## Studies of stigma receptivity of tomatoes during tomato flower developmental process and under heat stress conditions

Tomato is a self-pollinated fruiting vegetable. Fruit quality and yield depend on the success of pollination, fertilization, and fruit set. The stigma is the primary site for pollen capture, hydration, and germination, which promotes the growth and fertilization of pollen tubes. The optimal temperature for tomato reproduction is 25-28 °C. Under heat stress, tomato flowers can produce extruded stigmas, interfering with the pollination process. However, our previous studies have shown that tomato flowers may abort even in the presence of viable pollen under mild heat stress (HS) conditions (30-32 °C), even without extruded stigmas. Research has identified that the papillae cells of the stigma secrete small peptides that interact with pollen to activate pollen tube growth. However, little is known about the molecular mechanisms regulating stigma receptivity during mild heat stress.

In this study, we developed an assay procedure for stigma receptivity based on the Baker's method (Galen & Plowright, 1987). A receptive stigma turns purple due to the reduction of nitroblue tetrazolium (NBT), while non-viable tissues retain their original color (green for tomato stigma). Stigma receptivity was assessed in three tomato varieties: LA4044 (heat-sensitive), LA3242 (medium heat-tolerant), and LA2662 (heat-tolerant). Results confirmed that the LA4044 stigma exhibited the lowest stigma receptivity rate under both optimal (27 °C) and HS (30 °C) conditions.

To understand the molecular mechanisms affecting stigma receptivity, flower buds at the non-receptive stage (closed flower buds, 5-7 days before reaching the receptive stage) and the optimal receptive stage (first day in full bloom) were collected from LA2662 under both optimal and HS conditions. Papillae cells were harvested using fresh-frozen sectioned stigma (20  $\mu$ m thick) and laser capture microdissection. Single cell-type transcriptomics and proteomics were conducted to identify the molecular mechanisms underlying stigma receptivity and the impacts of HS.

In conclusion, this study developed a reliable assay method for evaluating stigma receptivity to investigate genetic differences and the effects of HS. It also provided novel insights into the molecular mechanisms regulating stigma receptivity under HS conditions. This project was supported by NIFA grant 2022-38821-37339.