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: INVESTIGATING THE ROLE OF GLUCURONIC ACID IN ARABIDOPSIS SEED COAT MUCILAGE

NAME OF APPLICANT: OYEYEMI AJAYI

STATUS: ( ) Undergraduate (✓) Graduate ( ) Medical

CAMPUS/LOCAL ADDRESS: 315 PORTER HALL, OHIO UNIVERSITY, ATHENS, OH, 45701
E-MAIL ADDRESS: oa715216@ohio.edu
DEPARTMENT: ENVIRONMENTAL AND PLANT BIOLOGY

EXPECTED GRADUATION DATE (Month and Year): MAY, 2021

RE-SUBMISSION: ( ) YES (Original Submission Date_____) (✓) NO

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

BUDGET: Total Request $5,994.21
(May not exceed $6,000)

FACULTY MENTOR INFORMATION:
NAME: DR. ALLAN SHOWALTER
E-MAIL ADDRESS: showalte@ohio.edu
DEPARTMENT: ENVIRONMENTAL AND PLANT BIOLOGY
DEPARTMENT ADMIN./E-MAIL: CONNIE VARGO / vargoc@ohio.edu

IRB AND IACUC APPROVAL:
To ensure that the University is in compliance with all federal regulations, complete the checklist below. *Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.*

Yes	No	Office of Research Compliance	Policy #
( )	(⊗)	Human Subjects in Research (including surveys, interviews, educational interventions): 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	Ajayi	Signature	Allan M. Showalter
Name	OYEYEMI AJAYI	Name	Allan M. Showalter
Dept/School	ENVIRONMENTAL & PLANT BIOL	Unit	Environmental and Plant Biology
Date	1/22/2020	Date	1/22/2020

DEPT CHAIR

~~Dean~~ Name	Allan M. Showalter
Dept/School	Environmental and Plant Biology
Signature	Allan M. Showalter

☑ **Optional:**
If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises. (Sign below)
Signature: Ajayi Date: 01/22/2020

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 (Including the OHIO-Affiliated Travel Form, if applicable)
☐ 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: Ajaykumar

Abstract

When seeds absorb water, they release sugar-rich molecules called mucilage. Seed mucilage has been linked to lowering the risk of type-II diabetes and cardiovascular diseases and has antioxidant, emulsifying and anti-inflammatory properties. Similarly, seed mucilage function in seed germination and dispersal. Despite its agricultural and health benefits, little is known about the mechanisms involved in seed mucilage formation. My previous work identified two genes (*GLCAT14A* and *GLCAT14C*) that transfer glucuronic acid to arabinogalactan-proteins in the seed coat of Arabidopsis. Seeds obtained from the genetic knock-out of either *GLCAT14A* or *GLCAT14C* genes (single mutants) in Arabidopsis behaved like the wildtype seeds in mucilage formation; however, when I knocked out both *GLCAT14A* and *GLCAT14C* genes (double mutant), there was a significant loss of sugar-rich mucilage in the seed coat. Inspired by this novel discovery, I propose to determine the role of glucuronic acid in seed mucilage formation by completing two objectives set out in this proposal: 1) to determine what sugars were altered in the double mutant compared to wildtype and 2) to determine the distribution of sugars in the double mutant seeds compared to wildtype**.** To accomplish these goals, I will extract the mucilage in the water-soluble and adherent layers from wildtype and double mutant seeds and analyze their sugar composition and abundance. I will also visualize the distribution of these sugars using antibodies directed at their targets. Results from this study will deepen our understanding of the structure-function relation-ships in seed mucilage formation. As a fourth year PhD Candidate, I am nearing the end of my degree and need this funding to complete my research and dissertation. I will be presenting my results at the Plant Biology 2020 meeting, and these results will also contribute to the manuscript that I plan to publish in a peer-reviewed journal.

Project Narrative

Goals, scope and context. During seed development, large amounts of sugar-rich **mucilage** accumulate within the seed coat. Hydration of a mature seed leads to an extrusion of the mucilage to form a gelatinous capsule that encapsulates the seed (1). Detailed analysis of the seed mucilage in Arabidopsis identified two layers: the water-soluble layer and an adherent layer, which is tightly attached to the seed coat **(Figure 1A)**. Both layers are composed primarily of pectin and cellulose with small amounts of arabinogalactan proteins that crosslink with other sugars in a highly regulated process (2, 3) **(Figure 1B).** Pectin is a complex sugar that is primarily composed of repetitive units of simple, smaller sugars (rhamnose and galacturonic acid), and forms 90% of the mucilage (2). Cellulose, a complex sugar, comprises of linear chain of several thousand units of simple sugars (glucose), while arabinogalactan-proteins is a bit more complex, consisting of simple sugars like galactose, arabinose, xylose and glucuronic acid sugars.

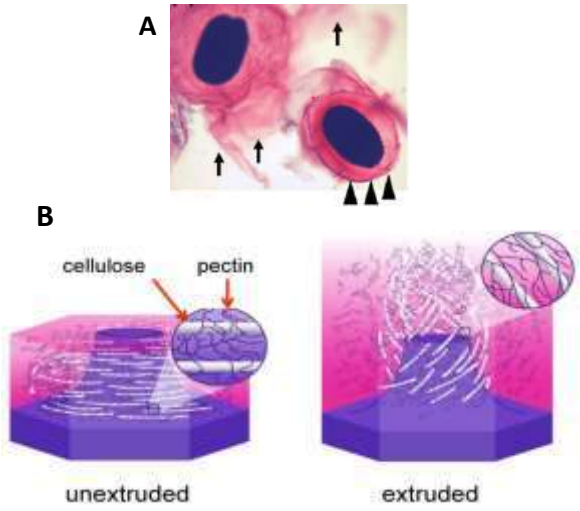

Fig 1: Arabidopsis seed coat mucilage (A) Staining for pectin showed the water-soluble (arrows) and adherent (arrowheads) mucilage layers (B) Pectin-cellulose crosslinking

Several genes/proteins called **glycosyltransferases** have been identified in the assembly and formation of the seed coat mucilage, and we are still trying to characterize their function. Three genes, *GLCAT14A*, *GLCAT14B* and *GLCAT14C*, are known to transfer glucuronic acid to arabinogalactan-proteins (5). Out of these three genes, only *GLCAT14A* and

GLCAT14C genes are expressed in high amounts in the Arabidopsis seed coat (6). Although arabinogalactan-proteins are **covalently** attached to pectin through glucuronic acid (7, 8), no *GLCAT* genes mediating this linkage has been identified**. I hypothesize that the genetic knock-out of *GLCAT14A* and/or *GLCAT14C* genes disrupts the structural assembly of pectin and cellulose components in Arabidopsis seed coat mucilage.** This is important because seed mucilage has antioxidant, emulsifying and anti-inflammatory properties (9), and function in seed germination and dispersal (10). Similarly, it has been linked to lowering the risk of type-II diabetes and cardiovascular diseases (11, 12).

Preliminary data: Using a **reverse genetics** approach, I generated **Arabidopsis** mutants lacking functional proteins either for GLCAT14A or GLCAT14C (single mutants) and both GLCAT14A and GLCAT14C (henceforth referred to as "double mutant") to investigate the role of these genes in seed mucilage formation. I observed that mutant seeds lacking in either GLCAT14A or GLCAT14C proteins (single mutants) were unaffected by the gene knockouts and performed similarly to the wildtype seeds. Surprisingly, the double mutant seeds lacking functional proteins for both GLCAT14A and GLCAT14C were severely altered in seed mucilage formation. I discovered the following: 1) double mutant seeds were more compacted that the wildtype when they absorbed water (**Figure 2A, B**). 2) When the seeds were stained with a dye that stains pectin, I observed a significant reduction in the amount of pectin in the adherent layer of the double mutant compared to the wildtype (**Figure 2C, D**). 3) Staining for cellulose showed a significant reduction in the adherent layer for the double mutant compared to wildtype (**Figure 2E, F**). These observations raise some interesting questions worth exploring: 1) Are the **sugar composition and abundance** of the water soluble and adherent mucilage altered in the double mutant compared to wildtype?

(Objective 1). 2) Is the **distribution** of sugars in the adherent mucilage of double mutant seeds altered compared to the wildtype? **(Objective 2).**

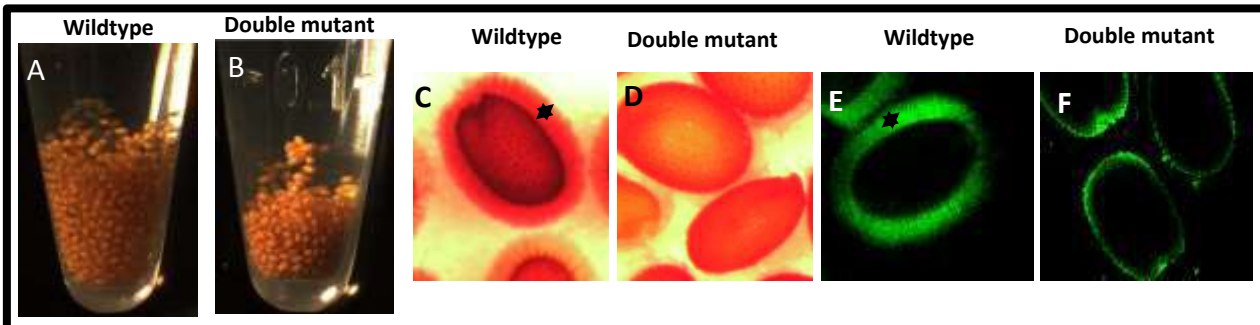

Figure 2. Arabidopsis seeds of wildtype and double mutant. Exactly 5mg of seeds imbibed in water (A, B); adherent mucilage stained with a dye for pectin (C, D) and a dye for cellulose (E, F) in wildtype and mutants. Adherent mucilage layer (C, E) is represented by the region indicated by the symbol (★).

1	2	3	4	5	6
Measure 100 mg of seeds in a 15mL falcon tube. Add 5ml of distilled water and vortex for 5 mins.	Transfer the supernatant to a new 15 mL falcon tube and label "**water- soluble mucilage".**	Rinse the seeds with 5 mL of distilled water and transfer the supernatant to the tube labelled **"water-soluble mucilage".**	Ultrasonicate the remaining seeds in 5 mL of distilled water for 20 secs. Transfer the supernatant to a new falcon tube and label **"adherent mucilage".**	Rinse the seeds with 5 mL of distilled water and transfer the supernatant to the **"adherent mucilage"** tube.	Place both tubes in a freeze drier and store in -80°C for the sugar analysis.

Figure 3. Method for isolating the water soluble and adherent mucilage in Arabidopsis seeds. Mucilage extraction is explained sequentially in steps 1-6 above.

Methods: For my objectives, I will harvest seeds from 10 wildtype and confirmed double mutant plants **(biological replicates)** grown under long-day conditions (16hr light, 8hr dark) in 22°C walk-in plant growth chambers. The seeds will be dried and stored at room temperature. The sequential steps for extracting the water soluble and adherent mucilage is presented in **Figure 3** following standard biology protocols (13).

Objective 1: Sugar composition analysis. The rationale for doing the sugar analysis serves to quantify the abundance of various sugars in both the water soluble and inner mucilage layers. Based on our preliminary data **(Figure 2)**, it is possible that some sugar(s)

(for example, rhamnose and galacturonic acid) in the adherent mucilage for the double mutant might have escaped into the water-soluble layer or was not synthesized at all; thus, impacting both the sugar composition and/or abundance in both layers. I will carry out this experiment using an established protocol (14). Briefly, 2mg of freeze-dried samples (outer water-soluble mucilage and inner adherent mucilage, see **Figure 3**) will be broken down into simple sugars using a strong acid (trifluoracetic acid). Nine sugar standards (Arabinose, Fucose, Galactose, Galacturonic acid, Glucose, Glucuronic acid, Mannose, Rhamnose and Xylose) will be used alongside my prepared samples. The composition and abundance will be estimated using a **Dionex** machine equipped with **CarboPac PA20** columns. I will run each sample five times on the Dionex machine **(technical replicates)** and conduct statistical tests to determine whether there is a difference in sugar abundance in the water-soluble and adherent mucilage samples. For my **negative control**, I will inject water instead of my sample.

Objective 2: Seed immunolabelling. The **immunolabelling** of seeds is a useful method to visualize the distribution of sugars in the seed mucilage using **antibodies**. Five primary antibodies will be used to accomplish objective 2 because they are well established standard antibodies used in the field to immunolabel whole seeds (15) **(Table 1)**.

Table 1: List of primary antibodies to determine the distribution of sugars in the seed mucilage.

Primary antibody	Target sugar (antigen)	Sugar family
[1]CBM3A	Crystalline cellulose	Cellulose
[2]LM2	Glucuronic acid	Arabinogalactan-protein
[2]JIM5	Methyl esterified homogalacturonan	Pectin
[1]CBM28	Amorphous cellulose	Cellulose
[3]CCRC-M36	Unsubstituted rhamnogalacturonan I	Pectin

[1] binds to Anti-His tag; [2] secondary antibody is goat anti-rat conjugated to AlexaFluor 488; [3] secondary antibody is goat anti-mouse conjugated to AlexaFluor 488.

Seed immunolabelling will be carried out using an established protocol (15). Briefly, wildtype and double mutant seeds (20 seeds) will be hydrated in water and labelled using both primary and secondary antibodies, and subsequently imaged using a **confocal microscope**. My **negative control** is to image seeds that have not been labelled with any antibody. I anticipate a significant alteration in the mucilage sugar distribution for the double mutants with concomitant effects on mucilage architecture, function and formation.

Significance. No study to date has examined the functional role of glucuronic acid in seed coat mucilage formation. I believe this study has the potential to delineate the role of glucuronic acid in seed mucilage formation, and insights gained can advance research on ways to improve seed mucilage composition for agricultural and health benefits.

Broader impacts. Seed mucilage has great potential in the food, agricultural and pharmaceutical industries. For example, chia mucilage is used as a food stabilizer and emulsifier (16) while sugars in psyllium mucilage serve as an efficient drug delivery system because of their non-toxic, chemically inert and biodegradable properties (17). In addition, seed mucilage is important in seed hydration, germination and dispersal (10). The insights gained from this study has the potential to favor the generation and synthesis of seed mucilage with unique features that can benefit food, agricultural and pharmaceutical industries. I have undergraduate students working with me on this project, and this provides a platform for them to acquire significant intellectual, hands-on skill that will enhance their understanding of how structure affects function in plant biology. I plan to present my findings at the Plant Biology2020 international conference in July to showcase my work to the larger scientific community. Also, as a fourth year PhD Candidate, the results from this work will help greatly in my dissertation and manuscript preparation.

Glossary

Mucilage: a sugar -rich substance extruded from plant organs e.g seeds

Glycosyltransferases: any of a group of enzymes that catalyze the transfer of sugar

Covalently: a strong bond that links two molecules together

Reverse genetics: is a method that knocks out a gene and study the effects of knocking out that gene in an organism of interest.

Biological replicates: biologically distinct samples that capture the biological variation

Dionex: is the piece of equipment used to detect and quantify simple sugars

CarboPac PA20: This is the column through which all the simple sugars pass through and are separated. In simplistic terms, it is a sugar separating column.

Technical replicates: repeated measurements of the same sample.

Immunolabelling: A method of identifying antigens (e.g sugars) by using antibodies.

Antibody: special proteins that recognize a unique part of the foreign target called an antigen (e.g sugars).

Antigen: a substance or molecule (e.g sugar) that is recognized by an antibody.

Confocal microscope: A powerful instrument with increased optical resolution for fixed images.

Arabidopsis: a plant that is used by plant biologist as a genetic model organism, to study the functional roles of genes in plants.

Bibliography

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linked to an arabinogalactan-protein. *Plant Cell*, 25: 270-287

9. Christos S, Gaiani C. and Hoffmann L (2018) Plant seed mucilage as emerging biopolymer in food industry applications. *Curr. Op. in food sci.* 22: 28-42

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13. Zhao X, Qiao L. and Wu, A. (2017) Effective extraction of Arabidopsis adherent seed mucilage by ultrasonic treatment. *Sci Rep,* 7:40672

14. Voiniciuc, C. and Günl, M. (2016). Analysis of monosaccharides in total mucilage extractable from *Arabidopsis* seeds. *Bio-protocol* 6(9): e1801. DOI: 10.21769/BioProtoc.1801.

15. Voiniciuc, C. (2017). Whole-seed immunolabeling of *Arabidopsis* mucilage polysaccharides. *Bio-protocol* 7(11): e2323. DOI: 10.21769/BioProtoc.2323.

16. Munoz LA, Coboz A, Diaz J. and Aguilera M. (2012) Chia seeds: Microstructure, mucilage extraction and hydration. *Journal of Food Engineering,* 108: 216-224

17. Singh B and Chauhan N. (2009) Modification of psyllium polysaccharides for use in oral insulin delivery. *Food Hydrocolloids*, 23, 928–935.

Presentation of Results

The results of my research will be presented at the Plant Biology 2020 meeting in Washington, DC USA from July 25-29, 2020. The Plant Biology 2020 meeting, organized by the American Society of Plant Biologists (ASPB), is the international gathering of plant biologist from all over the world. The nature of the conference is to unite over 3,000 plant scientists and students (undergraduates, graduate students and postdocs), from nearly 35 countries, to present their multifaceted plant biology research. Furthermore, top scientists conducting research in seed mucilage biology will be attending the conference. As a fourth year PhD candidate, attending Plant Biology 2020 will greatly enhance my career by connecting me with other leading plant scientists in my field from diverse locations and disciplines. In addition, I plan to enhance my presentation skills by giving a talk with a tentative title, "The 800-pound Gorilla: functional role of two genes involved in seed mucilage biosynthesis in Arabidopsis.". The presentation of my work would enable me to get valuable feedback that I can incorporate into my dissertation and scientific manuscript. This could in turn lead to new collaborations and connections. Every year, my advisor and my lab mates present their research works at Plant Biology meetings and I have undergraduates working with me in the lab that will present part of this work at the Ohio University Student Expo, 2021. With several poster presentations, workshops, and exhibitors at the conference, Plant Biology 2020 will enhance my knowledge on important plant topics and technology coupled with extensive opportunities for networking with scientists from around the world.

Oyeyemi Olugbenga Ajayi

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Academic appointments

2016-present **Teaching Assistant,** Department of Environmental and Plant Biology, Ohio University, Athens, Ohio, USA.

2014-2016 **Lecturer**, Department of Animal Science and Animal Technology
Benson Idahosa University, Benin City, Edo State, Nigeria.

2012-2013 **Visiting Research Scholar**, Cornell University,
College of Animal and Life Sciences, Ithaca, New York, USA.

Academic background

PhD - Molecular and Cellular Biology, Ohio University, Athens, Ohio.
(Anticipated date of graduation: May 2021)
(GPA: 3.91)

M.S. - Animal Breeding & Genetics, Federal University of Agriculture
Department of Animal Breeding and Genetics, Abeokuta, Nigeria.

B.S. - Agriculture, Federal University of Agriculture
Department of Animal Production and Health, Abeokuta, Nigeria.

Relevant course work

Biochemistry I

Biochemistry II

Molecular Biology

Laboratory genomics

Statistical Methods in Plant Biology

Plant genetics

Awards

2019 **College of Arts and Sciences Graduate Student Research Proposal** grant (Amount funded $1000).

2018 First place Ohio University Student and Research Creativity Expo.

2018 **Next Generation Delegate** for the Chicago Council on Global Affairs' Global Food Security Symposium

2012 **Norman E. Borlaug Leadership in Agriculture Program Fellowship** US Agency for International Development, Washington, DC. 2012.

Conferences Attended

2019 American Society of Plant Biologist, Midwest meeting, West Virginia University, WV

2018 American Society of Plant Biologist, Montreal, Canada

2017 American Society of Plant Biologist, Midwest meeting, Purdue University, IN.

Manuscript submitted and under review

Oyeyemi O Ajayi and Allan M. Showalter (2020). Systems Identification and Characterization of β-Glucuronosyltransferases Genes Involved in Arabinogalactan-Protein Biosynthesis in Plant Genomes (*Scientific Reports*)

Some selected conference presentations

1. Oyeyemi Ajayi, Ashton Smith, and Allan Showalter 2019. Genetic knock-out of three beta glucuronosyltransferase genes involved in glycosylation of AGPs impact growth and development. Presented at the student research and creativity expo, 2019, Ohio University

2. Sean McGovern, **Oyeyemi Ajayi**, Ashton Smith and Allan Showalter 2018. *In silico* analyses identify genes involved in pollen development in Arabidopsis. Student research and creativity expo, Ohio University

3**. Ajayi, O.O.**, Kaur**,** D., Vajdich, H., Hoffman, T., Torok, D and A.M. Showater 2017. Effect of Zinc nanoparticles (nZnO) on soybean growth, development, and yield.

Presented at Midwest ASPB Meeting, Purdue University, West Lafayette, Indiana (US), February 4-5.

Professional Affiliations

American Society of Plant Biologist (2017- present)

Service/Outreach

President, New Covenant Campus Fellowship (2018-present)

Science Fair Judge, the Southeast District Science Day, Ohio University, (Athens, Ohio, 2017-2019)

Language Skills

English, Yoruba (read/write)

Other Funding Sources

I have not applied for nor am I receiving funding from any other source at this time.

Budget

Consumable supplies

Objective 1: Sugar Composition analysis			
1	Corning™ Costar™ Spin-X™ Centrifuge Tube Filters		$561.12
2	2 ml Screw Thread Glass Vials, Flat Bottom		$265.36
3	Gold Electrode		$1, 068.21
4	CarboPac™ PA20 Columns		$416.02
	Objective 1 total		**$2, 310.71**
Objective 2: Seed immunolabelling			
1	Primary antibody (see Table 1)		
	CBM3A	($120 per ml, 5 mls)	$600.00
	CBM28	($120 per ml, 5 mls)	$600
	LM2	(5 mls)	$229
	JIM5	(5 mls)	$229
	CCRC-M36	(5 mls)	$540
2	Secondary antibody	(5 mg)	$710
3	Confocal microscope (Usage fee, $15/hr for 5hrs)		$75.00
	Objective 2 total		**$2, 983**

Conference Travel

Plant Biology 2020, Washington, DC July 24-29		
1	Flight (American airline, roundtrip CMH → DCA)	$193
2	Shared hotel room (5 nights, $104.5 + tax per person, per night)	$522.50
3	Conference registration	$535.00
4	Per diem	Provided by the student
5	PBIO Department travel fund	($550)
	Conference travel total	**$700.50**
	SEA proposal total	**$ 5, 994.21**

Justification

Consumable Supplies. *Objective 1* **–** During sugar composition analysis, I will use the **Corning™ Costar™ Spin-X™ Centrifuge Tube Filters** to remove bacteria and other contaminants from my samples. Samples to be analyzed will be placed in **2 ml Screw Thread Glass Vials, Flat Bottom** because the Dionex machine is equipped with robotic arm

autosamplers which are compatible with the vials. The **Gold Electrode** is the most important piece of equipment in the Dionex machine as it serves to detect the simple sugars and quantify their relative abundance. The gold electrode that is currently in the Dionex machine is currently "worn off" and would interfere with the sensitivity of detecting and quantifying simple sugars. Following the advice of my mentor, we agreed to purchase a new one to avoid generating unreliable results. I am using the **CarboPac™ PA20 Columns** because it is compatible with the Dionex machine and it is quick and efficient in separating simple sugars without compromising resolution. The price quotes given were quotes obtained from the supplier's website and are as cost efficient as possible. All other unlisted consumables and equipment necessary to accomplish objective 1 are available in my mentor's lab.

Objective 2 – Funds requested are for the purchase of both primary and secondary antibodies and imaging of the distribution of sugars in the seed coat mucilage. The primary antibody (**see Table 1**) targets specific sugars in the seed coat mucilage. The secondary antibody targets the primary antibody and emits certain signals that can be imaged using the confocal microscope which I already have access to in my department. The amounts of primary and secondary antibodies requested will be enough for the optimization of the protocols and conducting the actual experiments. The price quotes for both primary and secondary antibodies were obtained from the supplier's website and are as cost efficient as possible. I budgeted five hours for visualizing the seeds because I have used the same microscope in other experiments and have had adequate expertise in the use of the microscope. Any unlisted consumable supplies are available in my mentors' labs at no additional cost.

Conference Travel. The flight costs for the Plant Biology 2020 meeting were derived from expedia.com and represents the most cost-efficient prices available. Costs of lodging and conference registration are based on conference hotel rates and fees listed on the Plant Biology 2020 website. The Department of Environmental and Plant Biology annually provides $550 to graduate students for conference travel, which I will use towards travel expenses. The conference provides some beverages and meals and I will pay for the rest with my personal finances.