Hydrogen Peroxide Accumulation Regulated by LjRbohD Modulates Nodulation of *Lotus japonicus* Under Blue Light

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Abstract

Abstract In many legumes, roots that are exposed to blue light do not form nodules, and blue light induces the biosynthesis of hydrogen peroxide (H₂O₂). The mechanism of blue light restraining nodulation is poorly understood. Whether H₂O₂ induced by blue light inhibits nodulation needs to be further studied. In this work, blue light could promote the production of H₂O₂, activate the expression of LjRbohD and LjRbohE, while inhibit the expression of LjRbohB. After applying exogenous H₂O₂ and diphenyleneiodonium chloride (DPI), the results show H₂O₂ induced by blue light represses the nodulation of MG20. The accumulated H₂O₂ may be generated by LjRbohD, which supported by Q-PCR. Cryptochrome 1A, a blue light photoreceptor, is high expression under blue light. However, there seems to be no direct relationship between LjRbohD and LjCry1A. On the contrary, LjRbohB, a positive governor in the regulation of nitrogen fixation activity in *L. japonicus*, may be negatively regulated by LjCry1A according to the hairy roots transformation results. Therefore, the mechanisms of regulating the nodulation in *L. japonicus* by LjRbohB and LjRbohD are quite different under blue light. Keywords: *Lotus japonicus*, accumulated H₂O₂, blue light, nodulation, LjRbohD.

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Figure 1. The development of nodules and infection threads in *L. japonicus* was inhibited by blue light.

Mesorhizobium loti strain NZP2235 tagged with a constitutive *hemA::LacZ* reporter gene fusion was used for inoculation and roots were stained for β -galactosidase activity at 14 days after inoculation (DAI) to reveal the location of bacteria (dark blue color).

(A, B, C) The symbiosis phenotypes of wild-type MG20 at 14 DAI under white light;

(E, F, G) The symbiosis phenotypes of wild-type MG20 at 14 DAI under blue light;

(D, H) Number of infection events and nodules at 14 DAI in wild-type MG20;

Values are means $\pm$ SD (n=10). Asterisks indicate statistically significant differences using Student's *t*-test ($P < 0.001$, or $P < 0.01$).

Figure 2. ROS content.

The leaves and the roots were stained for DCFH-DA probe at 14 DAI to reveal the location of ROS (green fluorescence).

(A, B) The distribution of ROS in leaves. (A) under white light, (B) under blue light;

(C, D) The distribution of ROS in roots. (C) under white light, (D) under blue light;

(E, F) The average ROS fluorescence intensity in leaves and roots. Note ImageJ was used for fluorescence intensity conversion and quantitative analysis.

Values are means $\pm$ SD (n=10). Asterisks indicate statistically significant differences using Student's *t*-test ($P < 0.05$).

Figure 3. Activities of antioxidant enzymes and ROS content in roots.

(A) SOD activity in roots at 14 DAI;

(B) POD activity in roots at 14 DAI;

(C) CAT activity in roots at 14 DAI;

(D) The content of superoxide ion (O₂⁻) in roots at 14 DAI;

(E) The content of hydrogen peroxide (H₂O₂) in roots at 14 DAI;

Values are means ± SD. Asterisks indicate statistically significant differences using Student's *t*-test (*P* < 0.05).

Figure 4. Effects on nodulation of wild type MG20 by exogenous hydrogen peroxide and diphenylpicolonyl chloride (DPI).

(A, B) The H_2O_2 content and the nodulation of wild type MG20 in roots at 14 DAI when exogenous 10 mM H_2O_2 and 15 μM DPI were applied respectively;

(C) The nodulation phenotype of wild type MG20 under white light;

(D) The nodulation phenotype of wild type MG20 applying with 10 mM exogenous hydrogen peroxide under white light;

(E) The nodulation phenotype of wild type MG20 under blue light;

(F) The nodulation phenotype of wild type MG20 applying with 15 μM exogenous DPI under blue light;

Values are means $\pm$ SD. a, b, c indicate statistically significant differences using Duncan's multiple range test ($P < 0.05$).

Figure 5. The relative mRNA expression abundance of genes under blue light.

(A) The relative mRNA expression abundance of *LjRbohs* family genes;

(B) The relative mRNA expression abundance of *LjNIN*;

(C) The relative mRNA expression abundance of *LjCry1A*;

Values are means $\pm$ SD. Asterisks indicate statistically significant differences using Student's *t*-test ($P < 0.05$).

Figure 6. Effects of exogenous DPI on the relative mRNA expression abundance of *LjRbohs* in the roots of *L. japonicus* under blue light.

(A) the relative mRNA expression abundance of *LjRbohB*;

(B) the relative mRNA expression abundance of *LjRbohD*;

(C) the relative mRNA expression abundance of *LjRbohE*;

Values are means $\pm$ SD. a, b, c indicate statistically significant differences using Duncan's multiple range test ($P < 0.05$).

Figure 7. Hairy root transformed plants of overexpressing *LjCry1A*.

(A) the relative mRNA expression abundance of *LjCry1A* and *LjNIN* genes in the roots of *LjCry1A*-OE;
 (B) The hydrogen peroxide content in the roots of *LjCry1A*-OE;
 (C, D, E) The nodulation phenotype of wild type and *LjCry1A*-OE;
 (F) the relative mRNA expression abundance of *LjRbohs* family genes in roots of *LjCry1A*-OE;
 Values are means $\pm$ SD. * indicate statistically significant differences using Student's *t*-test ($P < 0.05$).