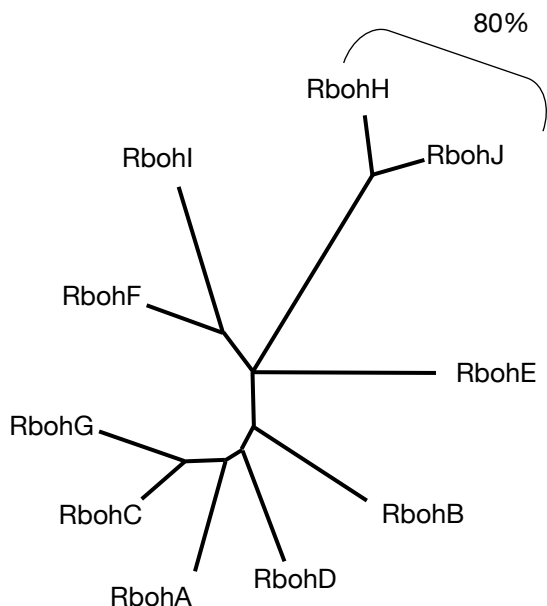


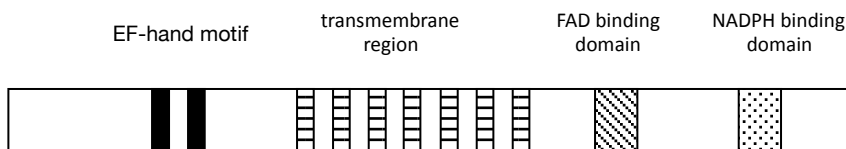
Ca²⁺-activated ROS production by *Arabidopsis* RbohH and RbohJ is essential for proper pollen tube tip growth

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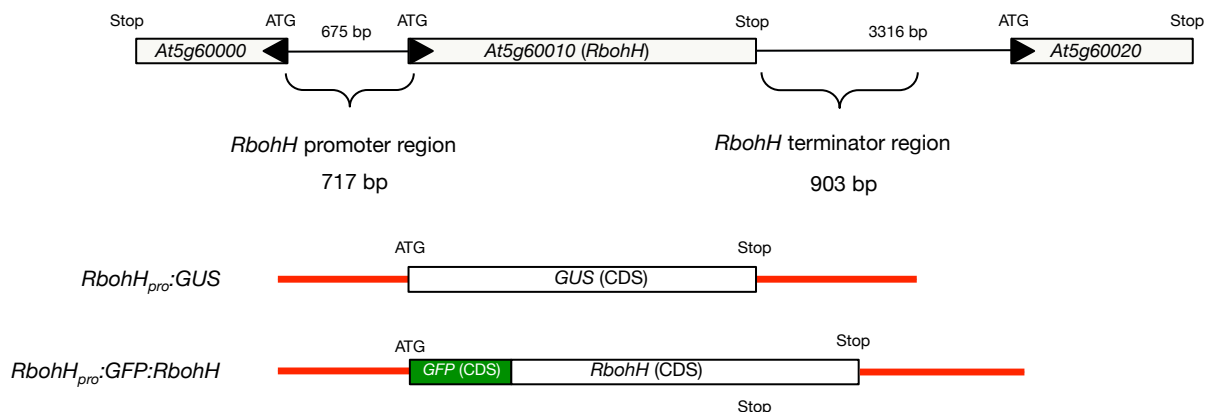
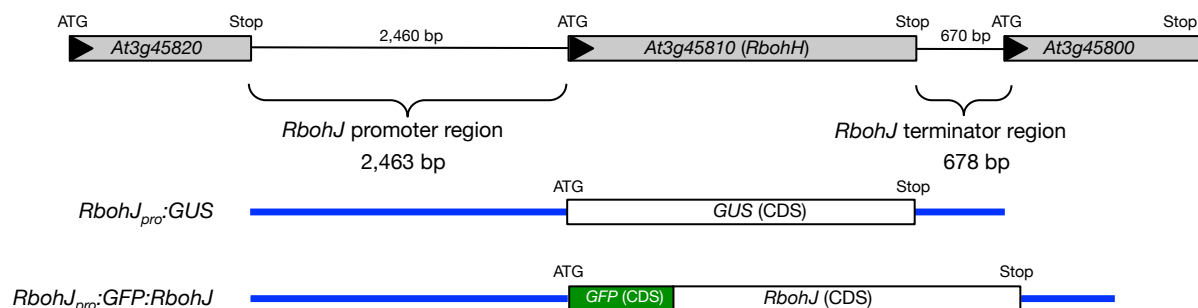
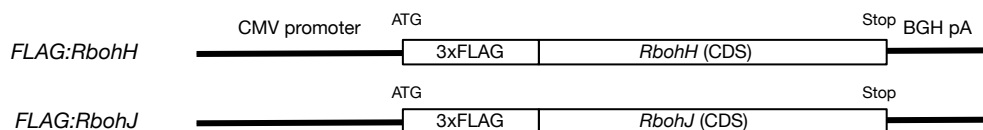
A



B



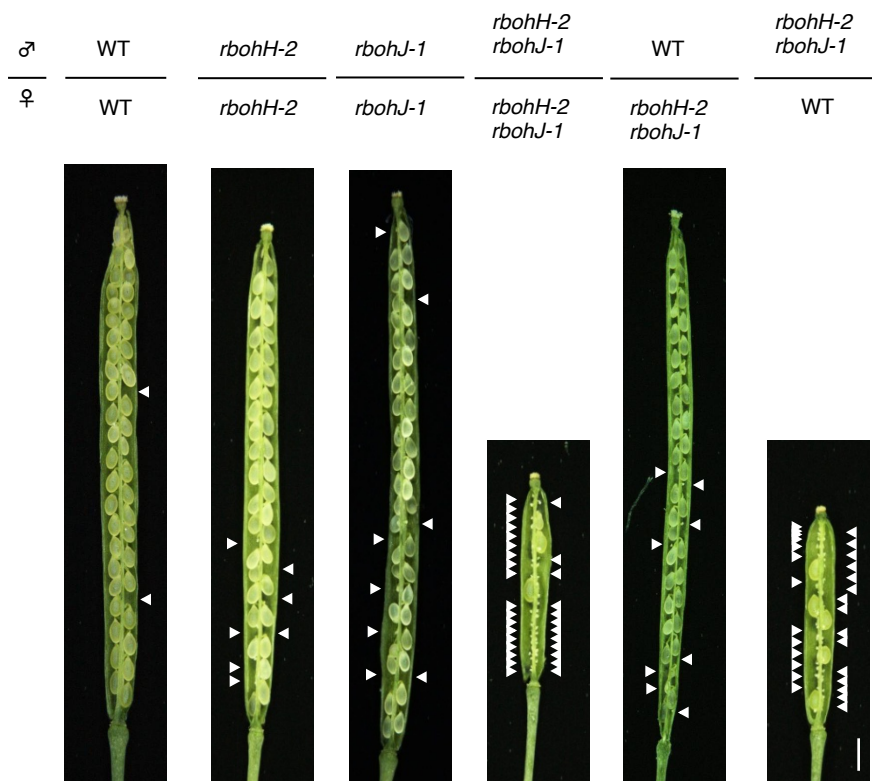
Supplemental Figure S1. Phylogenetic tree of *Arabidopsis* Rboh and the domain structure of Rboh (A) Phylogenetic tree of *Arabidopsis* Rboh based on the neighbor-joining algorithm in ClustalX. RbohH and RbohJ had the highest level of amino acid sequence similarity (80%). (B) Schematic representation of the domain structures of Rboh. All Rboh proteins are transmembrane proteins with six transmembrane helices. The N-terminal region of Rboh contains two EF-hand motifs, whereas the C-terminal region has FAD- and NADPH-binding domains. FAD, flavin adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

A**B****C**

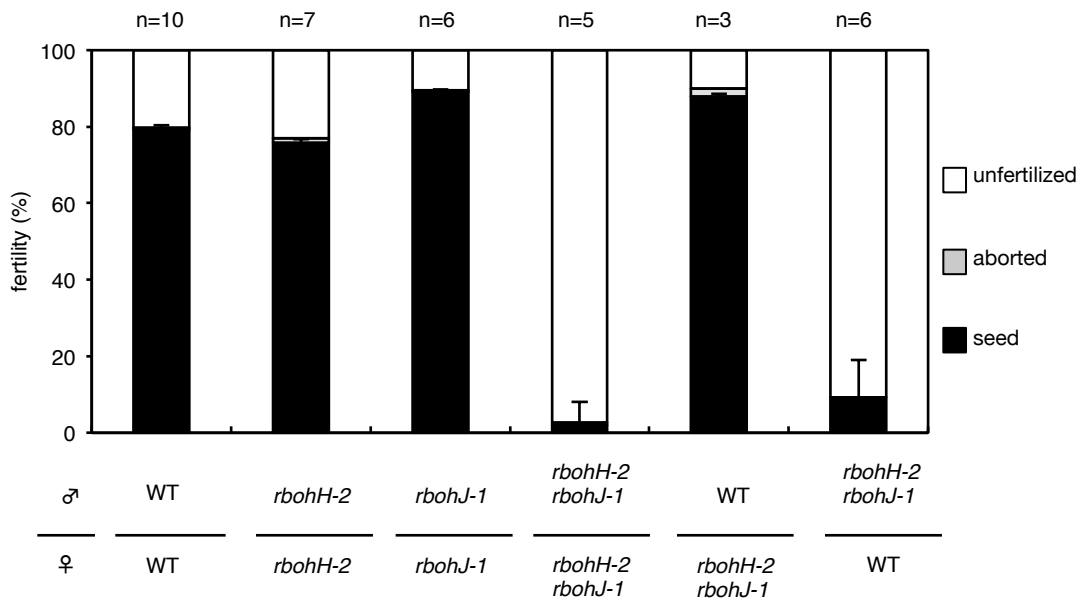
Supplemental Figure S2. Plasmid construction. (A) Genetic organization of *RbohH*. There are 675 base pairs between *At5g6000* and *RbohH*, whereas there are 3316 base pairs between *RbohH* and *At5g60020*. The 717 base pairs upstream of the start codon of *RbohH* were used as the promoter, and 903 base pairs downstream of the stop codon of *RbohH* were used as the terminator. Triangles indicate the direction of translation. Plasmid constructs (*RbohH_{pro}:GUS*, *RbohH_{pro}:GFP:RbohH*) were used to express the chimeric gene under the control of the *RbohH* promoter in *Arabidopsis thaliana*. The coding sequence (CDS) region of *GUS* and *GFP*-fused *RbohH* were cloned between the promoter and terminator (red lines) of *RbohH*.

(B) The genetic organization of the *RbohJ* region. There are 2460 base pairs between *At3g45820* and *RbohJ*, whereas there are 670 base pairs between *RbohJ* and *At3g4580*. The 2463 base pair region upstream of the start codon of *RbohH* was used as the promoter, and the 678 base pair region downstream of the stop codon of *RbohJ* was used as the terminator. The triangles indicate the direction of translation. Plasmid constructs (*RbohJ_{pro}:GUS*, *RbohJ_{pro}:GFP:RbohJ*) were used to express the chimeric gene under the control of the *RbohJ* promoter in *A. thaliana*. The CDS region of *GUS* and *GFP*-fused *RbohJ* were cloned between the promoter and terminator (blue lines) of *RbohJ*. (C) Plasmid constructs (*FLAG:RbohH*, *FLAG:RbohJ*) for expressing FLAG-tagged RbohH and RbohJ in HEK293T cells. The CDS region of *RbohH*, *RbohJ*, and their EF-hand mutants were cloned with a 3×FLAG sequence into the pcDNA3.1 (–) vector between the cytomegalovirus (CMV) promoter and the bovine growth hormone (BGH) polyadenylation signal (black lines). Each EF-hand mutant was generated by inducing point mutations in the EF-hand motif.

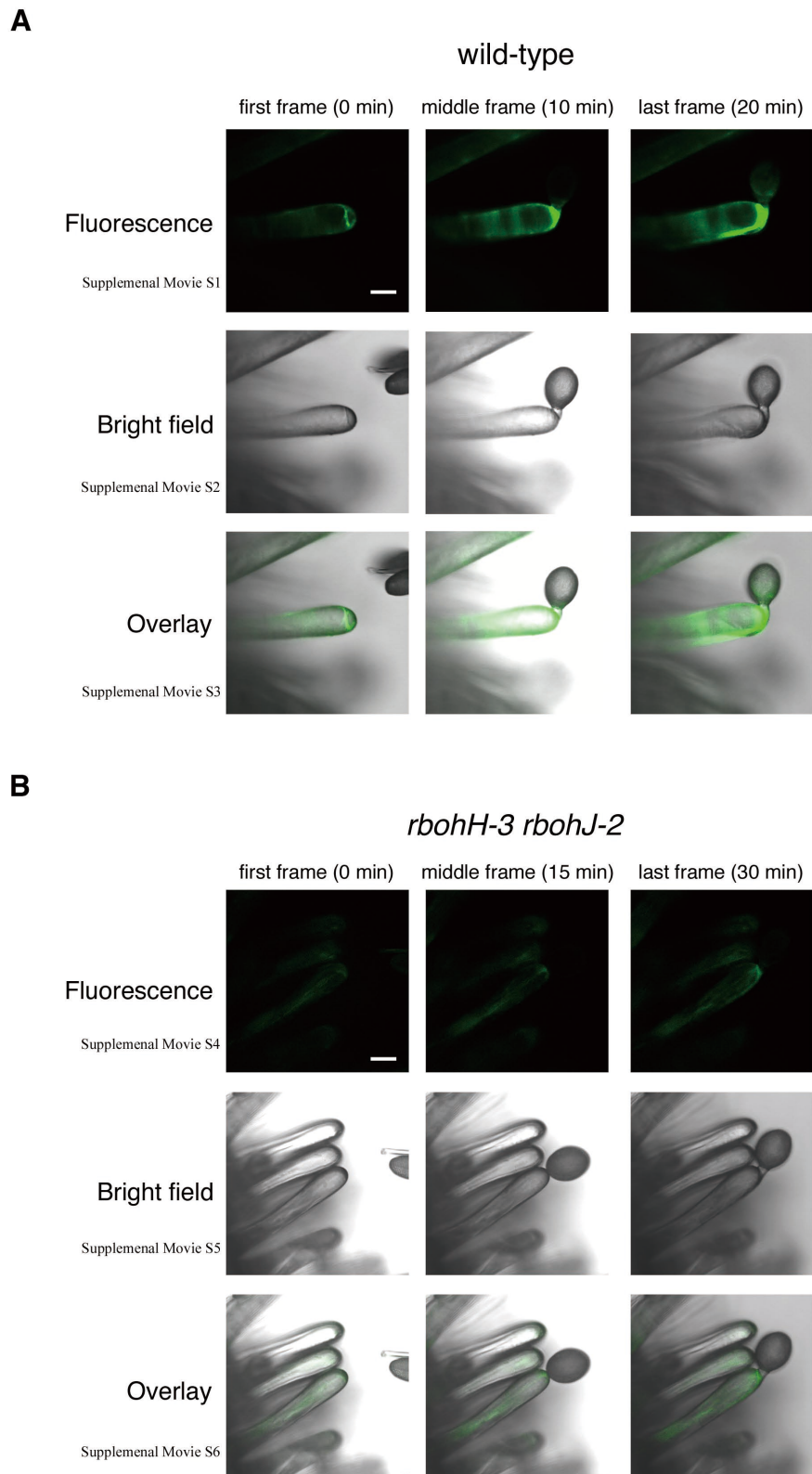
A



B



Supplemental Figure S3. The *rbohH-2 rbohJ-1* double mutant (Ws background) had reduced fertility. (A) Dissected siliques harvested 5–10 d after hand pollination. White arrowheads indicate unfertilized ovules. Scale bar = 1 mm. (B) Percentage of normal seeds, aborted seeds, and unfertilized ovules in siliques. Siliques were harvested 5–10 d after hand pollination. ♂ indicates the genotype of the pollen grain. ♀ indicates the genotype of the pistil. Closed bars indicate normal seeds; gray bars indicate aborted seeds; open bars indicate unfertilized ovules. Values are means ± S.E. (n = 3–10 siliques).



Supplemental Figure S4. The still images of Supplemental Movies S1–6. Detection of ROS on the wild-type stigmatic papilla cell after pollination with the wild-type (A; Supplemental Movies S1–3) or the *rbohH-3 rbohJ-2* double mutant (B; Supplemental Movies S4–6) pollen grain. ROS were visualized using the fluorescent ROS indicator Oxyburst Green (H₂DCFDA). Scale bar = 10 μ m.

Supplemental Table 1. Sequences of Primers Used in This Work.

primer name	Primer sequence	Purpose
RbohH-BamHI-F	5'- <u>GGATCC</u> AAAAGTAACAC <u>CCC</u> AACGGAGG-3'	Plasmid construction
RbohH-EcoRV-R	5'- <u>GATATC</u> <u>C</u> TA AAAATT <u>C</u> TCCTTGTGGAAAGT-3'	Plasmid construction
RbohJ-BamHI-F	5'- <u>GGATCC</u> AAAAACAACAAAAAGGT <u>G</u> GGGACC-3'	Plasmid construction
RbohJ-BamHI-R	5'- <u>GGATCC</u> TCAGAA ATTCTCTTTGTGAAA A-3'	Plasmid construction

Underlines indicate restriction enzyme site. Red colors indicate silent mutation sites.