

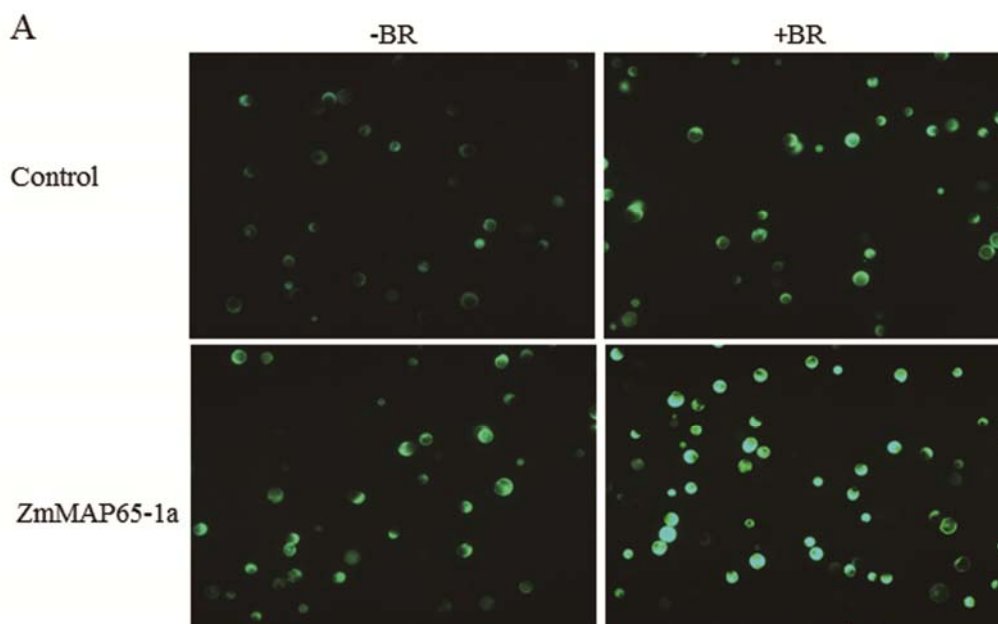
ZmMAP65-1a positively regulates H₂O₂ amplification and enhances brassinosteroid-induced antioxidant defense in maize

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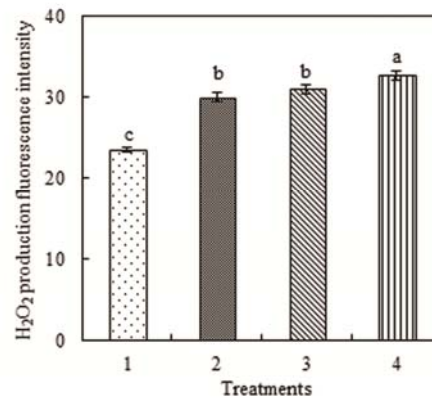
Supplementary Figure S1. Transient expression of *ZmMAP65-1a* enhances the BR-induced H₂O₂ production.

(A) H₂O₂-dependent fluorescence in protoplasts transiently expressing *ZmMAP65-1a*. Protoplasts transfected with 35S-*ZmMAP65-1a*-mCherry (*ZmMAP65-1a*) or empty vector (Control) were treated with 10 nM BR (+BR) or the incubation medium (-BR) for 10 min, and then loaded with H₂DCF-DA for 10 min. H₂O₂ was visualized by confocal microscopy. Experiments were repeated at least three times with similar results.

(B) Quantitation of the fluorescence intensity in (A). The protoplasts were treated as follows: 1, empty vector; 2, 35S-*ZmMAP65-1a*-mCherry; 3, empty vector + BR; 4, 35S-*ZmMAP65-1a*-mCherry + BR. Values are means \pm SE of three different experiments. Means denoted by the same letter did not significantly differ at P < 0.05 according to Duncan's multiple range test.



B



Supplementary Figure S2. Time course of changes in the expression of NADPH oxidase genes in response to BR treatment. Protoplasts were treated with 10 nM BR or culture medium (Control) for various times as indicated. Relative expression levels of *ZmrbobA-D* were analyzed by real-time quantitative PCR. Values are means \pm SE of three different experiments. Means denoted by the same letter did not significantly differ at $P < 0.05$ according to Duncan's multiple range test.

