Characterization of transgenic *Sorghum bicolor* overexpressing *SbFNR* via quantitative real-time PCR and metabolites profiling

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Introduction

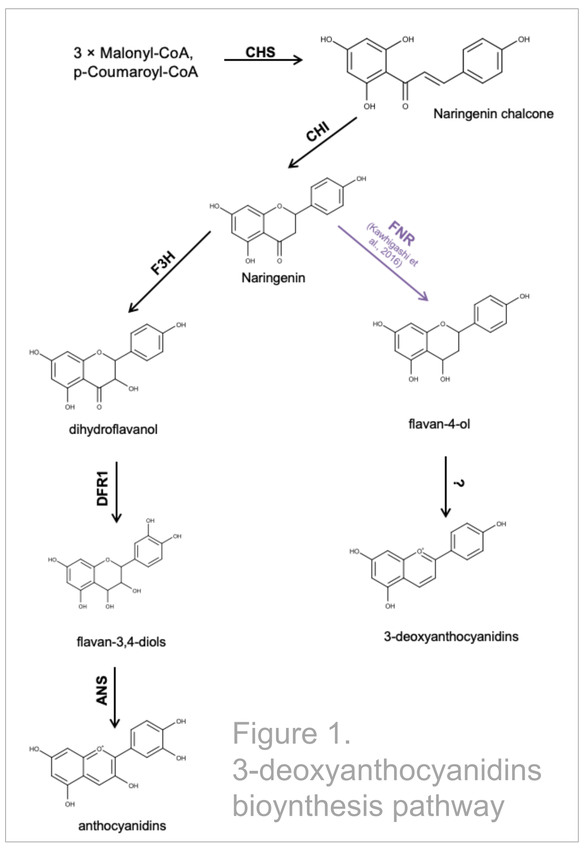

Figure 1. 3-deoxyanthocyanidins bioynthesis pathway

3-Deoxyanthocyanidins are a small group of phytochemicals that have been demonstrated to inhibit the proliferation of several cancer cell lines as well as the activities of some carcinogens. In response to mechanical wounding and fungal infection, some plants such as sugarcane, maize and sorghum, rapidly synthesize 3-deoxyanthocyanidins as phytoalexins. For instance, 3-deoxyanthocyanidins are known to exhibit antifungal properties against *Colletotrichum sublineolum*. *C. sublineolum* is a type of fungus that causes anthracnose, one of the most prevalent fungal diseases that limits the crop yield of sorghum. This is particularly unfavorable to the countries growing sorghum as a staple crop such as Ethiopia (Tsedaly et al., 2016). Thus, enhancing 3-deoxyanthocyanidin biosynthesis in sorghum may improve its resistance against anthracnose and thereby crop yield. Previously, a natural sorghum mutant defective in the *SbFNR* gene (which encodes a flavanae-4-reductase or FNR) was reported to not accumulate 3-deoxyanthocyanidins, indicating that SbFNR is likely involved in 3-deoxyanthocyanidin biosynthesis (Kawhigashi et al., 2016). Based on these premises, transgenic sorghum lines overexpressing the *SbFNR* gene were generated in collaboration with Professor Ian Godwin's research team from the University of Queensland. The objective of this study is to further characterize these transgenic lines by qRT-PCR and metabolites profiling.

Materials and Method

qRT-PCR for confirmation of overpression

Total RNA of *S. bicolor* overexpressing *SbFNR* was extracted from the leaves according to the manufacturer's instructions. Reverse transcription of mRNA was performed using PrimeScript RT reagent kit with gDNA eraser from Takara. Any genomic DNA that might be present in the sample RNA was removed using a gDNA eraser at 42°C for 2 minutes. Then the sample RNA was converted to cDNA using RTase. *In planta* expression of *SbFNR* in *S.bicolor* was then examined by quantitative RT-PCR using ABI StepOne Plus Real-Time PCR system and a EIF4a housekeeping gene was used as an internal control.

Metabolites profiling of OE-SbFNR sorghum

Approximately 50mg of leaves of OE-SbFNR sorghum and Tx430 for control were collected from the greenhouse. The leaves were lysed in a solution containing methanol then ultrasonicated in an ice water bath for 30 minutes. The supernatants were obtained by centrifugation at 10,000 rpm for 15 minutes and 50µl of them were transferred to 1.5ml test tubes containing 50µl of 2N HCl for acid hydrolysis. The suspensions were then subjected to analysis in a HPLC-qTOF-HRMS machine.

Figure 2. HPLC-qTOF-HRMS Machine

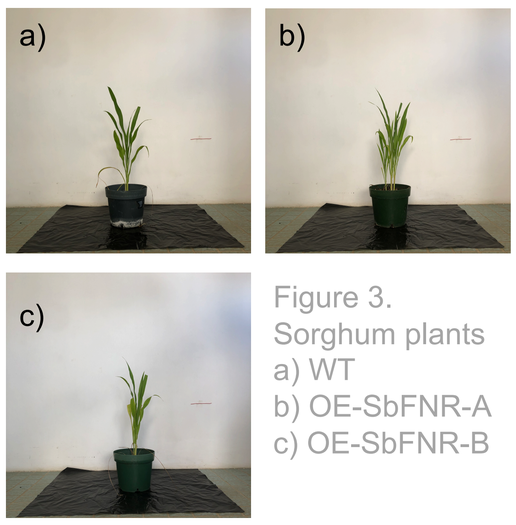

Figure 3. Sorghum plants
a) WT
b) OE-SbFNR-A
c) OE-SbFNR-B

Acknowledgement: I would like to thank Dr. Clive Lo, Mr. Andy Lui and Professor Ian Godwin's research team for their support and help throughout the project.

Results and Discussion

qRT-PCR results

qRT-PCR was performed to confirm the overexpression of *SbFNR* in transgenic sorghum. The fold change of mRNA expression level was calculated using the $2^{-\Delta\Delta_{CT}}$ method. The results confirmed that transgenic sorghum plants had successfully overexpressed *SbFNR*.

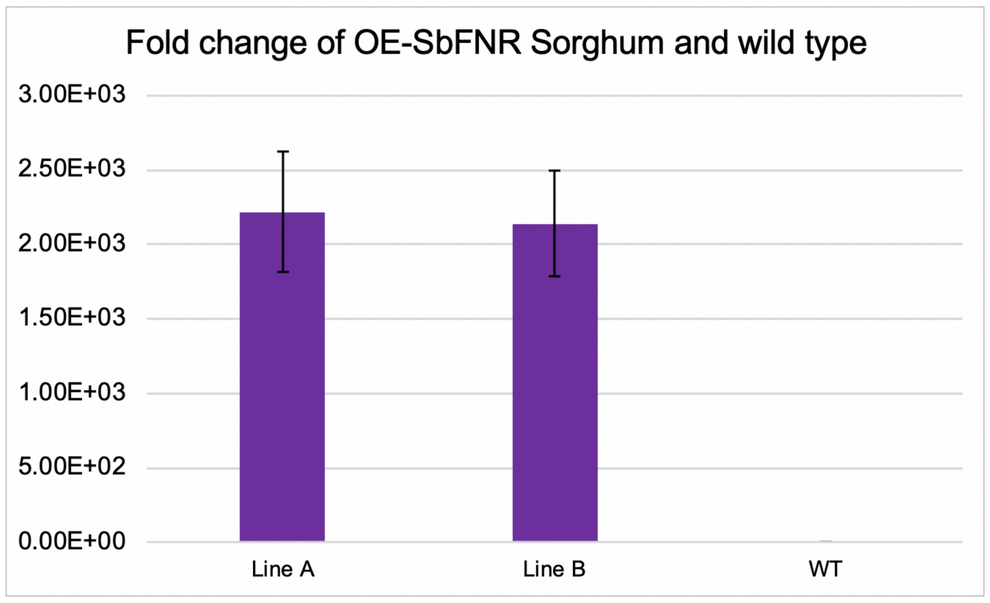

Figure 4. Fold change of OE-SbFNR Sorghum and wild type

HPLC-MS/MS Data of metabolites profiling

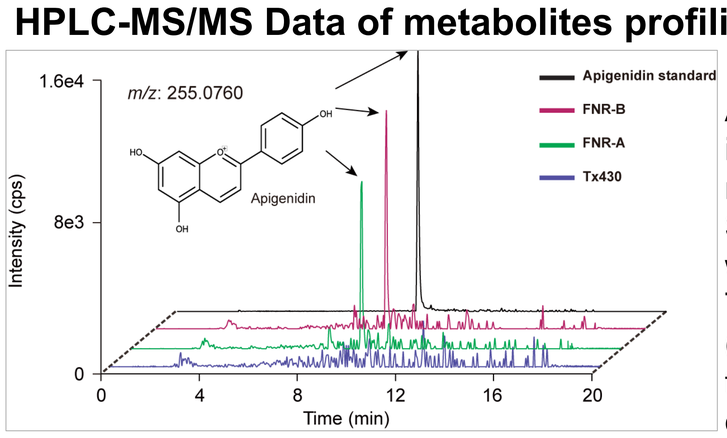

Figure 5. HPLC-MS/MS detection of apigeninidin

Apigeninidin was detected in the acid hydrolyzed metabolite extracts of OE-SbFNR-A and OE-SbFNR-B while it was not detected in Tx430 metabolite extracts (Figure 5). This suggests that transgenic sorghum overexpressing SbFNR accumulates apiforol and FNR is capable of converting naringenin into apiforol, which is transformed into apigeninidin by an unknown enzyme in the later step. Acid hydrolysis was necessary to replace the function of this unknown enzyme for the detection of 3-deoxyanthocyanidins. Additionally, apigeninidin instead of apiforol was subjected to analysis because standard flavan-4-ols are not commercially available due to their chemical instability, making it difficult to detect the peaks of flavan-4-ols.

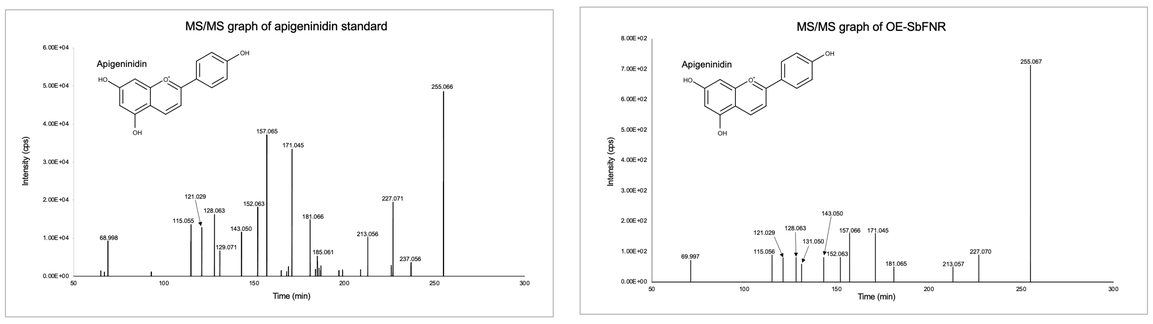

Figure 6. MS-fragmentation pattern graphs of apigeninidin standard and apigeninidin in OE-SbFNR Sorghum

The identification of a chemical compound producing a peak in OE-SbFNR-A and OE-SbFNR-B was done by comparing its retention time and respective MS-fragmentation pattern to an apigeninidin standard.

Conclusion

The overexpression of the target gene in transgenic sorghum plants was evident in the qRT PCR results and the accumulation of apigeninidin was detected in OE-SbFNR sorghum plants via metabolites profiling, indicating that FNR enzyme is responsible for the primary step of 3-deoxyanthocyanidins synthesis pathway. The future prospects of this study would be to test for the resistance to abiotic stresses and fungus of OE-SbFNR sorghum plants.

References

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