Mapping *in planta* Expression of Type III Effectors of the Bacterium *Acidovorax citrulli*

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Introduction

Bacterial Fruit Blotch (BFB) of cucurbits is a disease caused by the bacterium *Acidovorax citrulli*, which mainly affects melon and watermelon plants. There is a lack of knowledge about the pathogenesis of the bacterium. *Acidovorax citrulli* uses a type III secretion system (T3SS) to secret effectors into the plant cell. These type III-secreted effectors (T3Es) influence the plant cell metabolism and suppress defense responses. The expression of some T3E genes is regulated by the transcription factors HrpG and HrpX. Our hypothesis is that the expression levels of T3Es depend on the type of plant organ and the infection stage.

Methods

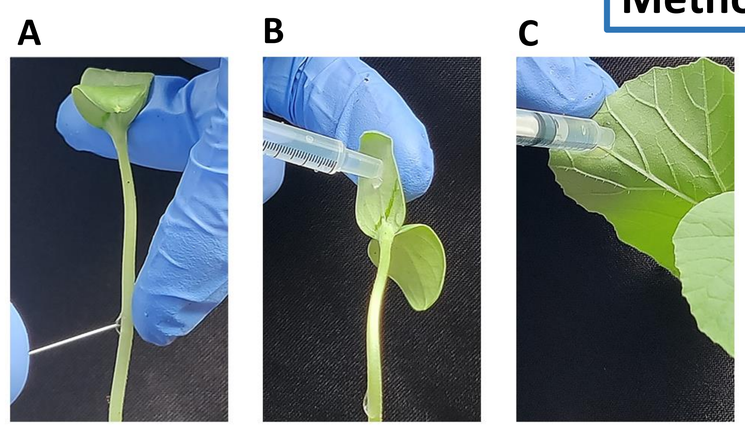

Fig 1. Inoculation methods. A, hypocotyl; **B**, cotyledon; and **C**, leaf.

Expression of T3E genes was measured by qRT-PCR. The Relative Quantification (RQ) was determined as:

$$RQ\ \Delta\Delta CT = 2^{-(\Delta CT_{treatment} - \Delta CT_{control})}$$

Cycle Threshold (CT), calculated for each gene; ΔCT - calculated between the target gene and the housekeeping gene *APS58_1610* encoding GAPDH. Gene expression was assessed from bacteria infecting different plant organs at different times. The reference was gene expression in bacteria grown in a rich medium (Nutrient Agar, NA).

Objective 1

To optimize the extraction of bacterial RNA from plant tissue for assessment of bacterial gene expression *in planta*.

Determination of optimal bacterial concentrations

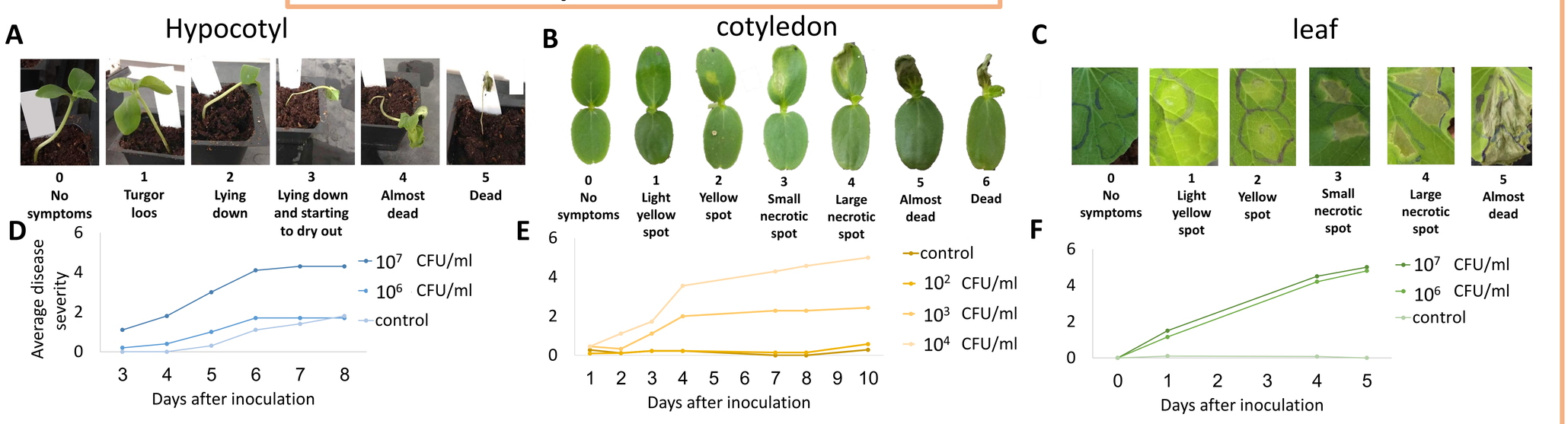

Fig 2. Assessment of various bacterial concentrations on different plant organs. Disease severity scales following inoculation of: **A**, hypocotyl; **B**, cotyledons; and **C**, leaves. **D**, **E** and **F** show progress of disease severity in the different assays following inoculation with different bacterial concentrations (controls were plants treated with 10 mM $MgCl_2$).

Objective 2

To map the RQ of selected *A. citrulli* T3E genes at different times of infection of hypocotyl, cotyledons and leaves of melon plants.

Mapping *in planta* RQ of T3E genes

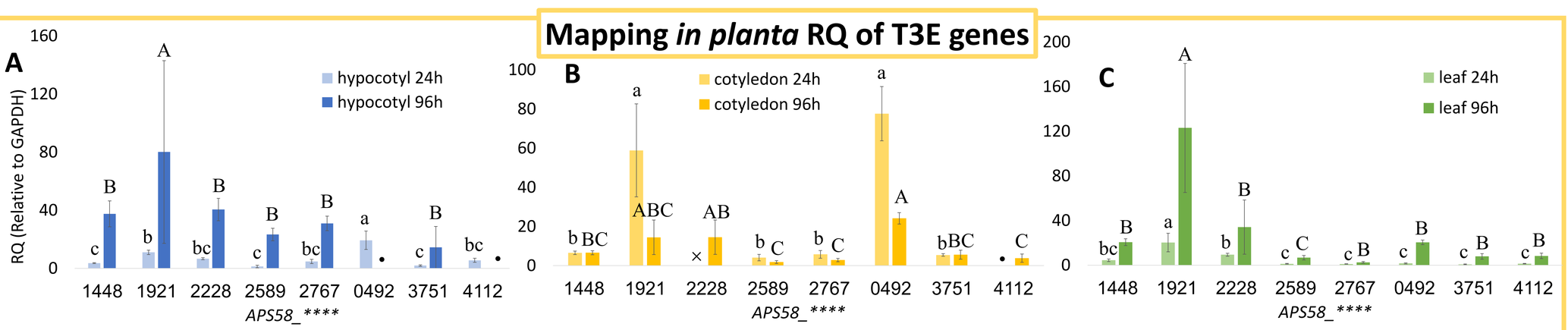

Fig 3. RQ of T3E genes in plant organs. A, hypocotyl; **B**, cotyledons; and **C**, leaves. RQs were determined 24 and 96 h after inoculation. Different letters indicate statistically significant differences among T3E genes in given organ, at the same time, Student's t test (p<0.05). Gene IDs, *APS58_***** where ****** is the ID no.

Objective 3

To compare results on expression of genes of *A. citrulli* grown in minimal medium (XVM2) with previous results based on RNAseq (Jiménez -Guerrero et al., 2020) and to examine the correlation of T3E gene expression under regulation of HrpX *in vitro* versus *in planta*.

Comparative analyses of expression of T3E genes, *hrpX* and *hrpG*

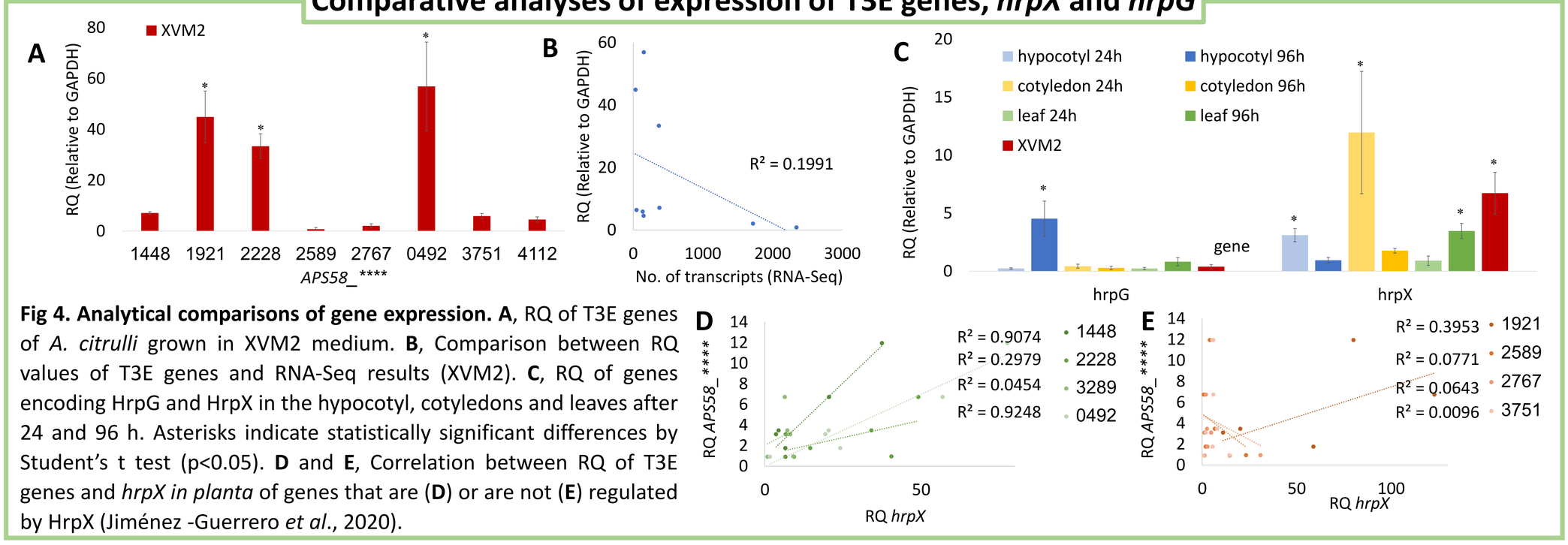

Fig 4. Analytical comparisons of gene expression. A, RQ of T3E genes of *A. citrulli* grown in XVM2 medium. **B**, Comparison between RQ values of T3E genes and RNA-Seq results (XVM2). **C**, RQ of genes encoding HrpG and HrpX in the hypocotyl, cotyledons and leaves after 24 and 96 h. Asterisks indicate statistically significant differences by Student's t test (p<0.05). **D** and **E**, Correlation between RQ of T3E genes and *hrpX in planta* of genes that are (**D**) or are not (**E**) regulated by HrpX (Jiménez -Guerrero *et al.*, 2020).

Conclusions

1. There is variation between *A. citrulli* T3Es in terms of gene expression in different melon organs.
2. High expression was detected for *APS58_1921* in all organs. Relatively high RQ was also seen for *APS58_2228* and *APS58_0492*.
3. The RQ of most tested genes in hypocotyl and leaves were higher at 96 than at 24 h, while an opposite picture was seen in cotyledons.
4. The patterns of HrpX-regulation detected in the *in vitro* experiment (Jiménez -Guerrero et al., 2020) were similar in planta.
5. The expression of HrpX-regulated genes positively correlated with expression levels of *hrpX*.
6. Different methods for assessment of RNA expression (eg, RNA-seq and qRT-PCR) might provide different pictures. .

Reference

Jiménez-Guerrero, I., et al. (2020). Show me your secret(ed) weapons: a multifaceted approach reveals a wide arsenal of type III-secreted effectors in the cucurbit pathogenic bacterium *Acidovorax citrulli* and novel effectors in the *Acidovorax* genus. Mol. Plant Pathol. *21*, 17–37.