Agar-plating as a mechanism for screening low germination of T-DNA insert lines

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Background

• Certain situations call for sterile conditions to grow *Arabidopsis thaliana*, and can include but are not limited to:
  • lethal mutants
  • early stage root and shoot phenotyping
  • mutations that require high humidity[1]

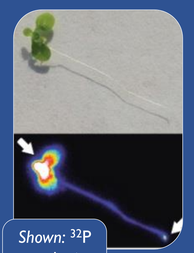
• Environment can be uniformly manipulated to include, but not limited to:
  • salt treatments
  • heavier metal treatments[2]
  • radioactive additives
  • nutrients

• Nutrient load within the medium can be evaluated before and after experiments to determine the uptake.

• Future work includes an examination of t-RNA using radioactive isotope ^{32}P via the agar growth methods here.

Special thanks to Dr. Renaud Geslain.

Shown: LED light system and agar wells.



Shown: ^{32}P uptake in germinant

In comparison to Pro-Mix

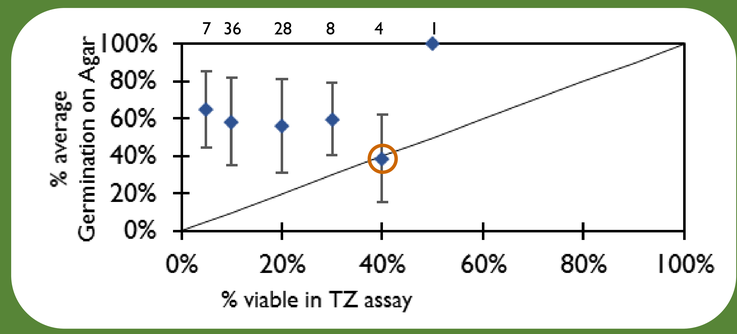
Pros	Cons
High germination rate	Requires increased time due to agar and seed preparation
Homogeneous medium facilitates greater testing uniformity	More expensive than traditional Pro-Mix germination
Provides more even water absorption by seeds	Not easily accommodated by all lab testing schemes
Media additives (e.g. Nitrogen, Phosphorus etc.) are easily incorporated during agar prep	For full plant growth, transplanting to traditional medium is required

Seed viability in farming Arabidopsis

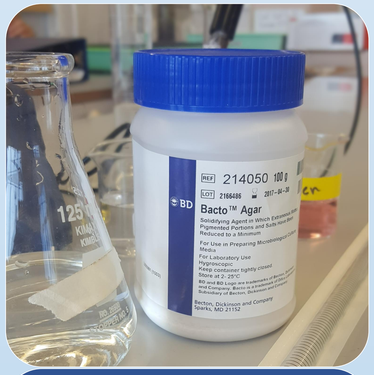
• SALK lines with no germination after three farming attempts were selected for the initial agar farms.

• Agar has been successfully used to grow Arabidopsis, yet was ineffective for tissue collection in prior attempts at this institution.

• Tetrazolium assays were performed to determine the viability of the seed stock according to the procedures set forth by Porter and Durrell[3].

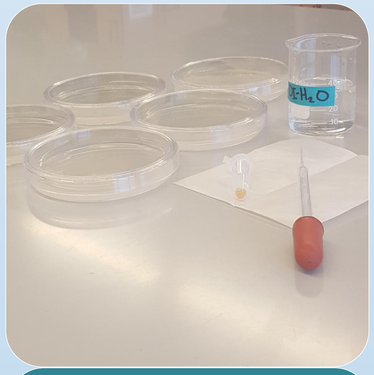


- Tetrazolium assays were not a sufficient prediction of seed viability in T-DNA insert lines.
- Red circle= contained a strain with a particularly low rate of germination.



Preparing Agar

- BactoAgar
- Murashige and Skoog Basal Salt Mixture (for nutrients)
- pH meter
- Autoclave access
- Laminar flow hood / Local exhaust vent access



Plating Seeds

- Sterilized seeds applied to agar using glass pipette.
- *Technique Tip*: Use a little water to transfer seed and remove water with pipette to ensure adhesion of seeds.

Grow Phase

- Seal edges with Micropore™ Tape.
- Grow at a 16 hour light cycle in growth chamber.
- *Problem Solving*: Use an overturned insert to hold the petri dishes.

Germination Phase

- The germinants are visible within 3 days.
- Take data at germinant phase.
- Small plants are ready for transplanting after 14 days.

Finish Project

- Small germinants can also be phenotyped at the two week stage for early root and shoot development.
- Transplant into potting mix and grow out for seed bulking.
- Take tissue sample if required by project plan.

[1]Bowling, S.A., Guo, A., Cao, H., Gordon, A. S., Klessig, D. F., & Dong, X. (1994). A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance. *The Plant Cell*, *6*(12), 1845-1857.
[2]van der Weele, C. M., Spollen, W. G., Sharp, R. E., & Baskin, T. I. (2000). Growth of Arabidopsis thaliana seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany*, *51*(350), 1555-1562.
[3]Porter, R., Durrell, M. and Romm, H. (1947). The use of 2, 3, 5-triphenyl-tetrazoliumchloride as a measure of seed germinability. Plant Physiol 22(2): 149.

Where did the Pirate sow his seeds?
In the ag-ARRRRRR.