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Collection of germinating tomato pollen using laser capture microdissection for identification of heat-induced proteomes

The primary objective of this study is to develop the technical workflow for collecting homogenous germinating pollen samples. Two tomato varieties, Black Vernissage (BV) and Micro Tom (MT), were grown in temperature controlled plastic greenhouses. For the non-heated control conditions, greenhouse temperature was set at 25C/21^{o}C (16/8 hours; day/night). For the heat-trearted greenhouse, temperature was set at 32/25 ^{o}C (16/8 hours; day/night). Supplemental light was provided using plant growth LED light. Ten freshly-opened flowers were collected at 9-10am and pollen were released into 2ml pollen germination medium (PGM) in 5-ml centrifuge tubes. After centrifugation, pollen pellets were washed three times in 2 ml 1X PBS. Pollen were resuspended in PGM and incubated for two hours at 37 C, 33 C, and 25C for pollen germination. Three replicates were included for each germination temperature conditions. The mixtures containing germinated and non-germinated pollen were centrifuged, and the pellets resuspended in PGM containing 2.5% carboxymethyl cellulose (CMC), and then loaded onto adhesive coated slide with chambers prepared using shurtape. The samples were cross-linked onto the slides using UV light using a Leica CM1950 Cryotome, and germinated pollen samples were harvested using a PALM laser capture microdissection (LCM) system. The collected samples containing only germinated pollen will be used to identified proteins and genes that are related to pollen germination under different temperature.