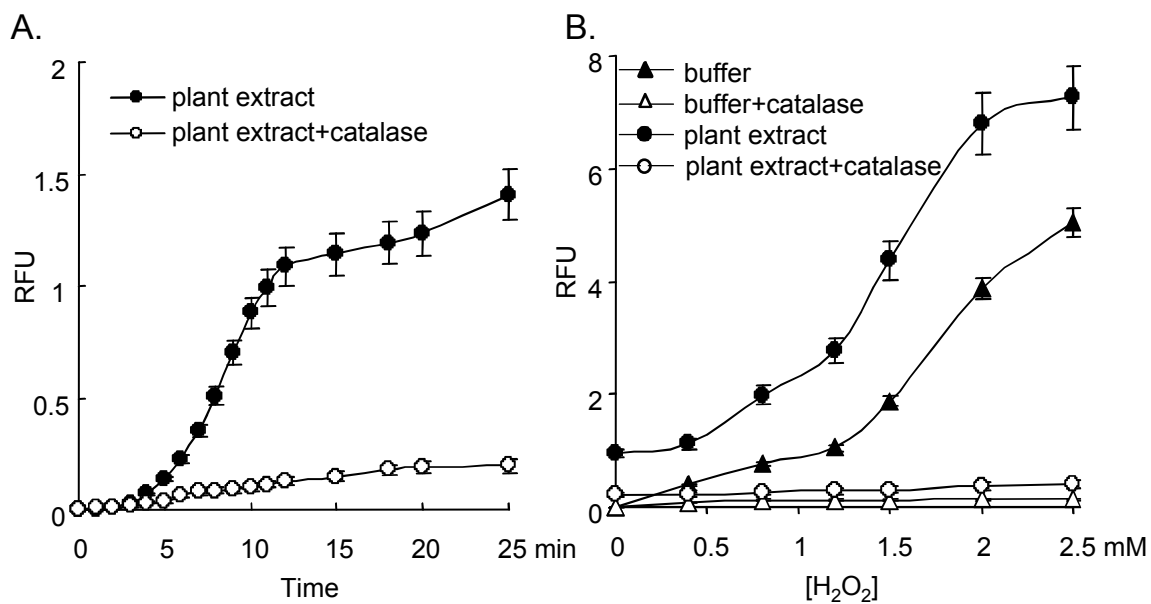


Different signaling and cell-death roles of heterotrimeric G protein α and β subunits in the *Arabidopsis* oxidative stress response to ozone.

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Supplemental Data



Supplemental Figure 4. Reactive oxygen species (ROS) assays using 2', 7'-dichlorodihydro-fluorescein diacetate (H₂-DCFDA). A. Frozen plant tissue was ground in liquid nitrogen. The powder was weighed and immediately taken up in 10 mM Tris-HCl buffer, pH 7.3. The extract was centrifuged twice at 15,000 rpm for 5 min. ROS production was assayed by adding 100 mM H₂-DCFDA in dimethyl sulfoxide (DMSO) to a final concentration of 10 μ M, incubating for the indicated time at room temperature, and measuring fluorescence using a VersaFluor fluorometer (Bio-Rad, Hercules, CA). Each measurement was carried out on two equal aliquots, to one of which catalase was added at a concentration of 300 U/ml. Total protein was quantified using a BioRad DC protein assay kit (Bio-Rad, Hercules, CA). The average fluorescence value obtained from 3 successive measurements was divided by the protein content and expressed as relative fluorescence units (RFU) per mg protein. B. H₂O₂ was added to the indicated final concentration to each aliquot of plant extract prepared in 10 mM Tris-HCl buffer, pH 7.3, with or without added catalase. The ROS assay was carried out using H₂-DCFDA as described in A.