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Single cell proteomics analysis of tomato pollen microspore under heat stress.

Abstract

Pollen development is very crucial step as it contributes to the proper fertilization and yield of the fruit. However, exposure of high heat can affect the development of pollen leading to changes in protein structure. In this study, we compare the proteins involved in microspore stage when exposed to heat with non-heat treated tomato. Two different variety of tomatoes were selected based on previous pollen viability test, which include heat sensitive "Micro tom" and relatively heat tolerant "Black vernissage". Tomato flowers were embedded in OCT compound and sectioned in to 20 um thickness cross-section using Leica CM1950 Cryotome. Pollen developmental stages were identified by DAPI staining of nucleus structure. The microspore was isolated using laser capture microdissection (LCM). Pressure cycle-based technology was used to extract the protein from the LCM collected cells and labelled with 16 Plex TMT tags. The sample were processed with LC/MS and its data were analyzed with Proteome discoverer 2.5, Sequest HT software against tomato database iTAG3.0. Protein showing significant difference in relative protein abundance between heat and non-heat treated samples are taken as differentially expressed proteins (DEPs). Bioinformatics analysis was used to identify biological processes enriched in DEPs that play essential roles in pollen development against Heat Stress.