

# SCW Assay 2 with Ovules

EXP22000556

**Project:** GALY USA**Author:** Hannah Ambrose**Auditors:** Prince Zogli, Luisa Bermudez**Status:** Needs review**Entry Created On:** 06 Sep 2022 16:19:02 UTC**Entry Last Modified:** 16 Sep 2022 14:55:00 UTC**Export Generated On:** 03 May 2023 15:01:19 UTC

## Review History

	User	Timestamp	Action	Comment
1	Hannah Ambrose	16 Sep 2022 14:55:45 UTC	Sent for review	

TUESDAY, 9/6/2022

**Table 1: Complete this table before requesting for Review**

	A	B
1	Quarter	Q3
2	OKR # and title	OKR1- Produce 100kg of Lab Grown Cotton Fiber
3	KR # and title	KR3 - In parallel with KR1 & KR2, develop a protocol to increase SCW content/fiber maturation for cell line screening
4	Name of the Responsible Scientist	Eric G
5	Sprint team members	Tyler M, Hannah A
6	Project Title/Experiment Title	SCW Assay 2 (with Ovule Cultures)

## Scientific Summary

Fibers have been successfully elongated from ovules using the BTOFE media formulation in the past (i.e., the infamous baby clothes), however these fibers have been shown to have little to no SCW development. This experiment aims to utilize this elongation process to obtain fibers that are primed for SCW development. These elongated fibers will be treated with BTOFE media (excluding GA3 & NAA) with added hormones that are thought to induce SCW deposition (H2O2, SHAM, and jasmonic acid).

## Hypothesis

Ovules elongated with BTOFE (GA3 & NAA) will show SCW thickening when treated with SHAM, H2O2, and jasmonic acid as compared to a hormone free control.

**Material and Methods** (List all relevant materials used to accomplish this project. Also provide detailed description of methods used or a link to an existing protocol/SOP. If an established protocol is being used, update this form with any modifications you made while using this protocol).

**Biological Materials** (List all biological materials used for the project)

<b>Table 1 (Biological Materials)</b>				
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
1	<b>Genotype</b>	<b>Cell line (name or description)</b>	<b>Cell type</b>	<b>Media type</b>
2	** unsure of the genotype	BTOFE+ GA3+NAA for ~14 days	Ovule	BTOFE
3				

**Chemical Materials** (List all chemical materials used for the project). It is OK to provide a link to an SOP when available.

<b>Table 2 (Chemical Materials)</b>		
	<b>A</b>	<b>B</b>
1	<b>Chemical</b>	<b>Source</b>
2	Melatonin	<a href="#">Bio Basic</a>
3	Hydrogen Peroxide	<a href="#">Bio Basic</a>
4	6BAP	<a href="#">RPI</a>
5	Ethepron	<a href="#">Sigma Aldrich</a>
6	Jasmonic Acid	<a href="#">Sigma Aldrich</a>
7	Abscisic Acid	<a href="#">Bio Basic</a>
8	Salicilic Acid	<a href="#">Sigma Aldrich</a>
9		

**Methods**

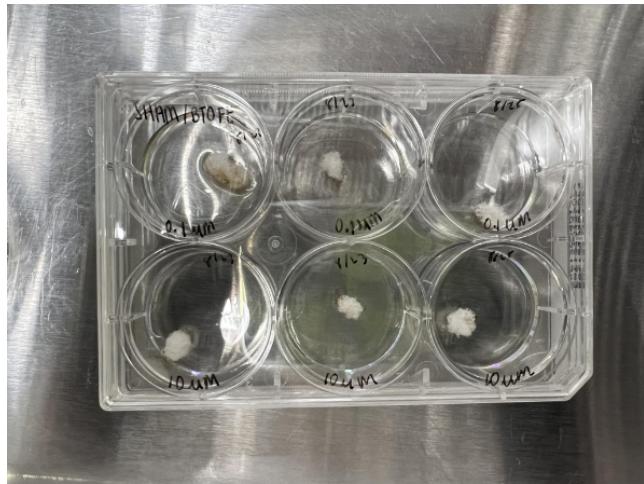
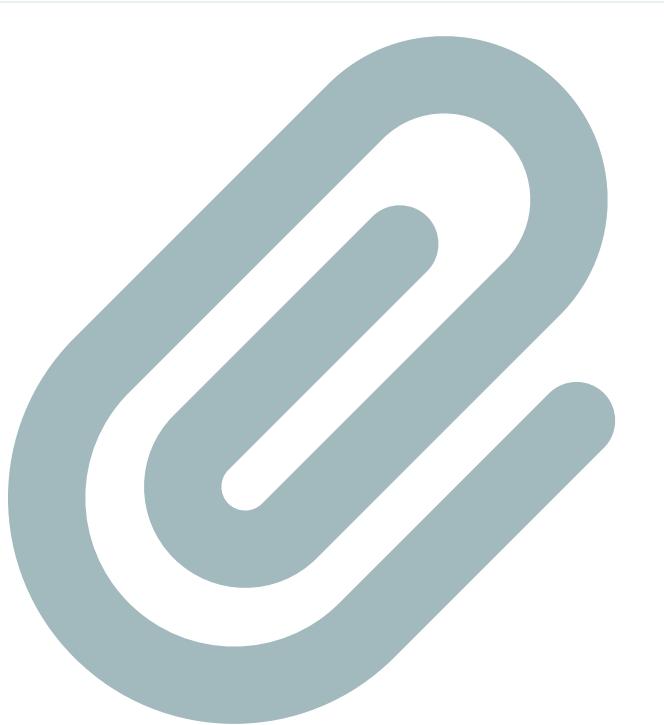
1. Media Preparation
  - a. BTOFE, Modified BT Media with GA3 & NAA (for Elongation)
  - b. BTOFE, Modified BT Media excluding GA3 & NAA (for SCW)
2. Ovule Harvesting
  - a. Ovules were harvested at 0, 1, and 2 DPA
  - b. Common sterilization process was used
    - I. ethanol for 2 min
    - II. 10% bleach for 15 min
    - III. wash with autoclaved water 3x
3. Ovule Elongation
  - a. Ovules were dissected and placed in 24 well plates with BTOFE Media with GA3 & NAA
  - b. Ovules were left in incubator at 34 C for ~2 weeks (14-18 days)
4. Ovule SCW Deposition (hydrogen peroxide, SHAM, and jasmonic acid)

- a. media was prepared at the following concentrations
    - I. BTOFE with 0.1 uM and 10 uM SHAM
    - II. BTOFE with 1 uM and 100 uM H2O2
    - III. BTOFE with 0.1 uM and 10 uM jasmonic acid
  - b. ovules were transferred from elongation to SCW media mentioned above
  - c. ovules were left in the incubator at 34 C for 1 week
5. From each treatment, one ovule was used for anthrone quantification and 1 was used for birefringence

\*\* Note: moved onto SCW media on 9/6/22



image of control ovules, harvested on 8/19, 8/25, and 8/23







**3. Results (Please include conclusions from both positive and negative data, do not omit anything).** Tables, figures, and relevant observations. Also provide link(s) to raw data as needed.

**Part 1: Data outputs/Deliverables: List all key data outputs required to achieved Objectives**

Anthrone

Birefringence

**Part 2: Provide the key result(s) as listed in part 1**

Anthrone Quantification of Glucose in Each Sample			
	A	B	C
1	Treatment	Concentration (uM)	Avg. Glucose Concentration (nM)
2	Jasmonic Acid	0.1	674.2
3	Jasmonic Acid	10	1064.9
4	Hydrogen Peroxide	1	936.7
5	Hydrogen Peroxide	100	1204.733
6	SHAM	0.1	378.4
7	SHAM	10	326.1
8	Untreated Control	NA	438.1

^



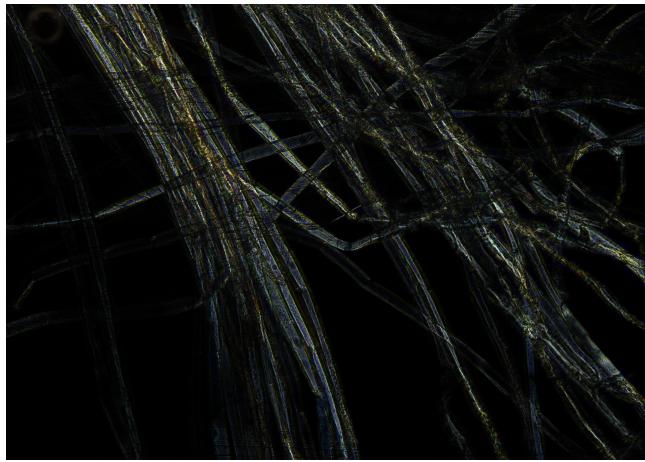
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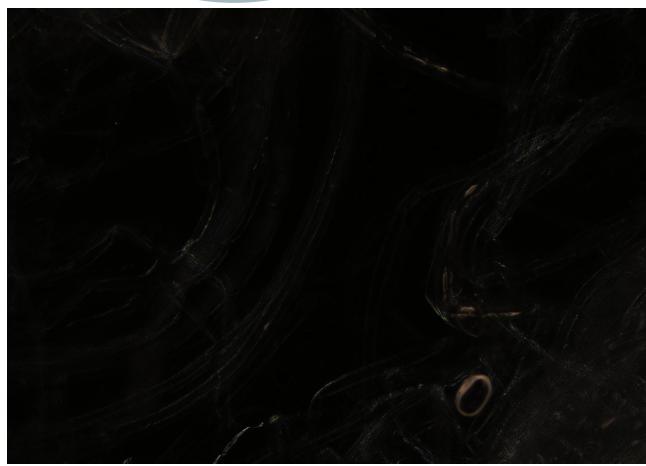


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#### 4. Major conclusions (Provide conclusions based on the results above).

Jasmonic acid at 10 uM and H<sub>2</sub>O<sub>2</sub> at 100 uM gave the best birefringence and highest glucose concentration.

#### 5. Outline the next steps (for final progress reports, include any fiber development process that resulted from this study and what the next steps are)

- Performing a follow up experiment with ovules at higher concentrations of hydrogen peroxide and jasmonic acid (100-500uM H<sub>2</sub>O<sub>2</sub> and 10-50uM JA) because the higher concentrations showed more SCW development (see [SCW Assay 3, Ovules](#) )
- SHAM will also be tested in the follow up experiment, because literature states a much higher concentration of SHAM than what was used in this experiment to induce SCW development (1-5mM)

**6. Scientist contributions(Please complete this table)**

List all scientist involved in this project and extent of their contribution towards completion of the objective(s)

**Table 3: Scientist role and effort towards achieving research objective**

	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
1	Name	Title	Role on Project	% Effort /extent of contribution
2	Hannah Ambrose	Research Associate	Experimental Design/Set up/ Analysis	50
3	Tyler Maxwell	SRA	Experimental Design/Set up/Analysis	50
4				
5				