

Title: Flavonol antioxidants maintain ROS homeostasis during pollen development within tomato anthers

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Heat stress impairs pollen performance, hindering plant reproduction and crop productivity. Elevated temperatures increase reactive oxygen species (ROS) levels in pollen grains and tubes, leading to reproductive failure. Flavonoids are plant-specialized metabolites with antioxidant activity, which include flavonols and anthocyanins. We have examined in vitro pollen performance at optimal and high temperatures in tomato genotypes with varying levels of flavonol synthesis. The mutant *anthocyanin-reduced* mutant (*are*) has impaired flavonol synthesis due to a defect in the gene encoding the enzyme *FLAVANONE 3-HYDROXYLASE (F3H)*, resulting in elevated ROS and impaired pollen performance, relative to the VF36 parental line. These defects are accentuated at high temperatures. The VF36-*F3H*-T3 transgenic line overexpresses *F3H*, increasing flavonol synthesis and enhancing pollen production and thermotolerance. We are now examining pollen within the anther to understand the role of flavonols in vivo. We have used the fluorescent probe DPBA, which binds flavonols, revealing that the flavonol kaempferol accumulates in mature pollen grains within anther locules, whereas quercetin accumulates in the anther epidermis. In pollen grains within the anther of *are* there is elevated ROS compared to VF36, while VF36-*F3H*-T3 has lower ROS levels. Current experiments are underway to determine the mechanisms of flavonol accumulation in developing pollen. Transcripts encoding some, but not all, enzymes of the pathway are detected in mature pollen, but all are detected in anthers. This suggests either transcription changes as pollen matures or that anthers may transport these metabolites or their precursors into pollen during development. We are currently exploring anther-localized accumulation of transcripts encoding flavonol biosynthetic enzymes and potential flavonol transporters over the course of flower development. We are also examining the effect of heat stress applied to whole flowers on pollen performance. Furthermore, we are investigating whether cultivars with thermotolerant pollen have increased flavonol levels. Supported by USDA NIFA (2024-09661).