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OVEREXPRESSION OF THE MOLECULAR TARGET OF APPLE PROLIFERATION PHYTOPLASMA EFFECTOR IN APPLE

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INTRODUCTION

Apple Proliferation (AP) is a severe disease widespread in apple-growing areas within Europe. Typical symptoms comprise shoot proliferation, small leaves with altered shape and undersized, tasteless and colorless fruits. Causal agent of the disease is the phytoplasma '*Candidatus* phytoplasma mali' ('*Ca*. P. mali'). The genome analysis of different strains of '*Ca*. P. mali' revealed the presence of one effector, **SAP11CaPM** (Siewert *et al.*, 2014), which has been observed to target at least two apple class II **TCP transcription factors** (TF), namely **MdTCP24** and **MdTCP25** (Janik *et al.*, 2017)

The SAP11-TCP binding leads to the **deactivation of the TFs** (Sugio *et al.*, 2011), although direct molecular evidences of the symptoms induction by the TFs deactivation are still lacking.

Goal of this work was to induce a stable **overexpression of MdTCP25** in apple to shed light on the role of this gene in the apple physiology and in the development of the disease.

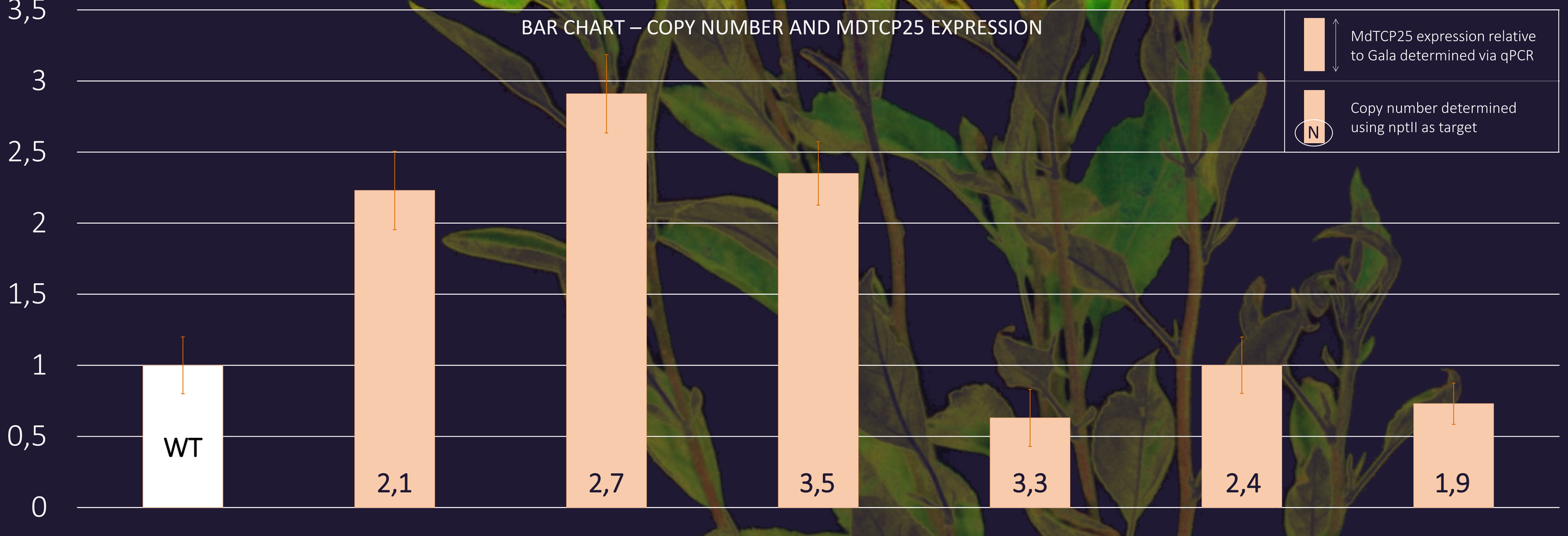
MATERIALS AND METHODS

The full length MdTCP25 gene was subcloned in the destination vector pK7WG2.D for *in planta* expression (35s promoter, KanR as selection marker) used for *A. Tumefaciens*-mediated transformation of apple, Cv. Gala. After the incubation with *Agrobacterium*, the leaves were put in regeneration on Kanamycin-containing media for several months.

Plantlets emerged from transformed calluses were propagated and chosen for further analyses: DNA was extracted and amplified using Agrobacterium-specific primers to exclude an ongoing infection and vector's backbone/ TCP-specific to confirm the successful transformation.

RNA was extracted from the leaves of 6 independent lines, retrotranscripted and used for MdTCP25 expression profiling. Ub and EF1 were chosen as housekeeping genes. Results, normalized such that the Wild Type expression is equal to 1, are displayed in the graph below

Confirmed transgenic lines were resampled and DNA was extracted from leaves. The number of copies of 35S::MdTCP25 integrated in the genome was determined as described by Dalla Costa and collaborators (2019) using the marker gene nptII as target. Results are displayed in the graph below.



In the bar chart is plotted the expression of MdTCP25 in transgenic lines calculated via qPCR, compared to the wild type (white bar). Error bars are calculated on biological and technical triplicates. The expression was normalized on two housekeeping genes (Ubiquitin and Elongation Factor 1). **The purple numbers** inside the bars represent the copy number for each line.
In the picture below the transgenic acclimatized lines are shown compared to the Wild Type Gala (first plant on the left).

Ca. P mali (AP-MLO) symptoms, where the typical ''witches brooms'' and enlarged stipules are clearly visible.

Photo by Biologische Bundesanstalt für Land- und Forstwirtschaft

CONCLUSIONS AND FUTURE PERSPECTIVES

The expression of TCP25 in transgenic lines is lower than expected and, in some cases, even lower than the basal WT expression. Given that the TCP family is comprised of more than 50 genes regulated at a very fine level, we suppose that only individuals with a low level of overexpression were able to survive.
Nonetheless, a slight change in the TCP25 expression seems to be sufficient to induce some phenotype effects: the MdTCP25 overexpressing plants tend to display loss of apical dominance, smaller leaves and shorter stem compared to the Wild Type.
Some selected transgenic lines are currently being infected with *Ca*. P. Mali *via* micrografting and an extensive analysis of their expression will follow.

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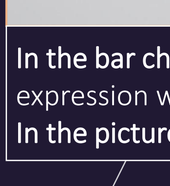

Thank you very much for reading my poster!
For any comment or question you are very welcome to contact me at:
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Have a nice day ☺

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