INNOVECTIS
Ein Unternehmen der
Johann Wolfgang Goethe-Universität
Frankfurt am Main

Inserted Stigma enhances Tomato Yield and Thermotolerance

Background

Stigma exsertion is a thermosensitive trait affecting many modern tomato cultivars. Although stigma exertion was selected against during tomato domestication and breeding, and several genes controlling this trait have been identified, the mechanisms by which some modern tomato cultivars still exhibit stigma exertion in response to high temperatures remain unknown. Scientists at Goethe University Frankfurt, Germany have now identified a heat stress-induced gene, named HES (Heat Exserted Stigma), which regulates this trait by controlling style length in response to high temperatures.

Technology

A novel CRISPR/Cas9 mutant line of HES exhibits an exserted stigma phenotype, reduced fruit and seed set under high temperatures, and significantly lower yield compared to wild type plants (cv. Moneymaker). Conversely, transgenic lines expressing HES under the CaMV35 promoter result in flowers with inserted stigma, showing enhanced fruit yield and seed set under both control and heat stress conditions, without having any trade-offs for plants. HES functions as a transcription factor capable of acting both as a repressor and activator.

In this project, we therefore propose, as a logical next step, to develop gain-of-function CRISPR lines for HES with two specific objectives: (1) to induce HES expression in response to elevated temperatures and (2) to enhance HES activity in order to promote flower development and fruit set.

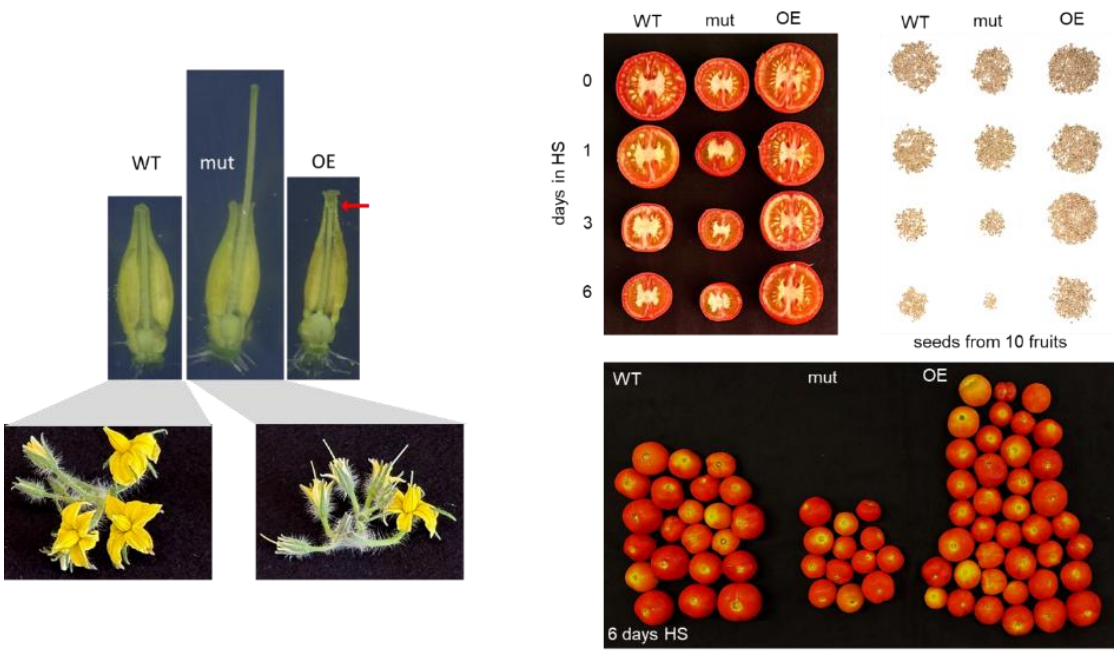

Figure 1. HES regulates style length in response to high temperatures. Left: flower of WT, HES knockout mutant (mut) and transgenic overexpression (OE) from plants exposed for 3 days to heat stress as described in the text. Right: Effects of different periods of heat stress treatments on fruit size, seed production and fruit yield in WT, mut and OE lines.

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Summary of Results

- **HES is a Transcription Factor**: Our research has confirmed that HES functions as a transcription factor, playing a critical role in regulating gene expression in response to environmental cues. We have identified the cis-elements recognized by HES and based on transactivation reporter assays (GUS), HES can act as repressor or co-activator of heat responsive genes when expressed with other members of the same gene family that act as activators. We have identified both the amino acid motifs that confer the repressor and co-activator functions, and we have accomplished to switch the function of the factor by mutations in these motifs.
- **Heat Stress Induced Gene**: HES is induced by heat stress in both leaves and styles, indicating its broad role in the plant's response to elevated temperatures. We have identified cis-elements in the promoter of HES that contribute to the induction of HES under heat stress conditions.
- **Generation of HES tomato lines**: Tomato transgenic (overexpressors carrying a PCaMV35S::3xHA-HES cassette) and knockout (CRISPR/Cas9 homozygous T-DNA free) lines were generated by Agrobacterium-mediated transformation using the pICH86966 and pICSL002208 (carrying Cas9) binary vectors as backbones, respectively. All lines are in the background of cv. Moneymaker, a semi-thermotolerant genotype. The ectopic expression in leaves and styles has been confirmed by qRT-PCR and immunoblot analysis using an anti-HA antibody. The mutation (38 nucleotide deletion in the 5´-primer region of the CDS) results in a premature termination codon and knockout of the gene.
- **Heat Stress Treatments**: Plants were grown under standard condition in a greenhouse (25°C day and 22 °C night temperatures under long day conditions (14 hours light). Plants with first open flower were transferred to Percival growth chambers (CLF) where they were exposed to 35°C / 16 h day – 28°C / 8 h night cycles for 1, 3, or 6 days. Control plants were maintained in the greenhouse under standard conditions for the same time. Preliminary results suggested that 1 day treatment was sufficient to induce stigma exsertion in the CRISPR line, while flowers of wild type plants had a higher frequency of flushed stigmas. Following experiments were done using the 3 days treatment.
- **CRISPR/Cas9 HES mutant analysis**: The mutation leads to a loss-of-function phenotype characterized by exserted stigmas, reduced fruit and seed set, and significantly lower yield under high temperature conditions.
- **Transgenic overexpression HES lines:** Lines carrying a PCaMV35S::3xHA-HES cassette exhibit slightly reduced growth, increased percentage of flowers with inserted stigma, enhanced fruit and seed set and improved yield under both control (no stress) and heat stress conditions.

Patents

Priority EP Patent application no. 25166407.4 „MODIFIED TOMATO PLANTS"
Filed in March 2025, pending

Status

The technology can be licensed or assigned. Moreover, collaborations regarding further development are welcome.

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