Sorghum stalk rot pathogens affect transcription of key genes involved in chlorophyll metabolism of a non-staygreen, disease susceptible genotype.

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INTRODUCTION

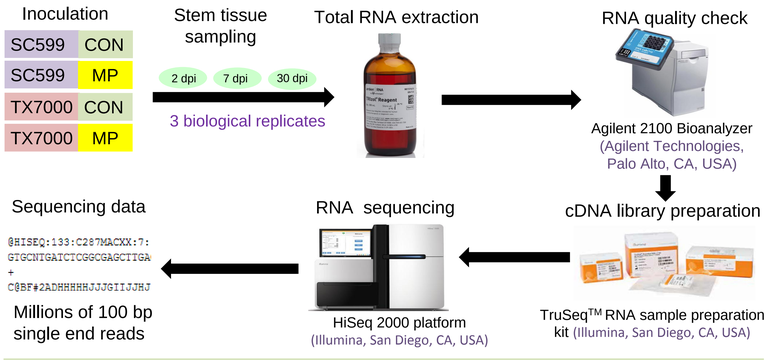
Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop in the world. It is the staple for millions of people, perticularly in the sub-saharan African countries. Fusarium stalk rot (*Fusarium thapsinum*, FT) and charcoal rot (*Macrophomina phaseolina*, MP) are high priority sorghum diseases worldwide. They cause tremendous crop loss wherever sorghum is grown. Although experimental evidence is limited, delayed post-flowering senescence due to the staygreen trait is accepted as a physiological means of stalk rot resistance. Staygreen is defined as the ability of certain genotypes to retain green leaves and stalks through physiological maturity. Staygreen trait has been shown to be highly correlated with leaf chlorophyll content (measured by a soil and plant analytical development meter, SPAD). We hypothesized that stalk rot pathogens can decrease stalk chlorophyll content. We also hypothesized that staygreen genotypes are more resilient to stalk rot-mediated chlorophyll degradation (conditional on first hypothesis). Moreover, we suspect that stalk rot pathogens can affect leaf chlorophyll content. Here, we used SC599 (stalk rot resistant, staygreen) and TX7000 (stalk rot susceptible, non-staygreen) sorghum lines to test our hypotheses. We used data from our recently conducted RNASeq experiment to make inferences on the first two hypotheses at the gene expression level, while a dedicated field experiment was used to investigate the third hypothesis.

OBJECTIVES

(i) Investigate the differential expression of chlorophyll metabolism-related genes among staygreen and non-staygreen sorghum lines in response to FT and MP inoculation and (ii) to study pathogen effects on leaf chlorophyll content (measured by Soil and Plant Analytical Development meter, SPAD) at three maturity stages.

MATERIALS AND METHODS

A) RNASeq experiment



- Adapter trimming and quality filtering with “Cutadapt”
- Read mapping to the *Sorghum bicolor* reference genome using “GSNAP”
- Normalization and quantification of read counts
- Differential gene expression analysis between MP and control at each DPI and line using “DEseq2” with multiple testing correction, 5% FDR
- Metabolic pathway analysis using information from the “SorghumCyc” genome database

B) Field experiment

- **Treatment structure** – 2 × 3 × 3 factorial (sorghum genotype, inoculation treatment, and maturity stages at which SPAD measurements were acquired)
- **Design structure** – split-plot with randomized complete blocks (WPF = genotypes, SPF = pathogens with control treatment)
- **Inoculation** – at 14 d after flowering, 3 subsample plants in each sub plot by injecting 0.1 mL of conidia (FT) or mycelial fragments (MP) (concentration = 2×10^6 mL^{-1}) into the basal node of the stalk. Phosphate-buffered saline (pH 7.2) was used as the mock-inoculated control treatment.
- **Measurement of leaf chlorophyll content** – SPAD of the flag leaf at soft dough, hard dough, and physiological maturity stages at 7, 19, and 30 d after inoculation, respectively.
- **Statistical analysis** – SAS software, version 9.2 (SAS Institute, 2008), Analysis of variance using the PROC GLIMMIX procedure. As SPAD measurements were repeated measures, a time series analysis was conducted by modeling data for the most suitable variance-covariance structure. The most parsimonious model was obtained when data were modeled with first order antedependence covariance structure.

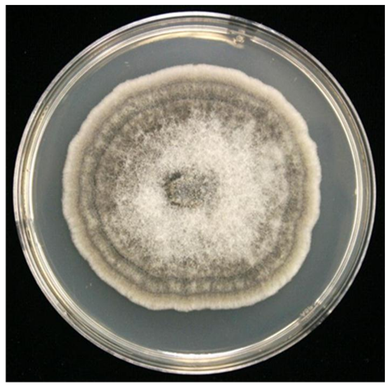


Fig 1. Morphology of *Macrophomina phaseolina* on potato dextrose agar

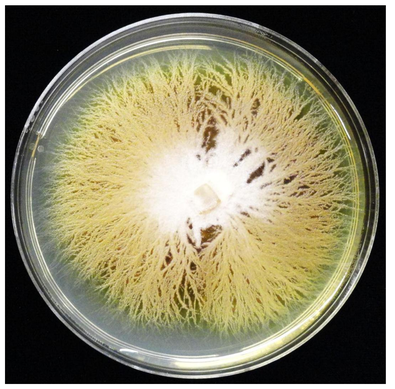


Fig 2. Morphology of *Fusarium thapsinum* on potato dextrose agar

Fig 3. Inoculation of sorghum plants using the injection method (see Materials & Methods)

Fig 4. Measuring leaf SPAD (see Materials & Methods)

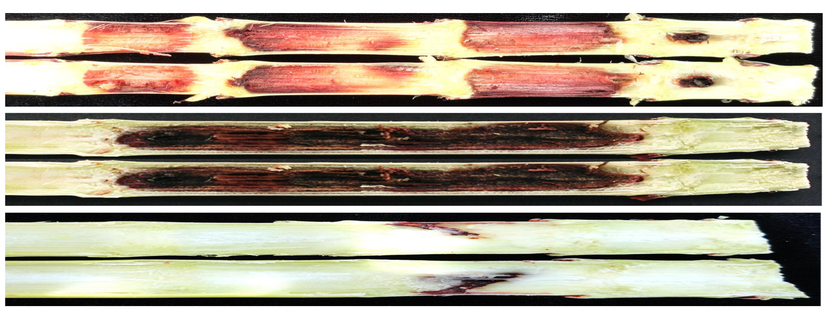


Fig 5. Typical lesions associated with Fusarium stalk rot (top panel), charcoal rot (middle panel), and mock-inoculated control (bottom panel).

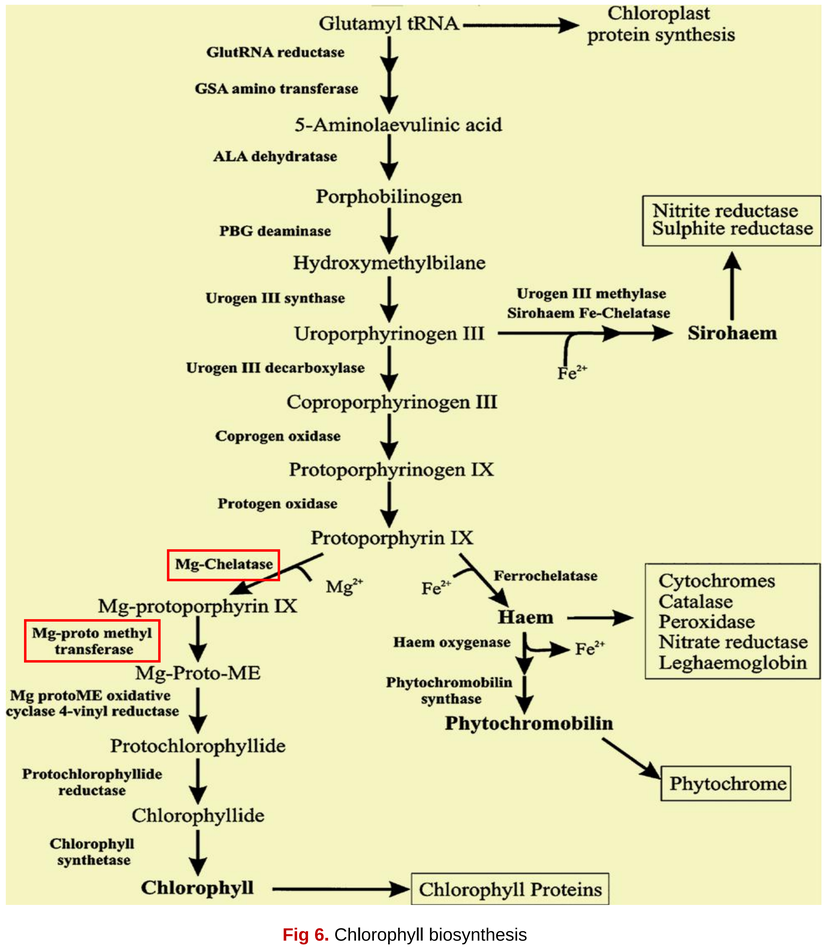


Fig 6. Chlorophyll biosynthesis

RESULTS

- At 7 d.p.i., a gene responsible for chlorophyll degradation, chlorophyllase-2 (*Sb02g012300*) was significantly upregulated in Tx7000 in response to MP while two key genes involve in chlorophyll biosynthesis, magnesium-protoporphyrin O-methyltransferase (*Sb10g002100*) and magnesium-chelatase (*Sb08g004300*) were significantly down-regulated in response of both pathogens.

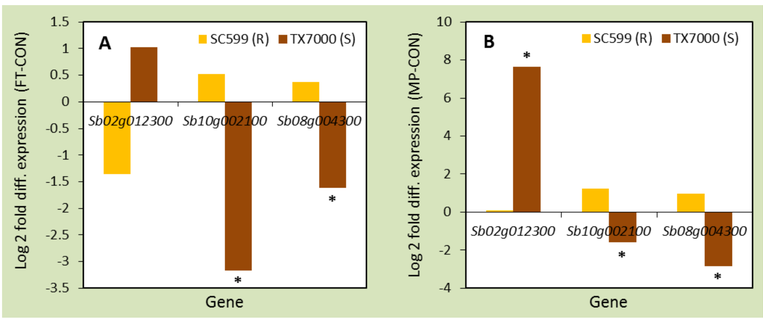


Fig 7. Log 2 fold differential expression of chlorophyllase-2 (*Sb02g012300*), magnesium-protoporphyrin O-methyltransferase (*Sb10g002100*), and magnesium-chelatase (*Sb08g 004300*) under (A) *F. thapsinum* and (B) *M. phaseolina* infection.

- Field experiment conducted in relation to the second objective did not reveal a significant SPAD reduction with TX7000 compared to SC599 at soft dough, hard dough, and physiological maturity upon inoculation

Table 1. *P*-values of F tests from analysis of variance (ANOVA) for SPAD readings measured at soft dough, hard dough, and physiological maturity stages; as measured with two sorghum genotypes after inoculation with *F. thapsinum* and M. phaseolina (α = 0.05). .

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	1	12.66	0.02	0.8820
Pathogen	2	12.66	19.84	0.0001
Genotype × Pathogen	2	12.66	0.17	0.8489
Growth stage	2	23.59	2.74	0.0853
Genotype × Growth stage	2	23.59	3.24	0.0570
Pathogen × Growth stage	4	23.80	2.27	0.0918
Genotype × Pathogen × Growth stage	4	23.80	0.96	0.4469

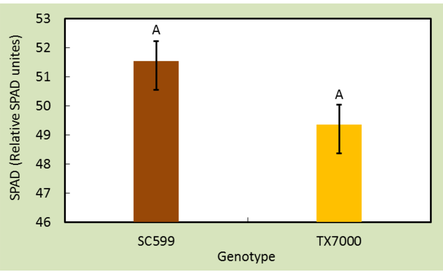


Fig 8. Comparison of the effects of genotype on SPAD (± standard error) across tested pathogens and growth stages using Tukey-Kramer’s test. Means followed by the same letters are not significantly different based on the adjusted *P*-value for multiple comparisons at α = 0.05.

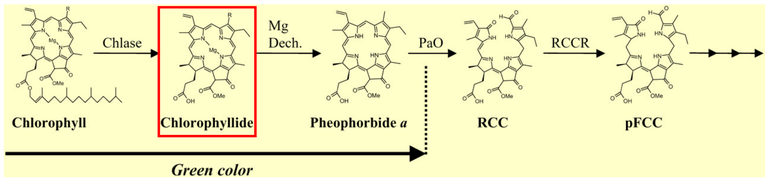


Fig 9. Chlorophyll catabolic pathway. A central reaction in chlorophyll catabolism is the opening of the porphyrin ring of “pheophorbide *a*” to form the primary fluorescent chlorophyll catabolite (pFCC). This requires Pheophorbide a oxygenase (PaO) and red chlorophyll catabolite reductase (RCCR), with red chlorophyll catabolite (RCC) as a presumably PaO-bound intermediate.

CONCLUSIONS

- Pathogens induce chlorophyll catabolism and suppress chlorophyll anabolism of the non staygreen, disease susceptible genotype, Tx7000.
- The staygreen genotype, SC599, is more resilient to stalk rot-mediated chlorophyll degradation.
- Transcriptional data were not reflected in the actual leaf SPAD data measured in the field.
- It is likely that Tx7000 leaves remain green under pathogen-induced chlorophyll degradation as chlorophyllide, the result of chloro-phyllase-2-mediated chlorophyll degradation, may contribute to the maintenance of leaf greenness.

ACKNOWLEDGEMENTS