THESIS SUMMARY (Investigation of SlDML2 promoter spatial and temporal expression pattern with reporter constructs in tomato)

Epigenetics refers to heritable changes in gene expression that occur without modification of the underlying DNA sequence. DNA methylation and demethylation are two of the many mechanisms by which epigenetic changes can occur. These changes can affect gene expression and contribute to plant development. DNA demethylation can occur either passively or actively. Active DNA demethylation is regulated by DNA demethylases. There are four DNA demethylases in tomato (SlDML1-4). SIDML2 controls fruit ripening. In line with this, its expression is induced during the breaker stage of ripening. Altering the cis-regulatory elements (promoters) of genes in vivo could result in phenotypes different from that of a null mutant. To map the regulatory elements that govern SlDML2 expression, a deletion series was created in the SlDML2 promoter by multiplex CRISPR/Cas9 genome editing.

This research is structured around four major goals. Firstly, wild-type and CRISPR mutant (A120) tomato plants were raised and characterized based on phenotype and genotype. A120 mutants contained a 2104 bp deletion and a 1 bp insertion in the proximal part of the SlDML2 promoter which was shown to be differentially methylated during ripening. The mutant had severe developmental defects that could be a result of the deletion in its promoter. We measured the expression of SlDML2 in the tomato shoots with RT-qPCR and found that the expression level was lower in the mutant. However, this method is not suitable to investigate the spatial and temporal expression in high detail. Therefore, we successfully created two, a mutant and a wild-type SlDML2 promoter driven GFP gene constructs to investigate the expressional difference of SlDML2 in plants in a non-invasive way. These constructs (pICH86966 D2pro-GFP and pICH86966 A120pro-GFP) were assembled using the Golden Gate assembly method which completed our second major goal. Thirdly, despite some delays, efforts are underway to deliver these gene constructs through Agrobacterium-mediated transformation in wild tomatoes.

Finally, upon successful transformation, expression analysis will be conducted in vivo to compare mutant and wild-type promoter-driven GFP and mCherry fluorescence in different tissues and developmental stages. The successful completion of these goals will provide valuable insights into the regulatory mechanisms governing gene expression and epigenetic modifications in tomato plants.