

Flavonols take the heat out of heat stress to protect pollen from elevated ROS

Joëlle K. Muhlemann¹, Trenton L.B. Younts¹, Gloria K. Muday¹

¹Department of Biology, Wake Forest University, Winston-Salem, NC, 27109

Plant reproduction requires long-distance growth of a pollen tube to fertilize the female gametophyte. Prior reports suggested that mutations altering synthesis of flavonoids, plant specialized metabolites that include flavonols and anthocyanins, impair pollen development in several species, but the mechanism by which flavonols enhanced fertility was not defined. We used genetic approaches to demonstrate that flavonols enhanced pollen development by reducing the abundance of reactive oxygen species (ROS). We further showed that flavonols reduced high-temperature stress-induced ROS accumulation and inhibition of pollen tube growth. The *anthocyanin reduced (are)* tomato mutant had reduced flavonol accumulation in pollen grains and tubes. This mutant produced fewer pollen grains and had impaired pollen viability, germination, tube growth, and tube integrity, resulting in reduced seed set. Consistent with flavonols acting as ROS scavengers, *are* had elevated levels of ROS. The pollen viability, tube growth and integrity defects, and ROS accumulation in *are* were reversed by genetic complementation. Inhibition of ROS synthesis or scavenging of excess ROS with an exogenous antioxidant treatment also reversed the *are* phenotypes, indicating that flavonols function by reducing ROS levels. Heat stress resulted in increased ROS in pollen tubes and inhibited tube growth, with more pronounced effects in the *are* mutant that could be rescued by antioxidant treatment. Using *flavonoid 3-hydroxylase* overexpression lines with increased levels of flavonols in pollen, we found that flavonol overaccumulation in these lines results in higher pollen viability than in the thermosensitive wildtype VF36 when developing flower buds are exposed to elevated temperature. A chemical complementation assay also showed that supplementation of the pollen germination medium with the flavonol quercetin rescues pollen tube growth in VF36 at high temperatures. Taken together, our results reveal that flavonol metabolites regulate plant sexual reproduction at both normal and elevated temperatures by maintaining ROS homeostasis.