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Dissecting the regulatory regions of the pleiotropic transcription factor *FUL2* in tomato

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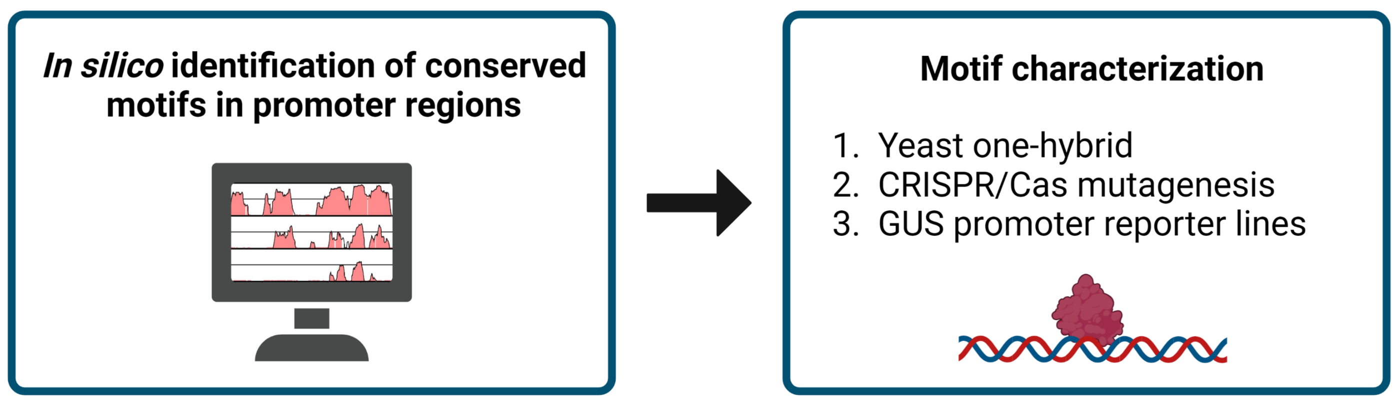
Introduction

Pleiotropic genes are genes that influence more than one trait and can therefore be problematic for plant breeding. Unintended effects can occur when breeding for a single trait, as one mutation in the coding sequence can disturb all traits at once. An example of such a gene is the **transcription factor *FUL2*** in tomato. *FUL2* is one of the two co-orthologs of the Arabidopsis *FRUITFULL* (*FUL*) gene and regulates **fruit development, fruit ripening, flowering time and inflorescence architecture**. To understand how *FUL2* is regulated and performs these different functions, we aim to identify and characterize the **cis-regulatory elements (CREs)** that are required for tissue-specific activity by using the following approach[1]:



1. *In silico* identification of conserved motifs in promoters

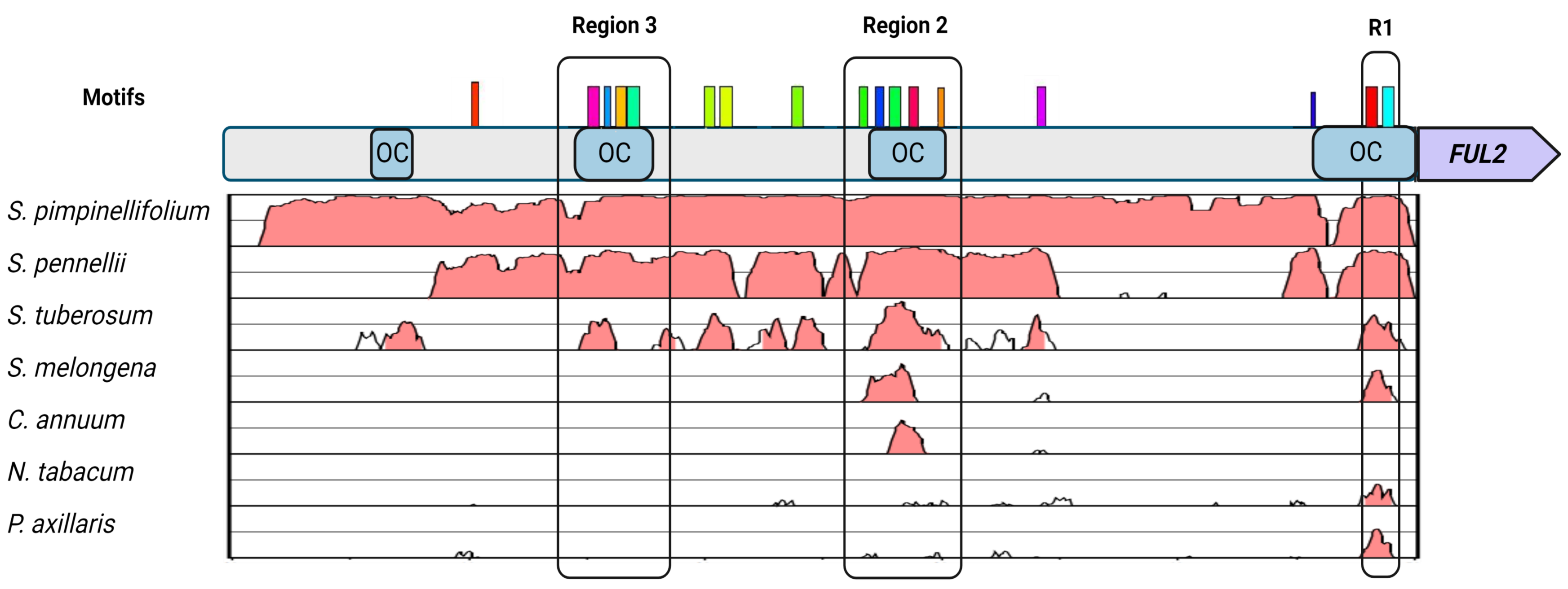


Figure 1. Results of phylogenetic footprinting for the promoter of *FUL2*. MEME[2] and mVISTA[3] were used for analysis and seven *Solanaceae* species were included. OC = open chromatin [1]

- Construct a phylogenetic tree to identify *FUL* orthologs
- Compare promoter regions of *FUL2* in different species to identify conserved motifs / regions (phylogenetic footprinting)
- Screen motifs against TF binding site (TFBS) databases such as JASPAR

2a. Motif characterization - Yeast one-hybrid (Y1H)

- Y1H with highly conserved R1 motif (Fig. 1)
  ➔ Identify binding TFs
- Screening against large *Arabidopsis* library (+-2000 TFs) with three replicates
- 9 interacting TFs were found in all screens

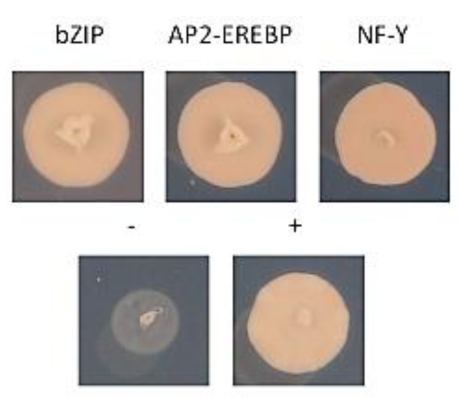


Figure 2. Three positive interactions (Y1H), next to a negative and positive control.

2b. Motif characterization - CRISPR/Cas12a mutagenesis

- A construct with 6 different gRNAs was made for each target (R2 & R3)
- Stable transformation with *Agrobacterium* in tomato
- Create large deletions to see effect on phenotype / expression level

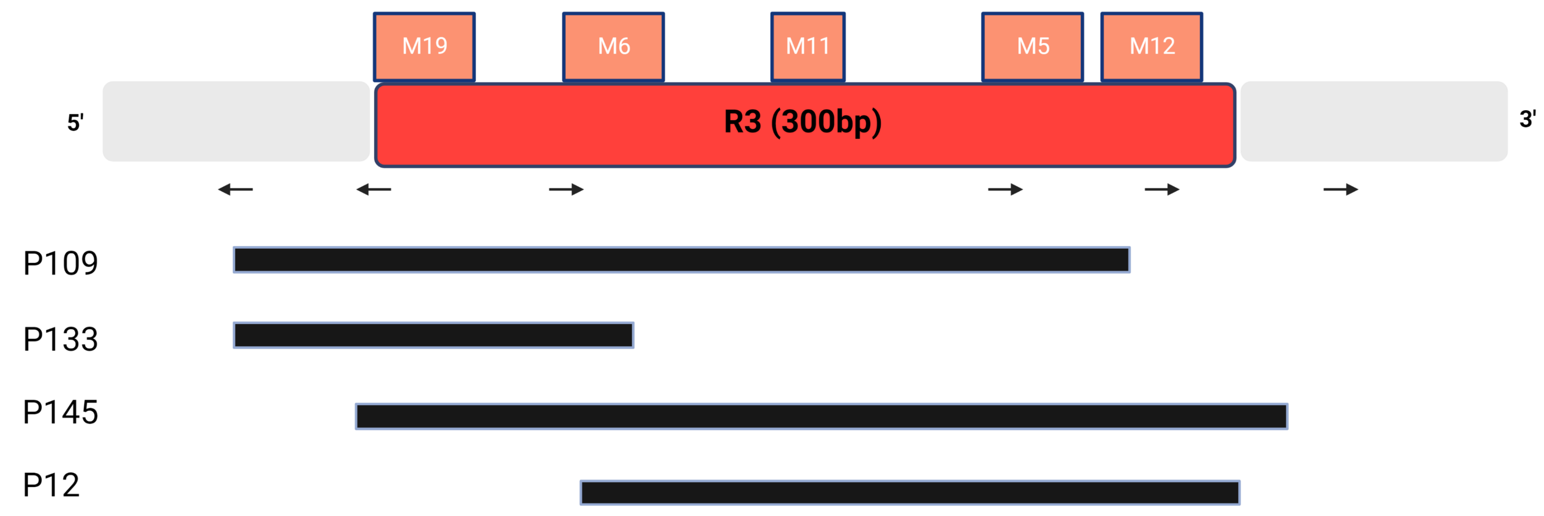


Figure 3. CRISPR/Cas12a mutagenesis of conserved region 3 in the promoter of *FUL2*. The obtained deletions are depicted in black bars and the gRNAs are shown as arrows.[1]

2c. Motif characterization – GUS promoter reporter lines

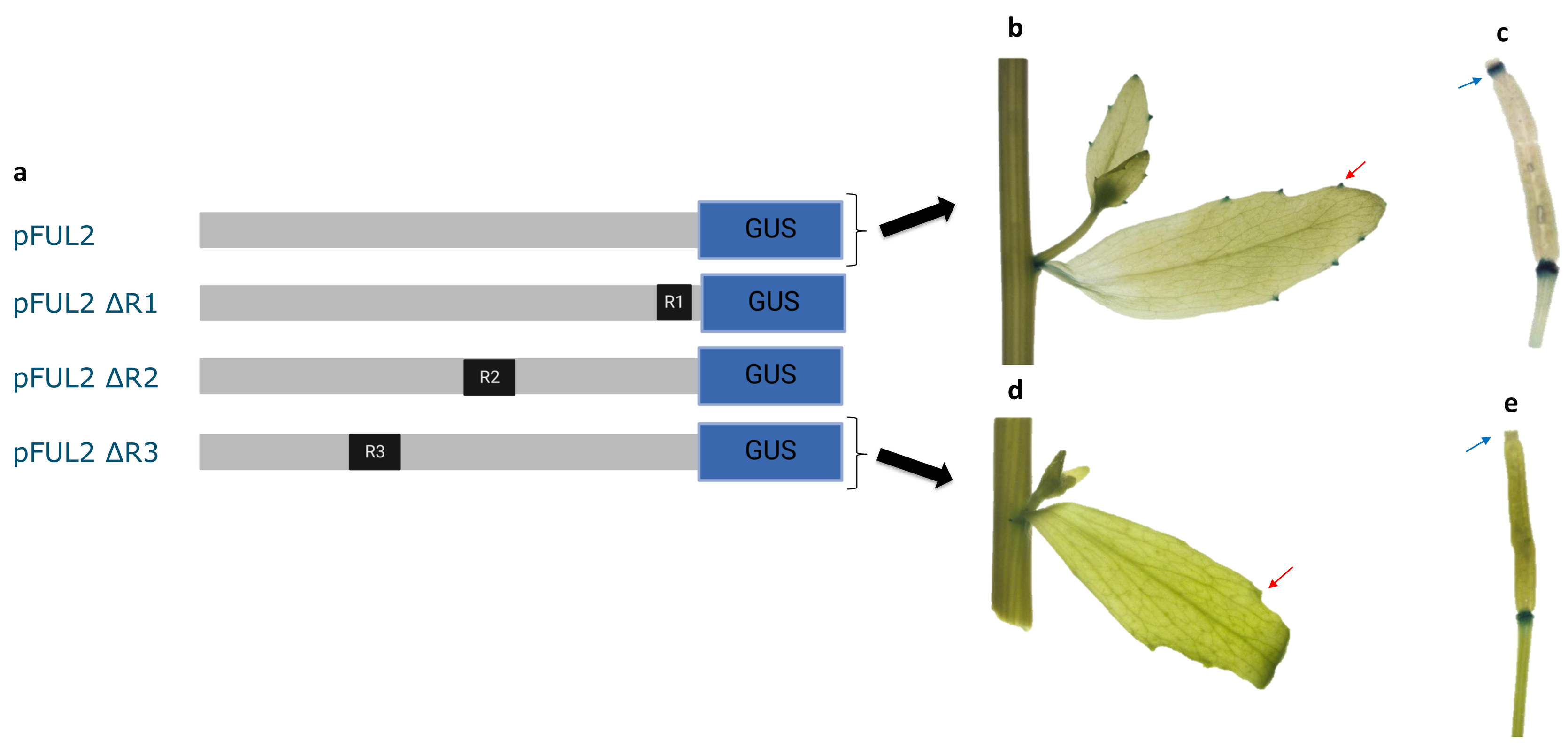


Figure 4. a, Four different promoter reporter lines **b-e**, GUS staining patterns for pFUL2 and pFUL2ΔR3 in the cauline leaves (**b,d**) and siliques (**c,e**). The red arrows mark the cauline leaf teeth and the blue arrows mark the style.[1]

- Four promoter reporter constructs were made in Arabidopsis to study the importance of three conserved regions (heterologous system)
- Similar expression patterns were observed for pFUL2, ΔR1 and ΔR2
- pFUL2ΔR3 lost expression in the cauline leaf teeth and in the style

  ➔ Tissue specific CREs are present in conserved Region 3

Conclusions and future perspectives

- *In silico* analysis revealed conserved motifs in the promoter of *FUL2*
- TFs interacting with conserved motif R1 were found with Y1H
- CRISPR/Cas12a mutants ➔ phenotyping + expression analysis
- pFUL2 reporter lines ➔ region 3 contains tissue-specific CREs
  - How does this translate into tomato?

References

[1] Created with BioRender.com

[2] Timothy L. Bailey and Charles Elkan, "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology, pp. 28-36, AAAI Press, Menlo Park, California, 1994.

[3] Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. VISTA: computational tools for comparative genomics. Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W273-9

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