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TRANSGENIC PLANT STRESS RESPONSE IN STRONG MAGNETIC FIELDS AND IN MAGNETIC LEVITATION (LOW GRAVITY) ENVIRONMENTS

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MOTIVATION

WHAT?

Establish: long-term [> 1 - 10 min. , < 8 hrs. (or 3.5 days!)],
low gravity [< 10 - 100 milli-g , $g = 9.8\ m/s^2$]
Earth-based environment for:
(a) plant growth? (b) protein crystallization? (c) other uses?

WHY?

To improve upon Earth-based low gravity experimental environments
(i.e. improve upon conditions available on NASA's KC-135 parabolic flight aircraft. Shuttle flights are costly!)

How?

Plants (*i.e.* mostly H_2O), Proteins, Plastics, many others are diamagnetic, so . . . .

Try Magnetic Levitation!

Assume (initially) that magnetic fields and gradients:
(a) do not effect plant growth and development?
[O.A. Kuznetsov, K.H. Hasenstein, Planta 198 (1996) 87]
[K.H. Hasenstein, O.A. Kusnetsov, Planta 208 (1999) 59]
(b) might help self-assemble macromolecules?

Biophysical Effects of Magnetic Levitation:
"Magnetic Levitation of *Xenopus laevis*: Toward Low Gravity Simulation", J.M. Valles Jr. *et al.*, *Biophys. Jour.* **73** (1997) 1130.
"Cleavage Planes in Frog Eggs Altered by Strong Magnetic Fields", J.M. Denegre *et al.*, PNAS 95 (1998) 14729.

MAGNETIC LEVITATION

Milligravity in a High Magnetic Field

Figure 1. Photograph of magnetically levitated plant (transgenic *Arabidopsis thaliana*), looking down the magnet bore.

|← ~ 50 mm →|

Control	Center Field Control	Magnetic Levitation
$B \leq 0.1$ mT $B\nabla B = 0$	$B = 18.9$ T $B\nabla B = 0$	$B = 14.4$ T $B\nabla B = 1708\ T^2/m$

(Reprint: www.phys.ufl.edu/~meisel/arabexp.htm)

Figure 2. Qualitative results from first run (Sept. 98) after staining of intact plants experiencing 2.3 hrs of the parameters given.

EXPERIMENTAL DETAILS

- Superconducting NMR magnet at UF: 0-9 Tesla (null results)
  Resistive magnets at NHMFL: 0-25 Tesla
- Plants 19-21 days old are placed in homogeneous magnetic field for a fixed time period (usually 2.5 hours).
- Plants are removed from field and frozen for later quantitative assay or stained for qualitative assay.
- Qualitative Assays performed 2/2000

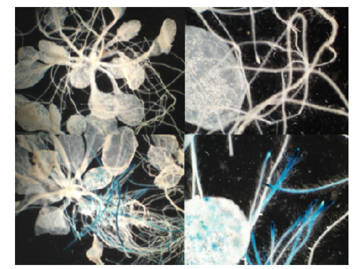
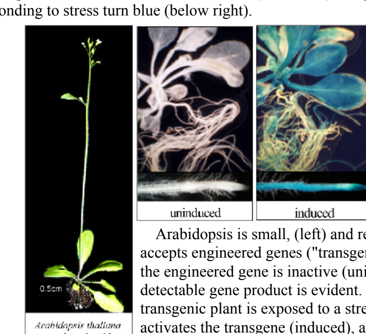


Figure 3. Top row: Control; bottom row: 21 Tesla for 2.5 hours

TAGES - Plant Biomonitors of Microgravity

Transgenic Arabidopsis Gene Expression System

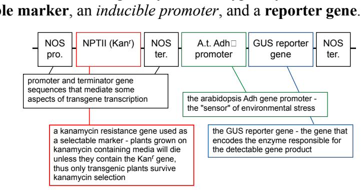
Arabidopsis plants have been engineered with a "sensor " (the *Adh* gene promoter) that monitors cellular physiology and stress and a "reporter gene" (encoding beta-glucuronidase - GUS) that can generate a signal visible to researchers. When stress, such as hypoxia or microgravity, is detected by the plants, the promoter induces the expression of the reporter gene which in turn makes a product that turns blue when incubated with the proper substrate (x-glucuronic acid). In other words, plants that are not stressed do not show a color change when incubated with the substrate (below left), but plants that are responding to stress turn blue (below right).



Arabidopsis is small, (left) and readily accepts engineered genes ("transgenes"). When the engineered gene is inactive (uninduced), no detectable gene product is evident. When a transgenic plant is exposed to a stress that activates the transgene (induced), a gene product is produced which can be visualized with histochemical reagents.

THE TRANSGENE OF THE TAGES PLANTS

Transgene–an artificial gene construct that has been introduced into a new host organism; a transgene is usually a combination of several different components that creates a gene system that typically contains three things: a **selectable marker**, an *inducible promoter*, and a **reporter gene**.



Magnetic Levitation: The Basics

(Not of the Superconducting or Similar Variety)

Diamagnetism: M. Faraday (1846)
Magnetic Levitation of Graphite: W. Braunbeck (1939)
Magnetic Levitation of Organic Materials: E. Beaugnon and R. Tournier (1991)

$$\vec{F} = (\vec{M} \bullet \vec{\nabla})\vec{B}$$

F ≡ force acting on the body M ≡ magnetic moment
B ≡ external magnetic field M = (c/mo) (Volume) B
ρ ≡ density of the material χ ≡ magnetic susceptibility

Force due to gravity: (mass) $g = \rho$ (Volume) g

Balances the magnetic force when material is diamagnetic (i.e. $\chi < 0$); and

$$B_z \nabla_z B_z = \frac{\mu_o \rho g}{\chi}$$

(assuming ideal conditions along the z-direction of solenoid magnet)
(hypergravity available too!)
H_2O levitates at [B dB/dz] ≈ 1400 T^2/m
Graphite levitates at [B dB/dz] ≈ 375 T^2/m

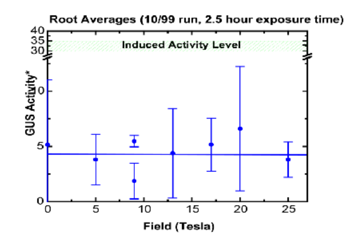


Figure 4.

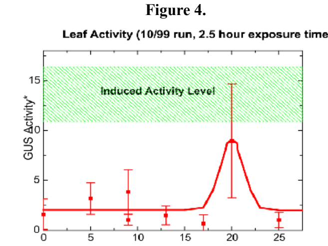


Figure 5.

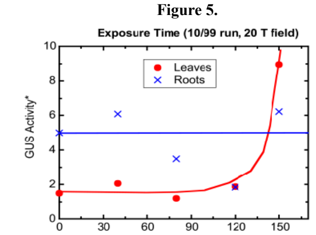


Figure 6.

QUANTITATIVE BIOCHEMICAL ASSAY DETAILS

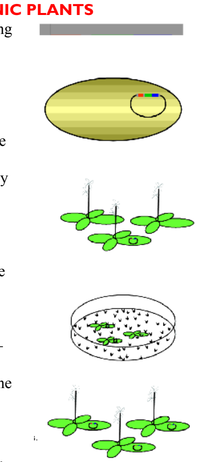
Leaf and root tissues for each plant are assayed separately, and each data point on the plot is an average of three plants. The induced activity level (generated by flooding) is indicated in green in the graphs. GUS activity units are nmol 4MU/_g protein/minute. See Figures 4, 5, and 6.

Root Activity: The GUS activity levels in the root tissues are approximately one sixth of the activity levels found in flooded plants. The observed response is relatively independent of magnetic field, as indicated by the blue line.

Leaf Activity: At 20 T, the activity levels in the leaves approach the level of the activity in flooded plants. The red line indicates the trend of the data to date. Qualitative data at 18.9 T and 21 T are consistent with the trend represented by the red line. The low response level at 25 T may be due to internal processes being completely disrupted by the magnetic field and simply shutting down.

Exposure Time Activity: The GUS activity in leaf tissue increases with the amount of exposure time. The trends in the data for the root and leaf activity levels are qualitatively represented by the blue and red lines, respectively.

THE BASICS OF MAKING TRANSGENIC PLANTS



1. Construct a transgene suitable for addressing the question of interest.
2. Clone the gene into the bacterium Agrobacterium tumefaciens – a type of bacteria that naturally infects many plants and introduces its own DNA into the plant host to create a suitable microhabitat for the bacterium. Commercial strains of Agrobacterium have been created especially for this type of gene transformation.
3. Infect Arabidopsis plants with the Agro. containing the transgene – the bacteria will introduce the transgene into the plants genome, creating a transgenic plant (we use the vacuum infiltration method).
4. Let all the putative transgenic plants make seed. Collect the seed, spread out on germination media containing kanamycin – only transgenic seeds will grow since a kanamycin resistance is part of the transgene contruct.
5. Grow the first generation transgenic plants and collect the seed; the second generation seed can be used for subsequent experients.

Figure 7. Typical specimen.

SUMMARY

- Evidence for a resonant-type stress response to homogeneous magnetic fields (17 T < B < 25 T) in leaves
- Null response in roots

PRELIMINARY HYPOTHESES

- Orientation of macromolecules during gene regulation?
- Interruption of electron transfer processes?

REFERENCES

"New Opportunities in Science, Materials, and Biological Systems in the Low Gravity (Magnetic Levitation Environment" J.S. Brooks *et al.*, to be published.

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Copies and Video: www.phys.ufl.edu/~meisel/maglev.htm, www.magnet.fsu.edu/levitation/index.htm
Plant Reprint: www.phys.ufl.edu/~meisel/arabexp.htm

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