This is provided as an example proposal.
It is important that you follow the
current guidelines.
The mentor letter has been removed.

A PROPOSAL TO STUDENT ENHANCEMENT AWARD REVIEW COMMITTEE

TITLE OF PROJECT: Elucidating Heteroxylan glucuronidation mechanism in Monocots

NAME OF APPLICANT: Akshayaa Venkataraghavan

STATUS: ◯ Undergraduate ☑ Graduate ◯ Medical

CAMPUS/LOCAL ADDRESS: 315 Porter Hall, Ohio University, Athens OH, 45701
E-MAIL ADDRESS: av420518@ohio.edu
DEPARTMENT: Environmental and Plant Biology

EXPECTED GRADUATION DATE (Month and Year): May 2024

RE-SUBMISSION: ◯ YES (Original Submission Date_____) ☑ NO

PROPOSAL CATEGORY (select one):
☑ Life/Biomedical ◯ Social/Behavioral
◯ Arts/Humanities ◯ Physical Sciences/Engineering

BUDGET: Total Request $5,982.92
(May not exceed $6,000)

FACULTY MENTOR INFORMATION:
NAME: Dr. Ahmed Faik
E-MAIL ADDRESS: faik@ohio.edu
DEPARTMENT: Environmental and Plant Biology
DEPT/COLLEGE ADMIN. NAME & E-MAIL: Kara Tabor, tabor@ohio.edu

IRB AND IACUC APPROVAL:
To ensure the University's compliance with all federal regulations, complete the checklist below. *Note: if your IRB/IACUC is not approved prior to submission, put "pending" or "to be submitted" instead of approval number. Note: funding will be withheld until IRB/IACUC notification of approval or exemption.*

Yes	***No***	***Office of Research Compliance***	***Policy #***
◯	☑	Human Subjects in Research: Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
◯	☑	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		**Faculty Mentor's Signature**	
Signature	Akshaya.	Signature	Ahmed Faik
Name	Akshayaa Venkataraghavan	Name	Ahmed Faik
Dept/School	Environmental and Plant Biology	Dept/School	Environmental and Plant Biology
Date	01/17/2023	Date	01/17/2023

School or Dept Chair's/Director's Signature

Signature	Allan M. Showalter
Name	Allan M. Showalter
Dept/School & Date.	Department of Environmental and Plant Biology, Arts & Sciences - 1/17/2023

■ **Optional:**
If selected for funding, I give permission to the Research Division to use my proposal as an example during training and workshop exercises. (Sign below)
Signature: Akshaya. Date: 01/17/2023

STUDENT ENHANCEMENT AWARD
APPLICATION CHECKLIST

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

☑ Cover page	use SEA form
☑ Checklist	use SEA form
☑ Abstract*	1 double-spaced page
☐ Resubmission Summary (**For Re-submissions Only**)*	1 double-spaced page
☑ Project Narrative	5 double-spaced pages
☑ Glossary/Definition of Terms* (**Not required**)	2 double-spaced pages
☑ Bibliography (**Not required**)	2 pages
☑ Presentation of Results	1 double-spaced page
☑ Mentor's Endorsement	1 page
☑ Biographical information (**Applicant(s) and key personnel**)	3 pages per person
☑ Budget and Justification	no limit specified
☐ Appended Materials/Multimedia Files	5 pages; and no more than 10 minutes of footage
☑ Electronic copy of proposal	Single Acrobat file, containing entire proposal and required signatures

Sections marked with a bullet (*) identify text sections that should be written in language understandable by an informed layperson to assist the Committee in its review.

****Please Note:** ***The committee has the right to return without review any proposals that do not conform to these format requirements*****

Applicant signature: 

Abstract

Plant cell walls (CWs) are highly complex and made up of interconnecting network of polymers and glycoproteins. Heteroxylans (HXs) represent the major hemicellulosic polymers in CWs of grasses and trees and have critical roles in CW integrity/recalcitrance and plant growth and fitness. They are also useful to humans in various commercial applications, such as food, feed, and renewable energy. Therefore, understanding the molecular mechanisms of HXs synthesis is crucial. Structurally, HXs have a backbone made up of a chain of β-linked xylose (Xyl) residues that are substituted with glucuronic acid (GlcA) (glucuronidation). Although the genes involved in the glucuronidation of HXs have been identified as *GUX1*, *GUX2* and *GUX3* (GT8 family) in dicots (e.g. Arabidopsis), the glucuronidation mechanism of HXs in monocots is currently unknown. The goal of my project is to identify genes responsible for the glucuronidation of HXs in monocots. Using phylogenetic analysis and multiple sequence alignment between Arabidopsis and rice members of the GT8 family, I identified three putative rice GUXs. Through an *in-vitro* enzyme assay, I observed that they indeed transfer GlcA from UDP-GlcA (donor) to xylohexaose (acceptor). To further validate my results *in-vivo,* I set up two objectives: 1. Generate a rice triple mutant having the three putative OsGUXs silenced using CRISPR/Cas9 technology; and 2. Analyze the CWs of mutant for HXs composition and structure. The CRISPR/Cas9 technology will be performed using rice calli and the HXs will be extracted using an established protocol. I will use HPAEC-PAD (Dionex instrument) to detect the presence/absence of GlcA residues in the extracted HXs, after total acid hydrolysis. This work is highly significant as it represents the first evidence about the genes involved in HX glucuronidation in monocots and help manipulate HXs for societal benefits. I will be presenting my results in the XVI plant cell wall meeting in June 2023, and I also intend to publish the data in a peer reviewed journal.

Project Narrative

Goals, scope and context: The plant cell wall (CW) is a highly complex structure, that provides first line of defense against various pathogens and stresses. Plant CW is made up of 90% carbohydrates classified as cellulose, hemicellulose, and pectin, which differ in structure and **monosaccharide** composition [1]. Heteroxylans (HXs) are major hemicellulosic **polymers**, that provide structural integrity of CWs, which directly impacts the growth and development of the plant. Apart from its significance in plant development, HXs are useful to humans in a variety of commercial applications like food, feed and it is a great source of renewable energy [2]. Being such commercially important polymers, the molecular mechanisms underlying their **biosynthesis** is poorly understood. The backbone of HXs is comprised of β-linked xylose (Xyl) residues with different substitution patterns (acetyl, glucuronic acid/methyl-glucuronic acid (GlcA/Me-GlcA) and/or arabinofuranosyl (Ara) residues [3]) (Fig1).

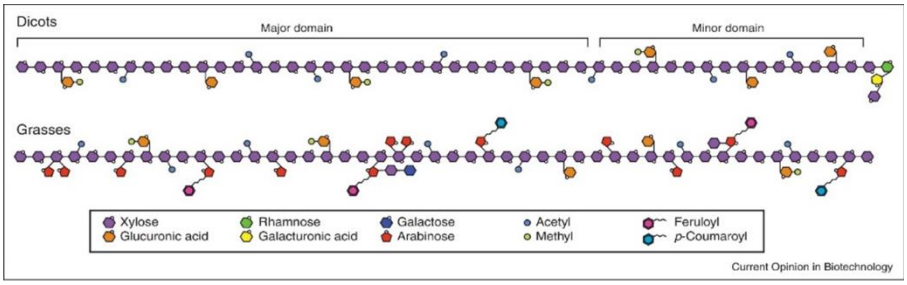

Figure1. Diagrammatic representation of the structure of heteroxylans observed in dicots and grasses (adapted from Rennie & Scheller, 2014 [3]).

Genetic studies in *Arabidopsis thaliana* (Arabidopsis, a **dicot** plant) have revealed that HXs are synthesized by at least 16 **genes** [4]. Among these genes, three Glucuronic acid Substitutions of Xylan (*GUX1, GUX 2* and *GUX 3*) genes are responsible for the addition of glucuronic acid (GlcA) onto xylan backbone (a process called glucuronidation) [5]. These three genes belong to the GT-8 family in CAZy database [4]. Despite this progress in dicots, not much

is known about the genes involved in glucuronidation of HX in **monocots**. In this proposal, *I aim to elucidate the molecular mechanism underlying glucuronidation of HXs in grasses*.

Preliminary data:

Identification of rice OsGUXs: To identify the putative *OsGUX* genes in rice (*Oryza sativa* subsp. *japonica*), I carried out **phylogenetic analysis** of Arabidopsis and rice members of the GT8 family (Fig 2a). The phylogenetic tree indicates that the three putative rice OsGUXs: Os02g0556000, Os01g0880200 and Os03g0184300 (hereby named as OsGUX-A, OsGUX-B and OsGUX-C) are clustered together with the Arabidopsis GUX1, GUX2, and GUX3 proteins. The putative rice OsGUXs were also found to share three conserved sequence **domains** (highlighted in red box) that were absent in other GT8 family members (Fig 2b).

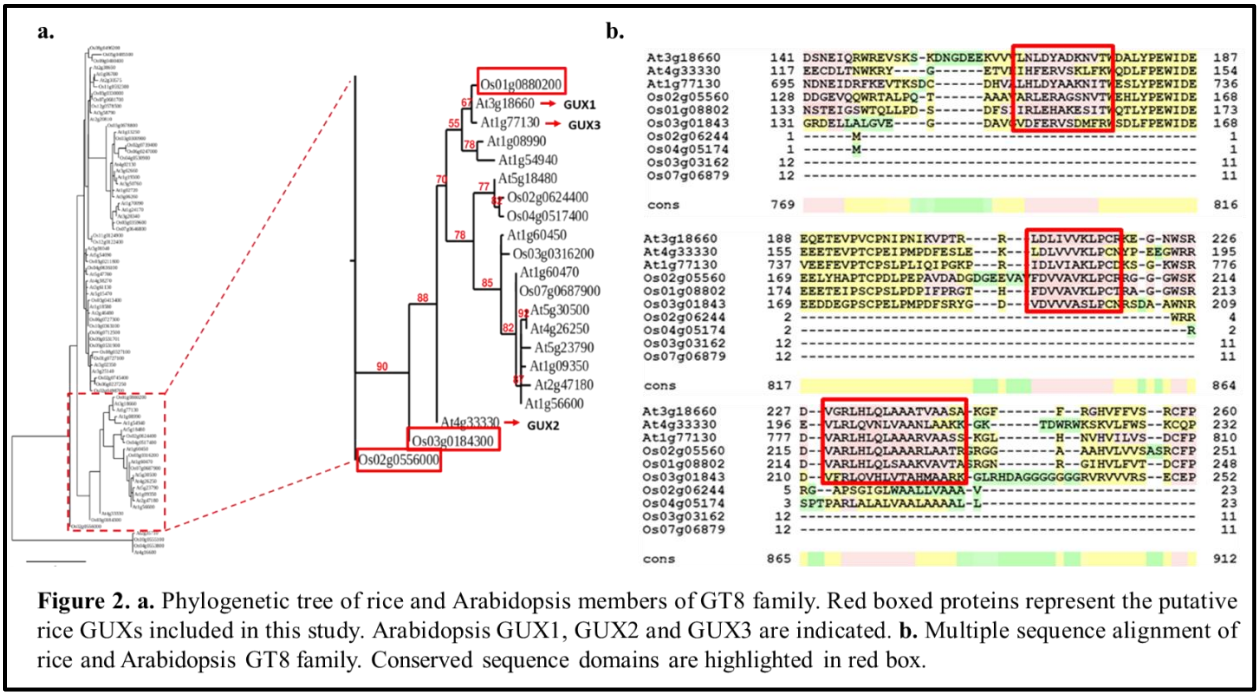

Figure 2. a. Phylogenetic tree of rice and Arabidopsis members of GT8 family. Red boxed proteins represent the putative rice GUXs included in this study. Arabidopsis GUX1, GUX2 and GUX3 are indicated. **b.** Multiple sequence alignment of rice and Arabidopsis GT8 family. Conserved sequence domains are highlighted in red box.

Enzyme activity of rice OsGUXs: An ***in-vitro*** enzyme assay was carried out to determine the glucuronosyltransferase (transfer of GlcA) activities of the identified rice OsGUXs. *OsGUX-A, OsGUX-B* and *OsGUX-C* genes were cloned as Halo-tagged versions at the N-terminal end. The plasmids were then used to synthesize the proteins using an *in-vitro* transcription translation (IVTT) system (Thermo fisher scientific). The Halo-tagged OsGUXs were captured on a 96 well plate coated with anti-halo **antibody**. After washing, the captured Halo-OsGUXs were tested for enzyme activity using UDP (Uridine di phosphate)-GlcA as the donor and xylohexaose as an acceptor, and the glucuronosyltransferase activity was monitored using GLO system, which measures the release of UDP from UDP-GlcA. If the OsGUX is able to transfer GlcA onto xylohexaose, then UDP will be released and can be measured as relative light unit (RLU) using GLO system (Thermo fisher scientific). As indicated in Fig.3, the three putative rice OsGUXs (OsGUX-A, OsGUX-B and OsGUX-C) showed high glucuronosyltransferase activity in the presence of the acceptor, which was absent in the absence of the acceptor. The activity is expressed as luminescence (RLU) values. Thus, *I hypothesize that these putative rice OsGUXs are involved in the addition of GlcA onto HXs in rice.*

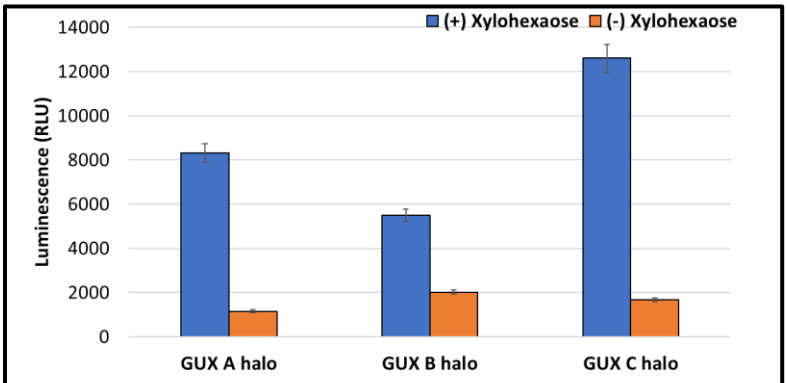

Figure 3. Glucuronosyl transferase activities of the putative rice GUXs measured using Glo system in the presence and absence of acceptor. The assay was performed in duplicates.

Objectives The following objectives will be carried out to validate my findings ***in-vivo***:

1. Generate a rice triple mutant in *OsGUX-A, OsGUX-B* and *OsGUX-C* genes using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 technology.
2. Extract HXs from the CWs of the triple mutant and analyze their composition and structure.

Method

Objective1: The three putative rice Os*GUX* genes (Os*GUX-A, OsGUX-B* and Os*GUX-C*) will be knocked out using CRISPR/Cas9 mediated technology by following an established protocol [6]. CRISPR/cas9 is a powerful tool which utilizes the help of a **guide RNA** (gRNA) that guides the Cas9 (an **endonuclease**) to the target gene in the **genome**. Cas9 acts as a molecular scissor to cut DNA at the specific gene, and while the genome tries to repair the DNA, it creates **mutations** which results in the gene being non-functional[6]. Hence by knocking out the putative rice GUX genes, I expect to see a reduction in the glucuronic acid content in the transgenic plants when compared to the wild type rice that has no mutation. Briefly, the method involves designing a gRNA for each gene using the software CRISPR-P 2.0 and a CRISPR construct with three gRNAs together will be cloned into pRGEB32 vector (a binary vector that contains Cas9 (under rice ubiquitin promoter) and rice snoRNA U3 promoter (to express the gRNAs)). The construct will then be transformed into Agrobacterium LBA4404 strain and will be ready for the transformation of rice **calli**. The overall procedure for rice calli transformation is summarized in fig.4. Currently, I have created the CRISPR construct and have performed calli transformation using the established protocol [7]. The transformed calli are in the pre-regeneration media. Once the transgenic plants are generated, genomic DNA will be extracted from them, and the plants will be sequenced to check for mutations in the target gene.

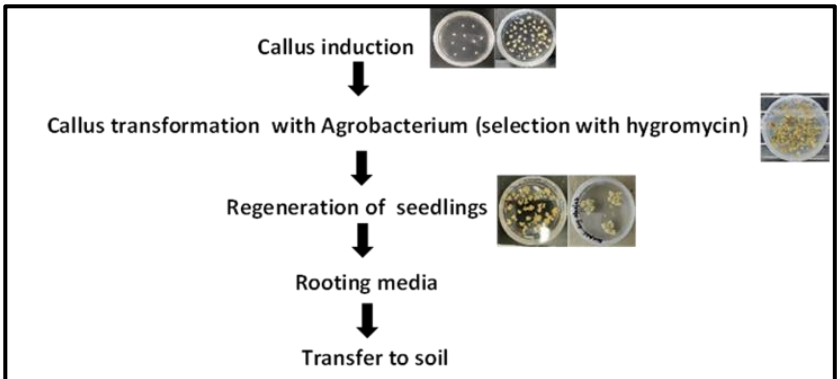

Figure 4. Schematic representation of tissue culture steps to generate the transgenic rice plants from calli.

Objective2: Cell wall HX will be extracted from **homozygous** and Cas9 free transgenic plants as previously described[8],[9]. In brief, Alcohol Insoluble Residue (AIR) will be prepared by grinding the plant tissue with liquid nitrogen and then resuspended with 80% ethanol. The pellet obtained after centrifugation is sequentially washed with absolute ethanol and acetone and the resulting residue is air dried for 24 hours. The AIR will then be subjected to sequential extractions with increasingly harsh reagents (50mM Ammonium oxalate, 50mM sodium carbonate, 1M KOH, 4M KOH) to isolate fractions enriched with various cell wall components. The 4M KOH extract which is rich in HX will be treated with strong acid like TFA (trifluoroacetic acid) to release the sugars and the sample will be loaded onto Dionex machine to check for the presence of glucuronic acid. I will use wild type rice plant as a positive control to compare it with transgenic plants.

Significance: The results are highly significant as they represent the first evidence for understanding the HX glucuronidation process in monocots/grasses. Since the presence of GlcA substitution affect HX depolymerization, understanding the mechanism of how GlcA is being added to HX in monocots will help manipulate the structure of HXs. This can further help to improve CWs digestibility for biofuel production.

Broader impacts: HX represents the third most abundant polymer on earth (after cellulose and chitin) and is of great importance in industries that produce food, paper, medicine, and biofuel. Recalcitration of biomass is one of the major problems in the conversion of plant biomass to biofuel. Understanding the genes involved in HX biosynthesis is crucial as it can help us to modify the pathway for better utilization of HX in industries. Completion of this project would allow me to include the results in my PhD dissertation and publish the data in a peer reviewed journal. I would also be able to share my research to public through Ohio University Student expo in 2024 and present my findings in the XVI Plant Cell Wall meeting in Spain (June 2023).

Glossary

Monosaccharide: Monosaccharides are carbohydrates that cannot be broken down (by hydrolysis) into simple units.

Polymers: Plant cell walls are composed of different types of polymers including polysaccharides, lignins and proteins, and they are a specific feature of plant cells. They provide mechanical support to the plant and constitute a physical protective barrier against environmental cues. They also enable cell-to-cell communication.

Biosynthesis: The production of complex compounds from simple precursors in a living organism.

Dicots: A class of plants that produce embryo with two cotyledons (first leaf to appear from a seed) and usually have floral organs arranged in cycles of four or five.

Genes: A gene is a sequence of DNA/RNA that codes for a protein molecule that has a function. For expression of a gene, the DNA is first copied into intermediate template mRNA, then coded into a protein which are the building blocks of life and performs a function in a cell.

Monocots: Monocotyledons commonly referred to as monocots are grass and grass-like flowering plants, the seeds of which typically contain only one embryonic leaf, or cotyledon.

Phylogenetic analysis: An analysis that provides in-depth understanding of how species evolve through genetic changes.

Domains: Domains are distinct regions of a protein that are responsible for a particular function or interaction.

In-vitro A process that is performed outside of a living organism (example: test tube, culture dish).

Antibody A protein that recognizes a particular part of an antigen.

In-vivo A process that is performed inside a living organism.

Guide RNA: A piece of RNA that functions as a guide for DNA targeting enzymes (example: Cas9)

Endonuclease: An enzyme that breaks down a nucleotide chain (DNA) by cleaving the internal bonds between them.

Genome: The complete set of DNA (genetic material) in an organism.

Mutation: Mutations refers to a change in the DNA sequence of an organism. It can occur either naturally or can be induced artificially (example: by using CRISPR/Cas9).

Calli: Growing mass of unorganized plant parenchyma cells.

Homozygous: A plant is called homozygous for a particular gene when both alleles (one from the male and other from female) at given locus are similar. A homozygous plant maintains a high degree of consistency for characters determined by the gene throughout the subsequent generations.

Bibliography

[1] D. C. Cosgrove and M. Jarvis, “Comparative structure and biomechanics of plant primary and secondary cell walls,” *Front. Plant Sci.*, vol. 3, 2012, doi: 10.3389/fpls.2012.00204.

[2] M. Pauly and K. Keegstra, “Plant cell wall polymers as precursors for biofuels,” *Current Opinion in Plant Biology*, vol. 13, no. 3, pp. 304–311, Jun. 2010, doi: 10.1016/j.pbi.2009.12.009.

[3] E. A. Rennie and H. V. Scheller, “Xylan biosynthesis,” *Current Opinion in Biotechnology*, vol. 26, pp. 100–107, Apr. 2014, doi: 10.1016/j.copbio.2013.11.013.

[4] C. Lee, R. Zhong, and Z.-H. Ye, “Arabidopsis Family GT43 Members are Xylan Xylosyltransferases Required for the Elongation of the Xylan Backbone,” *Plant & Cell Physiology*, vol. 53, no. 1, pp. 135–143, Jan. 2012, doi: 10.1093/pcp/pcr158.

[5] C. Lee, Q. Teng, R. Zhong, and Z.-H. Ye, “Arabidopsis GUX Proteins Are Glucuronyltransferases Responsible for the Addition of Glucuronic Acid Side Chains onto Xylan,” *Plant and Cell Physiology*, vol. 53, no. 7, pp. 1204–1216, Jul. 2012, doi: 10.1093/pcp/pcs064.

[6] K. Belhaj, A. Chaparro-Garcia, S. Kamoun, N. J. Patron, and V. Nekrasov, “Editing plant genomes with CRISPR/Cas9,” *Current Opinion in Biotechnology*, vol. 32, pp. 76–84, Apr. 2015, doi: 10.1016/j.copbio.2014.11.007.

[7] K. Xie, B. Minkenberg, and Y. Yang, “Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system,” *PNAS*, vol. 112, no. 11, pp. 3570–3575, Mar. 2015, doi: 10.1073/pnas.1420294112.

[8] S. Pattathil, U. Avci, J. S. Miller, and M. G. Hahn, “Immunological Approaches to Plant Cell Wall and Biomass Characterization: Glycome Profiling,” in *Biomass Conversion: Methods and Protocols*, M. E. Himmel, Ed. Totowa, NJ: Humana Press, 2012, pp. 61–72. doi: 10.1007/978-1-61779-956-3_6.

[9] W. Zeng, M. Chatterjee, and A. Faik, “UDP-Xylose-Stimulated Glucuronyltransferase Activity in Wheat Microsomal Membranes: Characterization and Role in Glucurono(arabino)xylan Biosynthesis,” *Plant Physiology*, vol. 147, no. 1, pp. 78–91, May 2008, doi: 10.1104/pp.107.115576.

Presentation of results

I plan to present the results of my research in the **XVI plant cell wall meeting** which will happen from June 18th- 22nd, 2023 at Málaga, Spain. The focus of this conference is to cover the latest discoveries in basic biology and applications of the plant cell wall. It will provide an excellent opportunity for me to network with scientists from all around the world and discuss the findings of my research. I will be able to learn about cutting edge discoveries in the plant cell wall biology and I would have the opportunity to collaborate with other researchers related to my work. This symbiotic relationship will greatly enhance my understanding of the project and as a fourth year PhD student, this will immensely help me to refresh my knowledge from all branches of biology and provide a new perspective on my research question. Presenting my work in national and international conferences will give me a valuable opportunity to gather feedback about my research from distinguished professors. It will allow me to overcome any caveats that might arise with my work. I also plan to present my findings in the **Ohio University Student Expo** which will be held on April 2024, at Ohio university. Students from different discipline will be presenting their research work in the expo. Since the student expo is open to Ohio and local community members, it would be a great platform for me to share my results to the general public. It would enhance my presentation as well as communication skills by interacting with students and faculty from various fields and allows me to gain insight on how different works are benefitting the society. I will also publish my research findings in a peer reviewed journal, and it will be a part of my PhD dissertation.

Akshayaa Venkataraghavan

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Academic Background

Ph.D - Department of Environmental & Plant Biology (anticipated date of graduation: July 2024), Ohio University, Athens, Ohio-45701.

M.Tech - Industrial Biotechnology (2017), SASTRA University, Thanjavur-613401, India.

B.Tech - Biotechnology (2015), Rajalakshmi Engineering College, Chennai- 602105, India.

Academic appointments

2019-present Teaching Assistant, Department of Environmental and Plant Biology, Ohio University, Athens, Ohio.

2017-2018 Junior Research Fellow, Indian Institute of Technology, Madras, India.

2017(January-June) Research Intern, Department of Agriculture, Food and Wine, The University of Adelaide, Australia.

Relevant Course work

PBIO 5310-Cell Biology (**A**)

PBIO 5500-Biotechnology (**A**)

PBIO 5240-Plant Physiology (**A**)

PBIO 5300-Plant Genetics (**A**)

PBIO 5010-Lab CaMPP (**A-**)

PBIO 5180-Writing in the Life Sciences (**A**)

PBIO 5150- Statistical methods in Plant Biology (**A**)

Cumulative GPA: 3.97

Technical skills

Cell and Microbiology: Microbial staining techniques, Antibiotic sensitivity assays, Bacterial and yeast culture techniques, Microbial characterization tests, Protein assays.

Plant Tissue culture: Generation of calli from rice seeds; regeneration of plants from callus.

Molecular Biology: Gene cloning, overlap PCR, quantitative real time RT-PCR, Co-expression of protein constructs in Tobacco epidermal cells, Agrobacterium-mediated genetic transformation, Membrane protein isolation, Bacterial and plant DNA isolation, Agarose gel electrophoresis, Competent cell preparation, Liposome preparation using rotary evaporation technique.

Analytical: Confocal microscopy, Colorimetry, UV-Visible spectrophotometry, fluorimetry, Stopped flow spectrophotometry, HPLC.

Awards

2021 (Spring) **Graduate Student Research Fund** ($1000), College of Arts and Sciences.

2021 (Fall) **Original work grant** ($1000), Ohio University.

2022 (Spring) **Graduate Student Research Fund** ($1000), College of Arts and Sciences.

2022 (spring) First place, Ohio University **Student and Research Creativity Expo**.

2022 **Travel grant** ($500), College of Arts and Sciences to attend IX cell wall research conference held at East Lansing, Michigan (June 13th - June 17th,2022).

2023 **Travel grant** ($500), College of Arts and Sciences to attend XVI Plant cell wall meeting to be held at Málaga, Spain (June 18th - June 22nd,2023).

Posters presented

Akshayaa Venkataraghavan and Ahmed Faik (2022) Elucidating Heteroxylan glucuronidation mechanism in grasses. Presented at the student research and creativity expo, Ohio University in April 2022.

Akshayaa Venkataraghavan, Matrika Bhattarai and Ahmed Faik (2022) Elucidating Heteroxylan glucuronidation mechanism in grasses. Presented at the IX cell wall research conference, East Lansing, Michigan from 13th- 17th June 2022.

Manuscript submitted

Tasleem Javaid, **Akshayaa Venkataraghavan**, John Schenk, Michael Held, and Ahmed Faik (2022). Uncovering specific protein-protein interactions between rice members of the GT43 and GT47 families and their implication in plant development (*Plant Physiology*).

Workshops and Conference

- Attended the IX cell wall research conference held at East Lansing, Michigan (June 2022)
- Organized a three-day Synthetic Biology workshop conducted at Indian Institute of Technology Madras-Research Park, India (July 2018).
- Attended one day hands on training on "Pharmaceutical and Cosmetic Formulations" conducted by VIT University, Vellore, India (February 2014).
- Attended a two-day workshop on "In silico drug design and DNA Finger printing" conducted by Rajalakshmi Engineering College, India (January 2014).
- Attended "International Conference on Bio Engineering" organized by Rajalakshmi Engineering College, India (January 2013).

Language Skills

English, Tamil, Hindi (read and write).

Other funding sources

I have neither applied nor am I receiving any other funding currently.

Budget

Consumable supplies

Objective 1: Generate a rice triple mutant in OsGUXA, OsGUXB and OsGUXC			
Item	**Supplier**	**Cost /Quantity**	**Total cost**
1. MS media with vitamins and glycine	Caisson Laboratories	$24.31/ 50L	$97.24
2. Phytagel	Millipore Sigma	$65.50/100g	$65.50
3. Petri plates	Sigma Aldrich	$125.07/100plates	$125.07
4. Hygromycin	Sigma Aldrich	$179.00/ 250 mg	$179.00
5. Kinetin	Thermo fisher	$98.78/ 5g	$98.78
6. NAA (1-Naphthylacetic acid)	Thermo fisher	$46.95/100g	$46.95
7. Excell Peters 15-5-15- mix - Call Mag Special (25 lb)	BFG	$44/25Lb	$44.00
8. Osmocot plus 15-9-12	BFG	$110.00/50Lb	$110.00
9. Custom made primers (for sequencing)	Invitrogen	$9.00 / primer	$54 (for 3 genes, forward and reverse)
10. DNA sequencing	Eton Biosciences	10.00$ per plant for sequencing 5' to 3' and 3'to 5' for two reactions ($5.00/reaction)	$600.00 (60 plants for growing 3 generations to screen Cas9 free and homozygous)
Objective2: Monosaccharide analysis of HX from the cell walls of transgenic plants			
1. Ammonium oxalate	Fisher Scientific	$390.50	$390.50
2. Sodium borohydride	Sigma-Aldrich	$46.90	$46.90
3. Sodium carbonate	Sigma-Aldrich	$88.20	$88.20
4. Endoxylanase-III	Megazyme International	$210.60	$210.60
5. Trifluoroacetic acid	Fisher Scientific	$506.50	$506.50
8. Sodium acetate	Fisher Scientific	$260.00	$260.00
9. Corning™ Costar™ Spin-X™ Centrifuge Tube Filters	Corning life sciences	$272.26/ case	$544.52
10. CarboPac^{TM} PA20 columns	Thermo fisher scientific	$528.00	$528.00
Consumable supplies total			**$3995.76**

Conference travel

XVI Plant cell wall meeting (June 18th- 22nd)2023 at Málaga, Spain	
Airfare (roundtrip)	$1367.00
Registration fee (with meals included)	$595.16
Hotel- 5 days ($105/ night)	$525.00
Travel funds provided by PBIO department	$500.00
Conference travel total	**$1987.16**
Total SEA funds requested	**$5982.92**

Justification

Objective1: To generate a rice triple mutant in *OsGUXA*, *OsGUXB* and *OsGUXC* genes, I transformed Agrobacterium LB4404 containing the CRISPR construct into rice calli. The calli are at present in pre-regeneration medium. The next step is to transfer them to regeneration media which will have phytagel (to solidify the gel), Hygromycin (antibiotic selection), Kinetin (hormone to regenerate shoots), NAA (hormone to regenerate roots). Once the shoots and roots are generated, it will be transferred to MS media with vitamins and glycine which will help strengthen the plant before transferring to soil. Petri plates are used for pouring both the regeneration and MS media. Excell Peters 15-5-15 and Osmocot plus 15-9-12 are fertilizers that are added to the soil while the rice plants are growing to help them get the appropriate nutrients. To sequence the generated transgenic plants, custom made primers (forward and reverse) will be designed in the three genes separately by flanking the guide RNA. The PCR amplified product using the primers will be sent for sequencing to Eton Biosciences company. Around 20 plants will be screened in each generation to check for mutation in the genes. The plants will be grown for at least 2 generations to get a stable homozygous line with no Cas9. Unlisted chemicals and materials which are needed to complete the objective1 are already available in the Faik lab.

Objective2: Alcohol insoluble residue will be extracted from the generated transgenic plants, which will further be subjected to sequential extraction with ammonium oxalate, sodium carbonate (with sodium borohydride added) to release HX. HX will be digested with endoxylanase III in sodium acetate buffer to separate them into individual xylose units. Acid hydrolysis is carried out by treating the digested HXs with trifluoroacetic acid. The samples are then centrifuged with Corning Costar Spin X filters to remove any contaminants and are fractioned on CarboPac PA20 columns in Dionex machine to help separate the sugars (Xylose and glucuronic acid). Unlisted chemicals and materials which are needed to complete the objective2 are already available in the Faik lab.

Conference travel: The airfare round trip for the XVI plant cell wall meeting was obtained from Kayak.com and the hotel rooms are shared accommodation for five nights. The flights and hotel accommodation options are chosen as cost effective as possible. I have received $500 from the PBIO department as a travel fund which I will also use towards my travel expense. Registration fee also includes lunches and other beverages, and the rest will be covered by my personal finance. Based on US department of state's report on Spain, I will ensure that I travel in groups in public places and be present mainly between my place of stay and the location where the conference is held. With regards to covid restrictions, currently Spain does not have any vaccination requirements, however, I am currently fully vaccinated and will take a covid test prior to departure if asked.