Characterizing Promoters for Terpene Production in Sorghum

Arkesh Das[1], Angel McKay Whiteman[2], Trine Andersen[3], Björn Hamberger[4]

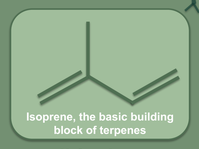
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA dasarkes@msu.edu[1], mckayang@msu.edu[2], ande2202@msu.edu[3], hamberge@msu.edu[4]

GREAT LAKES BIOENERGY RESEARCH CENTER

BIOCHEMISTRY & MOLECULAR BIOLOGY

What are Terpenes?

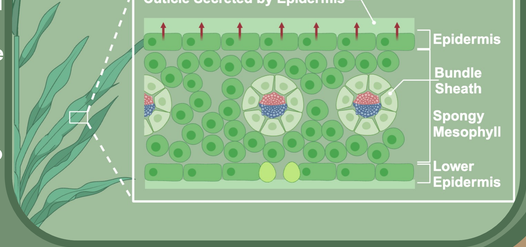
Terpenes are a class of organic molecules that come in all different shapes and sizes. Certain terpenes have applications in a variety of industries, from cosmetics to medicine, so there's is a large demand for these compounds [7]. Chemical synthesis of terpenes is often expensive and sometimes impossible; however, an alternative to chemical synthesis is extraction from plants.



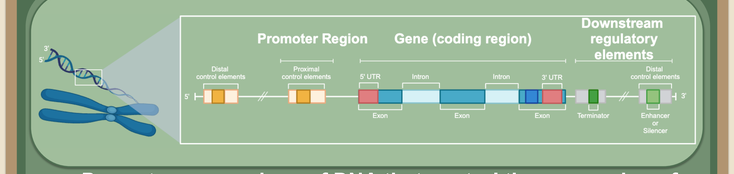
Isoprene, the basic building block of terpenes

Sorghum and Terpenes

Sorghum is a grass in the same family as corn that is commonly used as animal feed and as a source of biofuels. The *epidermis,* or 'skin' of sorghum secretes a waxy layer called the *cuticle*. The cuticle contains *cutin,* a lipid that protect sorghum and make its leaves and stalk hydrophobic. Like many other plants, the cuticle of sorghum contains terpenes. Plants naturally produce terpenes as a means of defense, attracting pollinators, and even communication. The cuticle of sorghum contains terpenes such α/β - amyrin and hopane, which have anti-microbial and anti-inflammatory properties [5].



What are Promoters?



Promoters are regions of DNA that control the expression of nearby genes. They are recognized by transcription factors in the nucleus which cause those genes to be expressed[8].

Project Goals

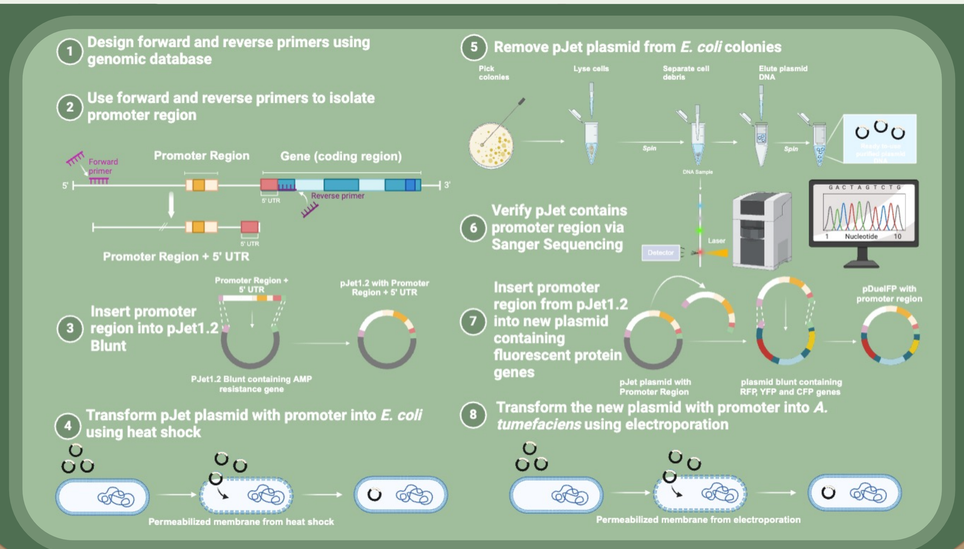
Aim 1: Identify the regulatory elements for gene expression to the sorghum cuticle.
- Find genes that have a high expression in the epidermis relative to other areas of the plant.
- Create primers specific to their promoter regions in the gDNA.

Aim 2: Transform those promoters into vectors to visualize their effect in the epidermis.
- Insert the promoter regions into pJet plasmid for easy replication using *E. coli.*
- Take the promoter regions and insert them into another plasmid, which contains genes that code for various fluorescent proteins.

Aim 3: Optimize Transient Expression Via Infiltration in *Sorghum bicolor*.
- Transient expression has already been streamlined in dicots such as *N. benthamiana* but has not been optimized in monocots like sorghum.
- Test how various conditions influence the efficiency of transient expression.

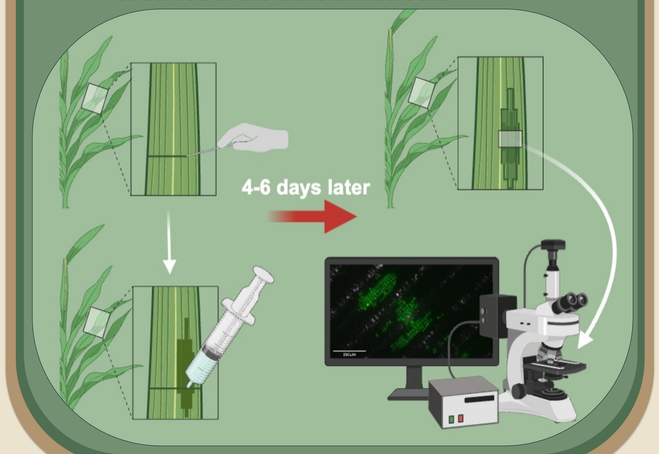
Promoter Cloning and Transformation

Gene Name	Cloned?	Function
SRD5A		Gene involved in wax biosynthesis; exact function unknown
ABCG11		Codes for member of ABCG family of proteins; Responsible for cutin and suberin export to the cuticle[6]
WSD1		Codes for Wax Ester Synthase and Diacylglycerol Acyltransferase enzymes, which synthesize cutin and suberin[6]
OSC9		Involved in the synthesis of the triterpenoids α-amyrin and β-amyrin which are expressed in the cuticle[5]
OSC15		Involved in the synthesis of triterpenoid derivatives of hopane which are expressed in the cuticle[5]
ABCT		Gene involved in leaf wax transport.
ATU		Gene involved in coding for an uncharacterized acyltransferase.
STKU		Uncharacterized gene in the Serine/Threonine Kinase Family.



Sorghum Infiltration

- After inserting the plasmid into *A. tumefaciens*, they are injected into the leaves of young sorghum plants.
- After 4-6 days the infiltration site is viewed under fluorescent microscopy. If successful, cyan and red or yellow fluorescence should be observed in the transformed cells



The Road Ahead

- Once regulatory elements of many genes expressed in the epidermis are found, *transgenes* (genes taken from other plants) that synthesize terpenes not found in sorghum can be expressed.
- Confirm transgenes are being expressed in the epidermis and their respective terpenes are being produced in the cuticle after infiltration.
- Potentially create stable lines of sorghum that have the transgenes for high-value terpenes.

References

[5] Busta et al. *A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry.* doi: https://www.pnas.org/doi/full/10.1073/pnas.2022982118

[6] Chemelewski et al. *Epicuticular wax accumulation and regulation of wax pathway gene expression during bioenergy Sorghum stem development, Frontiers in Plant Science* Vol. 14, 2023 ISSN=1664-462X

[7] K. B. Louie et al, 6.12.6.6 - *Terpenoids/Terpenes, Comprehensive Natural Products III*, Elsevier, 2020, Pages 263-306, ISBN 9780081026915

[8] Sharma et al. *Agrobacterium-mediated transient transformation of sorghum leaves for accelerating functional genomics and genome editing studies.* doi: https://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-020-04968-9

Images and figures made using BioRender