

Is *SIX1* an effector in the *Fusarium oxysporum* f.sp. *cabense*-banana interaction ?

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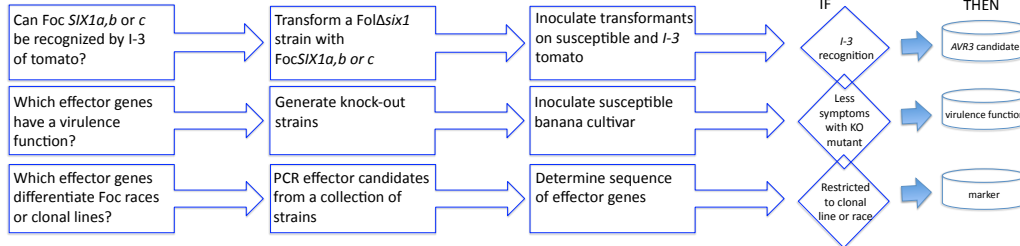


Introduction

Fusarium oxysporum f.sp. *cabense* (Foc) is the causal agent of Panama disease in banana. Foc has evolved into several different races (right panel). In the *Fusarium oxysporum* f.sp. *lycopersici* (Fol)-tomato system, small secreted proteins (Six, Secreted in Xylem) can act as virulence factors, but may also trigger resistance in the host. In tomato, three resistance genes are known, *I-1*, *I-2* and *I-3*, and the corresponding AVR genes *SIX4*(AVR1), *SIX3*(AVR2), and *SIX1*(AVR3) have been identified. In Foc, however, little is known about the effectors necessary to infect banana. We identified 11 candidate effectors in the genome sequence of a Foc TR4 strain (II5, sequenced and annotated by the Broad Institute) based on homology to *SIX* genes and other characteristics. Here we present results of our initial characterization of these candidate effector genes.

Foc Races	Host
Race 1 (R1)	Banana var. Gros Michel, Lady Finger, Ducasse, Sugar, Silk, but not Cavendish
Race 2 (R2)	Banana var. Bluggoe and Blue Java, but not Cavendish
Race 3	Heliconia, not bananas
Sub-Tropical Race 4 (ST4) (under specific abiotic condition)	Cavendish and other banana varieties
Tropical Race 4 (TR4)	Cavendish and other banana varieties

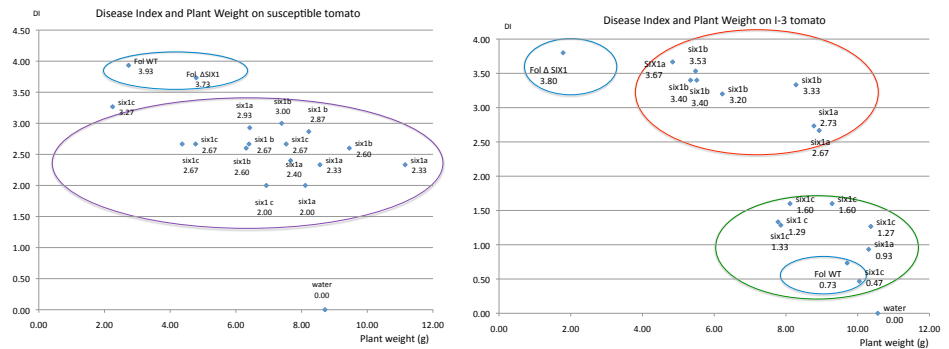
Research Pipeline



Foc effector candidates *SIX1c* and possibly *SIX1a* can be recognized by the *I-3* resistance gene of tomato

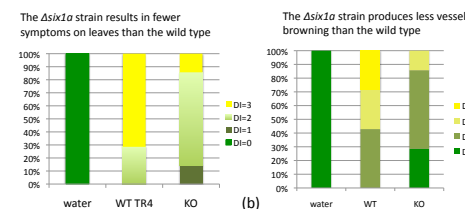
RIGHT: Several different transformants of Fol + FocSIX1a,b,c were generated. To test their pathogenicity those transformants were inoculated on I-3 tomato and also on susceptible tomato. The wild type strain (WT), the Δ six1 strain and water were also included as a control. Initial results show that *SIX1c* and possibly *SIX1a* can complement for avirulence, indicated by higher fresh weight on I-3 plants accompanied by lower disease index.

Blue circles indicate the control strains, the red circle indicates the transformants unrecognized by I-3, and the green circle indicates transformants that are recognized by I-3. Transformants that show reduced virulence on the susceptible plant line are indicated by purple circle. Each dot is the average of 15 plants. DI = Disease Index



The Foc *six1a* knock out mutant is compromised in virulence on banana

We inoculated susceptible Cavendish cv. Grande Naine with Foc TR4 wild type strain II5 and a Foc Δ six1a mutant. We observed symptoms internally by looking at brown vessels in the corm and externally by looking at yellowing, necrosis and wilting of the leaves.



DI=0, if % of yellowing, wilting and necrotic leaves = 0 %
 DI=1, if % of yellowing, wilting and necrotic leaves ≤ 25%
 DI=2, if 25% ≤ % of yellowing, wilting and necrotic leaves ≤ 50%
 DI=3, if 50% ≤ % of yellowing, wilting and necrotic leaves ≤ 100%

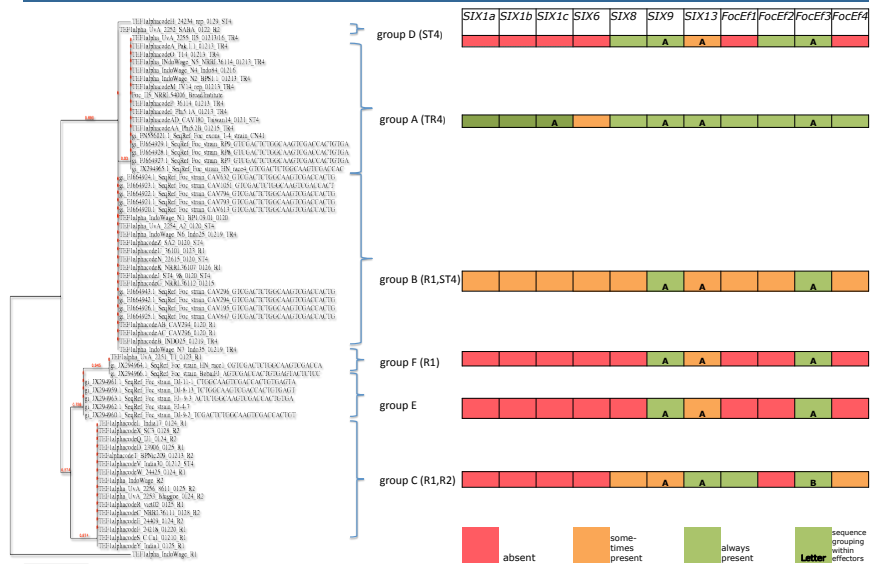
DI=0, if length of vessel browning in corm 0 – 0.5 cm ;
 DI=1, if 0.5cm-length of vessel browning in corm ≤ 1cm ;
 DI=2, if 1cm-length of vessel browning in corm ≤ 2cm ;
 DI=3, if 2cm-length of vessel browning in corm ≤ 3cm

Visual symptoms were measured as disease index at 13 weeks after inoculation.

Outlook

- RNA sequencing to identify more candidate effectors
- Gene knock-out and complementation of the candidate effectors
- Develop marker specific to a particular race of Foc

Foc *SIX1a*, *b* and *c* appear to be restricted to the TR4 clonal line whereas Foc *SIX9* and *FocE3* are present in all clonal lines



Six different TEf1a sequences were found in Foc isolates, assumed to represent clonal lines. The presence of candidate effectors in these clonal lines is shown to the right

Conclusions

- ✓ Some of the FocSIX1 transformants are recognized by the I-3 gene in tomato, indicating that Six1 homologs from Foc can have an avirulence function.
- ✓ The *SIX1a* knock-out mutant showed less virulence, suggesting that Six1a has a virulence function during banana infection.
- ✓ Foc *SIX1a*, *b* and *c* appear to be restricted to the TR4 clonal line.

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