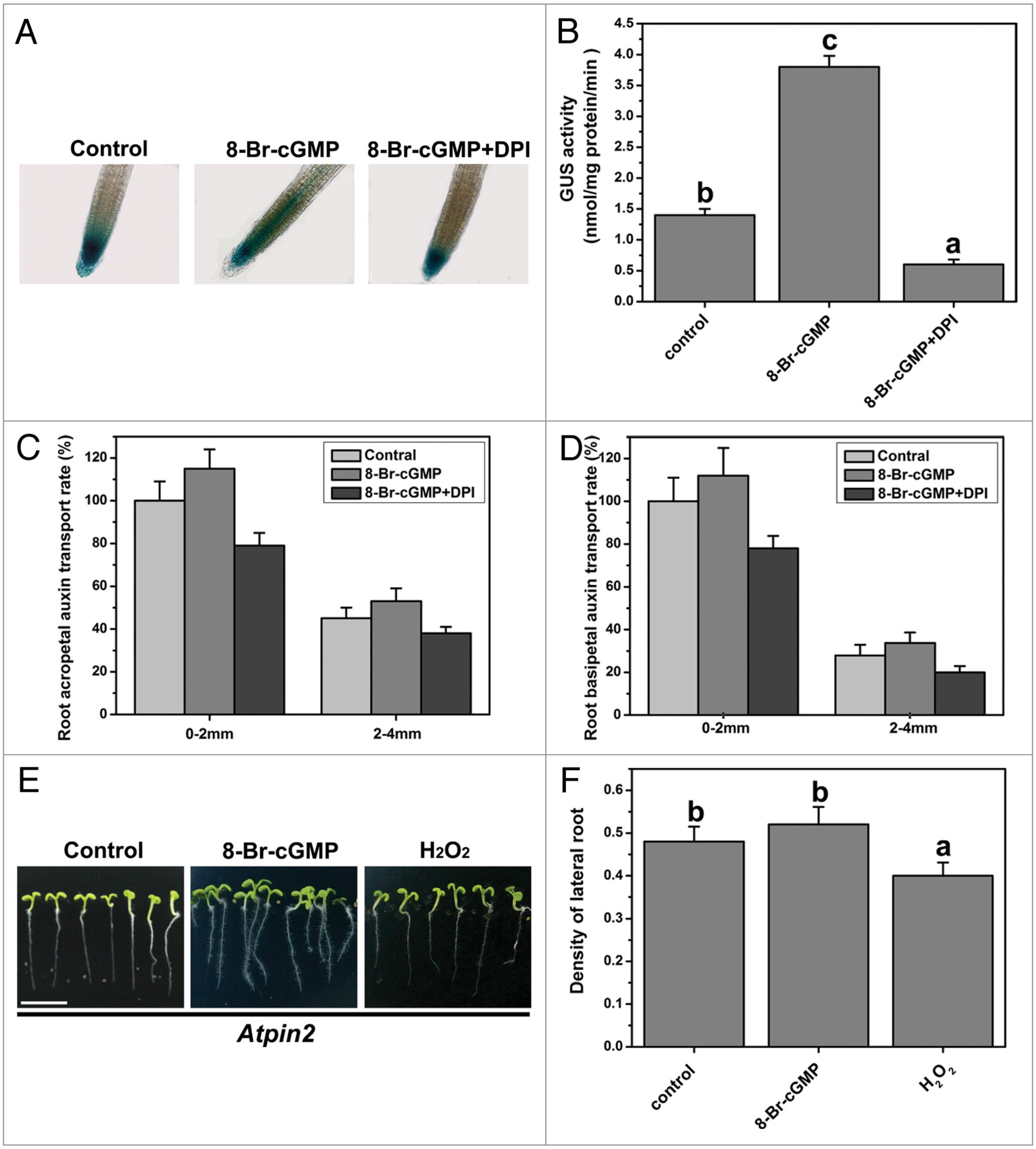

**Figure 2.** Effects of 8-Br-cGMP and $H_2O_2$ on polar auxin transport in *Arabidopsis* root. (**A**) Histochemical GUS staining patterns of *DR5::GUS* after various treatments for 3 h in apical zone of primary root. (**B**) GUS activity assays of *DR5::GUS* transgenic root after various treatments for 3 h. (**C and D**) Root acropetal auxin transport and basipetal auxin transport in wild-type *Arabidopsis* were assayed after various treatments for 12 h. (**E and F**) Effects of 8-Br-cGMP and $H_2O_2$ on the density of lateral root in pin2 mutant plant. Five-days-old seedlings to various treatments for 5 d. Ten μM $H_2O_2$, 40 μM 8-Br-cGMP 5 μM DPI and Ly83583 were used for various treatments. Bar = 1 cm. Mean values and SE were calculated from three replicates. Within each set of experiments, bars with different letters were significantly different at the $p < 0.01$ level.

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