Experiment-Transformation of protoplast derived from hypoctyl

EXP23000104

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WEDNESDAY, 2/22/2023

Title: Transformation of protoplast derived from hypocotyl

Objective:

Replicate existing protoplast transformation protocols

Justification:

We have been trying to transform protoplast derived from callus and liquid culture, with little to no success. Published cotton protoplast transformations are done with differnt cells, such as cultures derived from hypocotyl and root. Our cultures were derived from ovules many generations ago and may thus be far less competent than these other cultures.

List of Equipment and Materials

This field will have all of the company's used equipment and this will help us make a decision if we need more equipment or if some equipment is underutilized. If you know we don't have a specific material/equipment, please include a price range and a website or supplier where this item can be purchased. If you need a specific make or model, please include that as well. Try to reference reagents and equipment already present in our directory.

Detailed Methodology:

Seed Sterilization

Materials

- FJA seeds
- MS Media
- MS + Agar
- Petri dishes
- Soap
- 70% EtOH
- 3% hydrogen peroxide
- autoclaved diH2O
- Forceps

Protocol

1. Soap and water wash
    a. In the sink
2. Rinse with EtOH 70% for 1 minute
    a. In a 50mL Falcon tube in the Biosafety Cabinet

   b. Pour out EtOH
3. Hydrogen Peroxide (3%) sterilization
   a. Add hydrogen peroxide to the 50mL falcon tube
   b. Leave in biosafety cabinet for 4 hours
4. Rinse with autoclaved diH2O
5. Plate on MS+Agar media, wrap in aluminum foil, and place in 25°C incubator for 2-3 days
6. Unwrap plates, place in incubator with 12hr light/12hr dark

Data analysis:

Describe the steps of the analysis, preferably including citations from the literature.

Others:

This is space is for any other additional information.

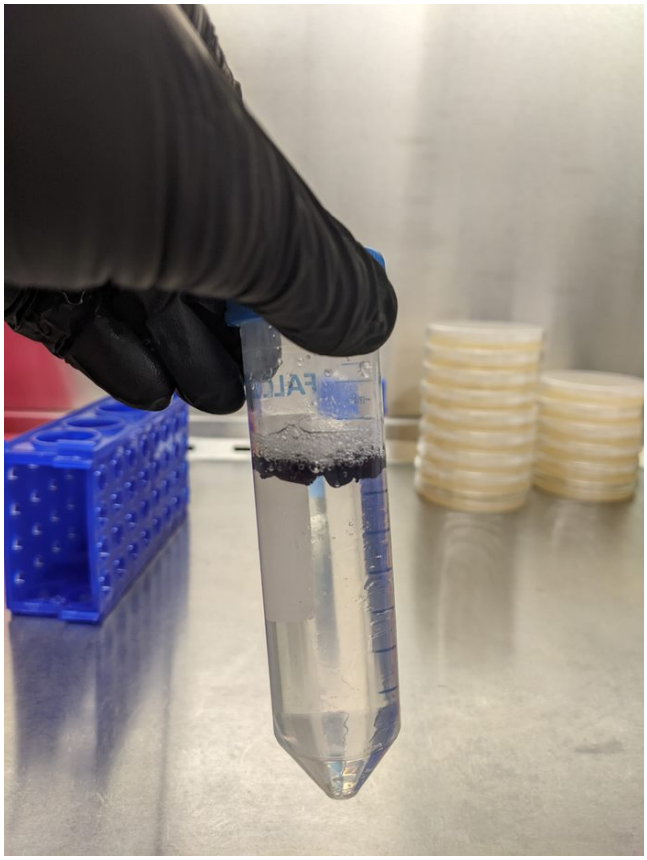
References:

Method adapted from:

Wang et al 2022. "A Rapid and Efficient Method for Isolation and Transformation of Cotton Callus Protoplast." doi: 10.3390/IJMS23158368

Results:

On 22-Feb-23 performed steps 1-5. Seeds were covered in some kind of purple powder when taken from the envelope. The powder didn't come off in the soap and water washing step, but some leached during the 70% ethanol wash.
Seeds also sank during the initial stages, generally considered to be a sign of viability, but after being in the hydrogen peroxide (3%) they floated, even after rinsing with distilled water several times. This may be due to hydrogen peroxide producing bubbles.



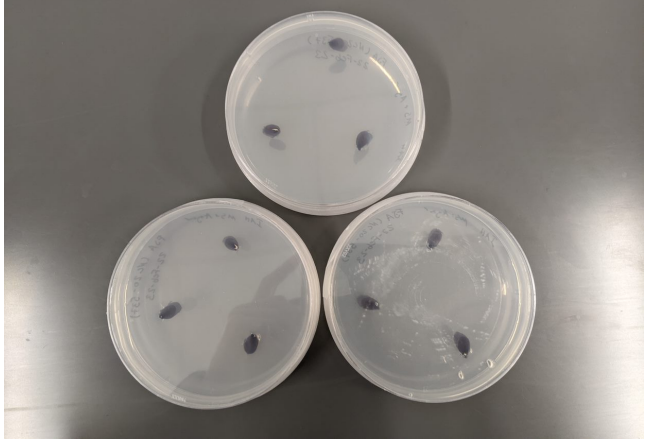
Seeds floating after the hydrogen peroxide incubation.

Time line:

Description of activities per day, following the mode (Use "Insert" > 'New day", or Ctrl + /

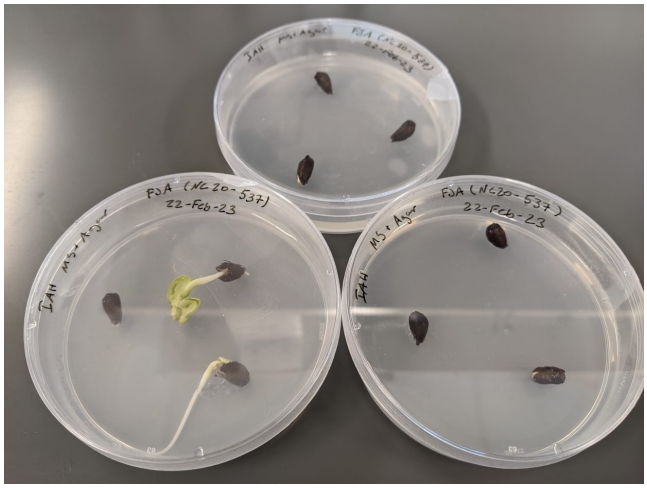
FRIDAY, 2/24/2023

At about noon, took the FJA seeds out of the foil and placed them in a 12hr/12hr incubator in the bioreactor room. Some seeds had begun to open.

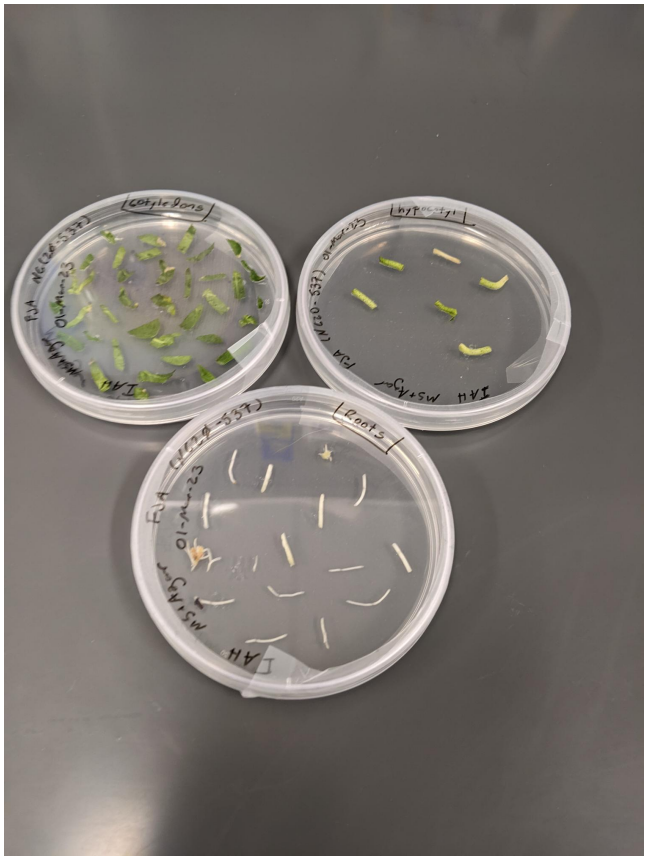


MONDAY, 2/27/2023

Took a look at the seeds. Some have sprouted and the hypoctyl has begun elongating. Others just emerging, and a couple still not emerging.



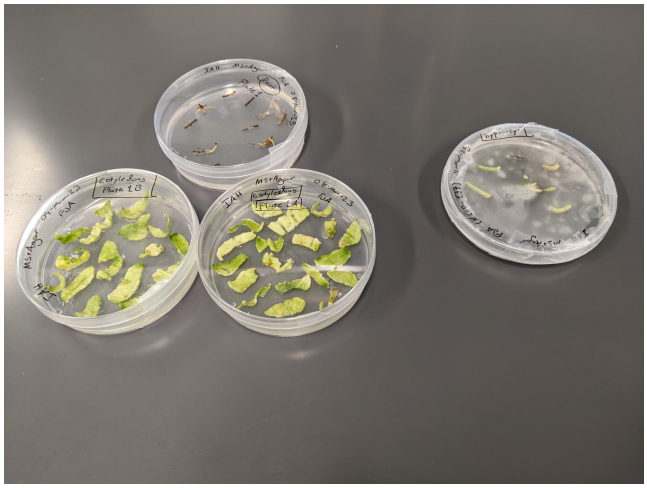
WEDNESDAY, 3/1/2023



Sliced up the first two seedlings and plated cotyledon, hypocotyl, and roots on separate plates. Plates were then put om the culturing room incubator where the callus cultures are stored.

Transferred the remaining seeds to magenta boxes with MSA. Wrapped those boxes with aluminum foil and placed back in 35°C incubator

WEDNESDAY, 3/8/2023

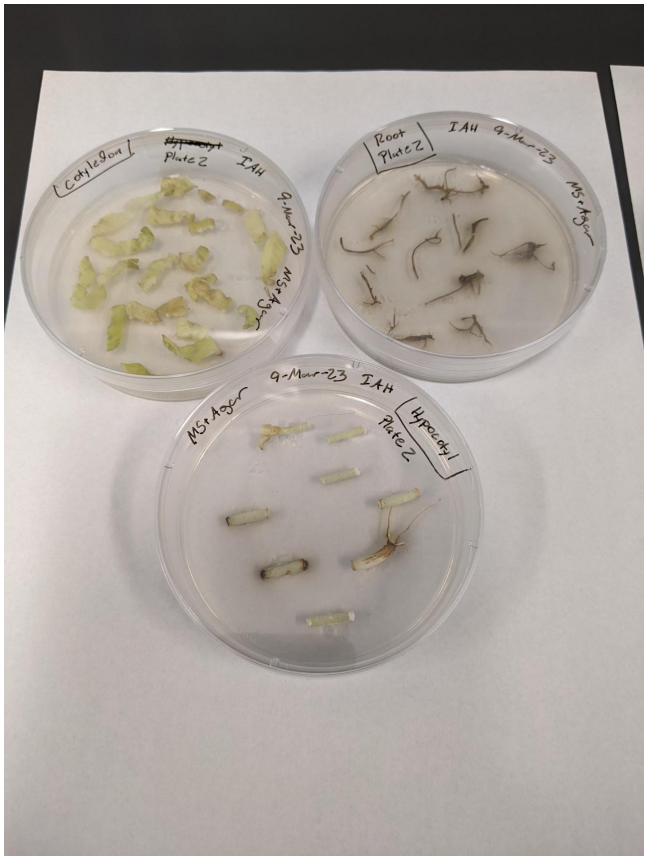


Transferred cotyledon and root cuttings to new media. Divided cotyledons into two plates because they had greatly expanded and gotten crunchy. Roots were discoloring the media. Left the hypoctyl as they seemed fine.

Two other seedlings got quite tall in the magenta boxes. Kept those wrapped in foil. Remaining seeds left unwrapped to see if that would encourage germination.

THURSDAY, 3/9/2023

Plated explants from the two additional seedlings


Cotyledon
Plate 2
IAH
MS+Agar
Root
Plate 2
IAH
MS+Agar
9-Mar-23
IAH
Hypocotyl
Plate 2
MS+Agar

MONDAY, 3/20/2023

Replated explants. On the hypocotyls cut off the cultures at the ends to encourage growth.


Plate 1 A
cotyledon
FJA
IAH
20-Mar-23
Plate 1B
cotyledons
Plate 1
hypocotyl
Plate 1
root
MS + 2% Suc


FJA
Plate 2
Cotyledon
20-Mar-23
I4H
MS+Agar
FJA
Plate 2
Hypocotyl
I4H
20-Mar-23
MS+Agar
FJA
Plate 2
Root
20-Mar-23
I4H
MS+Agar

Additionally, sterilized 20x seeds of both FJA and Coker 312 to produce more cultures. Left in diH2O overnight.

WEDNESDAY, 3/22/2023

Seems I screwed up the MSA media. I forgot to add hormones or vitamins. So made new plates (MS+KD) and transferred the explants.

Additionally, transferred sterilized seeds to Magenta boxes with MSA. Placed in dark incubator.

FRIDAY, 3/24/2023

Moved seeds in magenta boxes to lighted incubator.

MONDAY, 4/3/2023

Started new explant cultures from Coker 312 and FJA

TUESDAY, 4/11/2023

Started three additional Coker 312 explant cultures

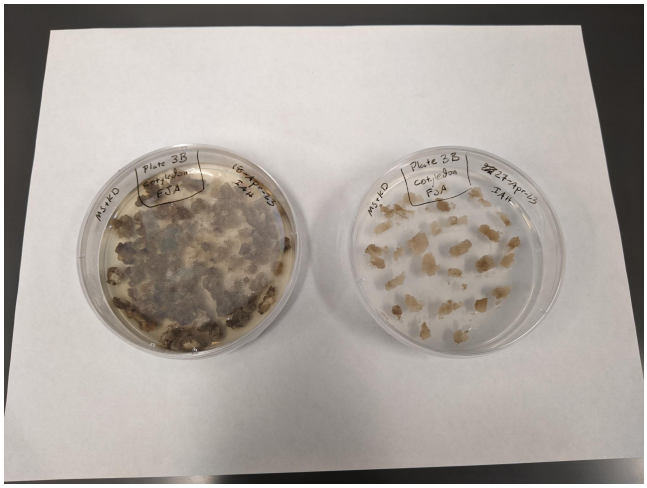
TUESDAY, 4/25/2023

First attempt at isolating protoplasts from seedling derived callus. Used Plate 2 (exp.proto.0035)
4x cotyledon, 2x hypocotyl, 2x root

Used Method 1 (Wang et al 2022) Protoplast Isolation SOP - 2023

Yields very dirty. Also ran out of W5 solution, so ended up washing in MMG media instead.

THURSDAY, 4/27/2023



Moving good-looking callus from the discolored stuff. Hopefully will get some cleaner results this way.