Transgenic Arabidopsis Plants as Monitors of Low Gravity and Magnetic Field Effects

T. F. Stalcup, J. Reavis, and J. S. Brooks
Department of Physics and the National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL 32306-4005, USA

A.-L. Paul and R. J. Ferl
Department of Horticultural Sciences and the Biotechnology Program, University of Florida, Gainesville, FL 32611-0690, USA

M. W. Meisel
Department of Physics and the National High Magnetic Field Laboratory, University of Florida, Gainesville, FL 32611-8440, USA

Magnetic levitation experiments have been performed on arabidopsis plants equipped with a stress-responsive reporter gene system. Seedlings were levitated in a magnetic field gradient ($B = 14.4$ T and $B\nabla B = 1708$ T^2 m^{-1}) for 2.3 hours, and control specimens were located at the center of the magnet ($B = 18.9$ T) and in a low field ($B \leq 0.1$ mT) region. The reporter gene qualitatively indicates that the levitated and the magnetic field control specimens were differentially stressed, while the low field controls indicated no stress. These results suggest the need for more extensive experiments to determine the molecular consequences of levitation and high magnetic fields on plant growth and development.

A number of significant problems must be solved before humans will be able to spend long periods of time in space. One major issue is the establishment of a sustainable food supply since, to date, plants cultivated in low gravity possess reduced growth characteristics, particularly with regard to seed set.[1] Consequently, a large number of plant growth experiments have been sponsored, and new programs are in queue.[2,3] For example, a group at the University of Florida is experimenting with arabidopsis (*Arabidopsis thaliana*[a]) as a model system for studying the effects of low gravity conditions on plant growth and gene regulation. Transgenic arabidopsis plants containing the *alcohol dehydrogenase* (*Adh*) driven GUS (β-glucuronidase) gene are being used to monitor the effects of reduced gravity on gene regulation of plant metabolism. In general terms, the reporter gene that has been engineered into the plant responds to stress (*e.g.* hypoxia, cold, reduced gravity) which initiates a chemical reaction that results in a color change (colorless to blue) when incubated with a specific

[a] *Arabidopsis thaliana* is the *Latin* name for a mustard plant which is fast-growing and easily transformed genetically. Consequently, this system is ideal for transgenic analyses and has been well-characterized at genetic and physiological levels.

exogenous substrate (x-glucuronide). By monitoring the color changes, specific stresses can be identified, and preliminary experiments have been performed on NASA's KC-135 turbojet, which provides a milligravity (milli-g) environment. Plants experiencing parabolas exhibited marked increase in expression of the reported gene, indicating the onset of stress inductions similar to those reported from shuttle missions. In fact, the degree of induction of reporter gene activity was higher than that reported for *Adh* induction during shuttle flights.[4] Due to the parabolic nature of the KC-135 flights, the experiments experience an equal amount of milli-g and 2-g forces, so control investigations have been performed at the high-g centrifuge at the Ames Research Center. The results of the experiments on the KC-135, which have provided nominally 25 minutes of total time in a milli-g environment, confirm the ability to test the activation of various genes via this transgenic reporter gene technique. However, longer term Earth-based testing would contribute to our understanding of plant growth in low-g conditions and would help optimize the design of experiments that will eventually fly on the space shuttle which experiences between 0.1 to 10 milli-g while in orbit.[1] Magnetic levitation is an established technique[5,6,7,8] which can supply the longest term Earth-based low-g environment [1] but, to our knowledge, has not been used previously to study plant growth as a part of a space biology program.

Naturally, the crucial difference between the magnetic levitation and space shuttle environments is the presence of a strong magnetic field ($B \leq 21$ T) and gradients ($B\nabla B \approx 1800$ T^2 m^{-1}).[5,6,7,8] Other researchers have studied the growth and development of wild-type and TC7 starchless mutant arabidopsis in strong gradients ($B\nabla B \approx 60 - 530$ T^2 m^{-1}) using medium strength magnetic fields ($B \approx 0.5 - 0.8$ T).[9] Some abnormal changes in root curvature were measured in wild-type arabidopsis, although no growth variations were detected in TC7 arabidopsis. These gradient strengths are similar to those needed for magnetic levitation, and this earlier work suggests that magnetic fields and gradients associated with levitation may not adversely effect some types of arabidopsis. With this background, we were motivated to conduct a magnetic levitation experiment using arabidopsis.

In preparation for the high magnetic field experiment, we measured the magnetic magnetization of wild-type arabidopsis (14 days old) in fields up to 5 T at 295 K. The studies were performed on whole plants, leaf/shoot tissue, roots, and distilled water. Using standard mathematical models,[6,7,8] the susceptibility values allow us to estimate the gravitational forces that the different parts of the plant might experience while being magnetically levitated. The results indicate that the leaf/shoots may experience about -50 milli-g (the negative sign represents levitation) while the roots may experience about

10 milli-g. These somewhat coarse estimates compare favorably with the low-g environment ($\leq$ 100 milli-g) that was established on the parabolic flights.

On 19 September 1998, three arabidopsis plants (4 weeks old) were magnetically levitated for a period of 2.3 hours using the Keck magnet of the NHFML in Tallahassee. This growth window represents a significant fraction of the development time of arabidopsis which goes from a seed to mature plant in approximately 6 weeks. Moreover, 2.3 hours is a meaningful time frame in which to study gene activation phenomena, as stimulus-dependent regulatory events can be detected within tens of minutes. Equilibrium levitation conditions required $B = 14.4$ T and $B\nabla B = 1708$ T^2 m^{-1} at the point where the plants were free floating. At the same time, three plants were located at the center of the magnet ($B = 18.9$ T), and an additional three plants were kept in a low magnetic field region ($B \leq 0.1$ mT). All three experimental regions experienced similar conditions with respect to lighting and temperature (nominally 295 K). After the 2.3 hours, the samples were stained and transported to Gainesville. After staining, the plants were placed in 70% ethanol to remove chlorophyll and other endogenous pigments, so the blue color of the reporter gene would be clearly visible. The results are shown in Fig. 1.

Figure 1: Typical results after staining of intact plants experiencing 2.3 hours of: (left) low field conditions of $B \leq 0.1$ mT, (center) constant magnetic field of $B = 18.9$ T and $B\nabla B \approx 0$, and (right) magnetic levitation in $B = 14.4$ T and $B\nabla B = 1708$ T^{2}m^{-1}. The plants experiencing the large magnetic field environments (center and right) show stress response as indicated by the blue (*i.e.* dark) stain, while the low field control (left) shows significantly weaker stress response. Furthermore, the magnetically levitated plants (right) possess a higher level of expression of the reporter gene than the specimens in the constant magnetic field (center). This last result is not obvious in these pictures, but it is clearly discernible in the full color versions, see http://phys.ufl.edu/$\sim$meisel/arabexp.htm.

Our experiments qualitatively suggest that the arabidopsis plants were differentially stressed by the high magnetic field and low-g environments. For plants, such a significant response to the presence of a strong magnetic field has not been reported in the literature. However, our results are reminiscent of the ones reported by Valles *et al.*,[7] who have reported that cell division planes in *Xenopus laevis* embryos align with a strong magnetic field ($B \geq 10$ T). Similar effects may be occurring in arabidopsis, but further studies are necessary to confirm this hypothesis. In conclusion, it appears that low gravity environmental effects may be induced by magnetic levitation, albeit on a "*background*" response generated by the strong magnetic field. The magnetic effects need to be studied and understood if high magnetic field ($B \geq 20$ T) MRI is going to be used to image *in vivo* gene regulation, an application proposed for the new generation of GHz NMR facilities.[10] Clearly, more extensive experiments are needed to determine the molecular consequences of levitation and high magnetic fields on plant growth and development.

This research was sponsored, in part, by the National Science Foundation, through support of the National High Magnetic Field Laboratory, and the W. M. Keck Foundation. The Space Biology research program of RJF is support by a grant from NASA (NAG 10-0145).

1. D. Moore and A. Cogoli, in *Biological and Medical Research in Space*, ed. D. Moore, P. Bie, and H. Oser (Springer-Verlag, Berlin, 1996) pp. 1-106.
2. *Program and Abstracts of the 13th Annual Meeting of the American Society for Gravitational and Space Biology*, Grav. Space Bio. Bull. **11**, 1-82 (1997). The ASGSB may be accessed at http:// baby. indstate.edu:80/asgsb/.
3. *Plant Biology in Space: Proceedings of the International Workshop*, ed. A. Sievers, B. Buchen, and T. K. Scott, Planta **203**, S1-S216 (1997), and references therein.
4. D. M. Porterfield, S. W. Matthews, C. J. Daugherty, and M. E. Musgrave, Plant Physiol. **113**, 685 (1997).
5. E. Beaugnon and R. Tournier, Nature **349**, 470 (1991).
6. E. Beaugnon and R. Tournier, J. Phys. III France **1**, 1423 (1991).
7. J. M. Valles Jr., K. Lin, J. M. Denegre, and K. L. Mowry, Biophys. Jour. **73**, 1130 (1997); and private communications.
8. M. V. Berry and A. K. Geim, Euro. Jour. Phys. **18**, 307 (1997).
9. O. A. Kuznetsov and K. H. Hasenstein, Planta **198**, 87 (1996).
10. *High Field NMR: A New Millennium Resource*, Conference sponsored by the NHMFL, Washington, D.C., 15-16 January 1998; see http://www.magnet.fsu.edu/whatsnew/nmrcoll.