Biomagnetics: Effects on transcription associated with *Adh*/GUS and *Adh*/GFP promoter-reporter systems in *Arabidopsis* due to static magnetic fields up to 9T

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1. Abstract

Static magnetic fields produced by an Oxford Teslatron Magnet at 9 T do not alter observable expression in genetically altered *Arabidopsis thaliana*. These plants are engineered to report transcriptional alterations by means of fluorescence or pigment expression. These visible signs are made possible by the transcription of the promoter, *alcohol dehydrogenase* (*Adh*) that promotes the reporting of Green Fluorescent Protein (GFP (fluorescence)) and β-glucuronidase (GUS (pigment expression)) [1,5]. The results of the experiments performed in the laboratories of Drs. Mark Meisel, Anna-Lisa Paul, and Robert Ferl show that a magnetic field of 9 T, applied over a period of 48 hours, does not provide a strong enough field to cause the transcription of *Adh*. If this promoter is not turned "on," then the activation of the reporting genes will not occur [1]. After performing four separate experiments, it was found that a magnetic field of 9 T does not affect the physical act of fluorescence; it also does not allow *Adh* to promote either GUS or GFP when initially inactive; and finally, a magnetic field of 9 T does not disable the ability of *Adh* to block the expression of GFP when initially fluorescing.

2. Introduction

One general inquiry that has been brewing around the scientific areas of physics and biology is the potential ability of magnetic fields to alter the genetic coding of living organisms. In the University of Florida laboratories of Anna-Lisa Paul, Robert Ferl, and Mark Meisel, the main concentration of this general premise has revolved around the plant species *Arabidopsis thaliana*. This species is a well-researched plant that has proven to have an effective protocol for regeneration, has a well detailed genetic map, and has had a significant portion of its genome identified [4].

Using this plant, the overall goal is to identify any transcriptional changes associated with an applied static magnetic field of 9 T or less. This goal can be accomplished through the use of two types of promoter-reporter systems. Past research has shown that the Arabidopsis plant can survive in certain stressed environments, such as darkness, flooding, coldness, and hypoxia through chemical processes. During such processes, the gene promoter, *alcohol dehydrogenase* (*Adh*) is triggered "on" through altered transcription and causes the reporter beta-glucuronidase (GUS) to be expressed in the first type of promoter-reporter system and Green Fluorescent Protein (GFP) to be expressed in the second type [1]. These systems are built through genetic engineering and allow for the physical macro-level observation of the presence of genetic and enzymatic processes occurring at the level of electron microscopy.

In my research, these systems will be utilized through a range of experiments that will allow for the detection of transcriptional changes. In the first experiment, I utilize Arabidopsis plants that have been engineered to always fluoresce. In this experiment, the primary objective is to identify any alterations in the actual emission of fluorescing light

in the presence of a magnetic field. If the intensity of the fluorescence dissipates to a great degree with increased magnetic field, then it will be impossible to decipher whether the change in fluorescence of subsequent experiments is due to transcriptional alterations or physical alterations of the light itself. In succeeding experiments, both promoter-reporter systems will be used in the presence of varying magnetic fields for varying times at differing stages of growth of the Arabidopsis plant. Imaging equipment and software will then be exercised to analyze any variations in the expression of GUS and GFP.

3. EXPERIMENTAL METHODS

In order to understand how magnetic fields affect processes in biological systems, a primary objective was to find a living specimen that met specific criteria to facilitate our research needs. The species chosen was the plant *Arabidopsis thaliana*. Using three strains of Arabidopsis, four experiments were devised to reveal any type of effect the magnetic fields had on our transgenic plants. In all experiments, an Oxford Teslatron Magnet is used to produce and sustain a static magnetic field with a possible maximum 9T field (Figure 1)[1].

[1] The Oxford Teslatron is a superconducting magnet that operates at temperature approximately equal to 4 K. In order to ramp the field, electrical leads are connected and current is passed through the coils of the magnet. Approximately 9.22 A corresponds to an increased magnetic field by 1T. Once the desired magnetic field is reached, the current leads are removed and the magnet is left to superconduct. Ramping the field can all be accomplished without affecting the location or orientation of samples present inside the bore.

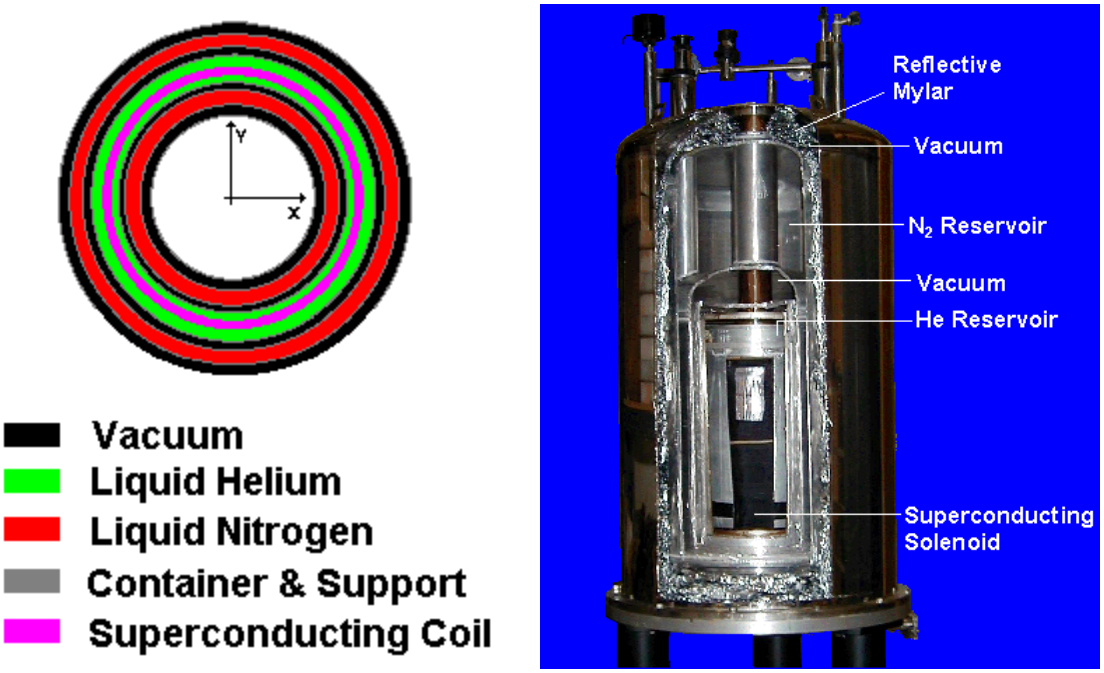

Fig 1 (a) Superconducting Magnet cross section and (b) cut away view

3. 1 Ultrabrite fluorescence test

One strain of Arabidopsis contains a genetic coding that allows the plant to fluoresce green under any circumstance, regardless of the amount or type of stress applied. This genetically engineered strain, ultrabrite Green Fluorescent Protein (GFP), was utilized to determine whether or not magnetic fields alter the fluorescence itself. On the quantum level, this enigma could be cast as a question: Do magnetic fields affect the spin, orientation, or ability of electrons to change energy states that would ordinarily result in fluorescence? The method by which this question was resolved can be broken down into four steps: growth, magnetic field application, imaging, and RNA analysis.

During the growth stage, approximately 20 individual ultrabrite-GFP plants were grown on agar in a Petri dish with a diameter of 87 mm. The Agar medium consisted of a gelatinous solution of sucrose, salt and enzymes. The ultrabrite seeds were then exposed

to temperatures above 4 ^{o}C, allowing for germination. During growth in the lab, the plants were grown vertically along the agar and were exposed to four individual 20-Watt Fluorescent Lights. The temperature of the system remained steady at lab temperatures between 23 and 24 ^{o}C. In the final trial effort, the plants were grown for a period of 19 days and then were directly placed 570 mm deep into an Oxford Teslatron Superconducting Magnet[2] through the use of a two-joint lowering system. A lighting system containing two light plates and an O-ring light source was fit around the 88 mm diameter bore and shined ~470 nm (blue) light onto the Petri Dish 570 mm deep. A notch band pass blue filter[3] was then placed above the lighting source so that the only light passing through the filter was the fluorescing green from the ultrabrites. Once in place at 0 T, digital images were taken with a Nikon 950 CoolPix Camera (shutter speed 8 sec., no flash, white balance set precisely to perfect white, and telephoto lens with a zoom setting at F4.0 x2.5). Without moving the camera or the sample inside the bore, the magnetic field was ramped to 9 T. Once 9T was reached, the plants were again digitally photographed under the same camera settings as previously described. Once the images were saved successfully, they were taken to a second lab to be quantitatively imaged and analyzed. We used Quantum One Imaging Software, which allowed us to compare the images at 0 and 9 T with respect to mean intensity, maximum intensity, minimum intensity, and intensity density for specific areas on the Petri Dish. Adobe Photoshop 6.0 was also used to measure the hue of the fluorescence in the two magnetic fields. Once these data were acquired, Microsoft Excel was used as the means for storing and

[2] The Oxford Magnet being used for all experimentation produces a uniform magnetic field at 570 mm deep. The Petri Dish is laid flat along this plane at 570 mm. Plant growth diverges away from this plane by +/- 2 mm. However, the Oxford Teslatron is still able to produce a uniform field within this range.
[3] The band pass notch filter allowed all colors of the visible spectrum to pass through with the exception of blue light(~470 nm)

graphing the data. For the final phase, RNA analysis, involved harvesting approximately 15 individual ultrabrite plants off of the agar and placing them into 2 mL vials surrounded by a chemical called "RNA later." These vials were then refrigerated at temperatures around $0^{o}C$. Anna-Lisa Paul, using highly sensitive Polymerase Chain Reaction (PCR) processing machines, then analyzed the transcriptional effects of the magnetic field at NASA and University of Florida laboratories. This experiment was run fully on three separate occasions.

3. 2 846/GFP exposure to Magnetic Fields

The design of this experiment included four phases, all of which followed the generally outlined phases of the Ultrabrite experiment (growth, magnetic field application imaging, and RNA analysis). Key differences do exist between the two experiments. During the 846/GFP experiment, Arabidopsis plants were grown in the exact same fashion as in the ultrabrite experiment with one important exception; the ultrabrites were replaced by 846/GFP, a genetic derivative of the ultrabrites. The magnetic field application stage involved placing approximately 20-day-old 846/GFP plants into a 9 T field for time increments of 1, 4, 8, 12, 24, and 48 hours. At each interval, the plants were photographed under an imaging apparatus consisting of a cylindrical tube placed above and around the sample Petri dish. The cylinder housed a 468nm (blue) O-ring light source and was topped with a blue filter eyepiece (Figure 2). A camera (manual setting, shutter speed 2 seconds, white balance calibrated to pure white, and zoom F4.0 x 1.60, no flash) was then attached to a tripod above the eyepiece and images were taken to

qualitatively determine the magnetic fields ability to stress the GFP plants. Finally, the plants were harvested just was they were in the ultrabrite experiment and were sent off to be analyzed at NASA and Fifield Hall at UF.

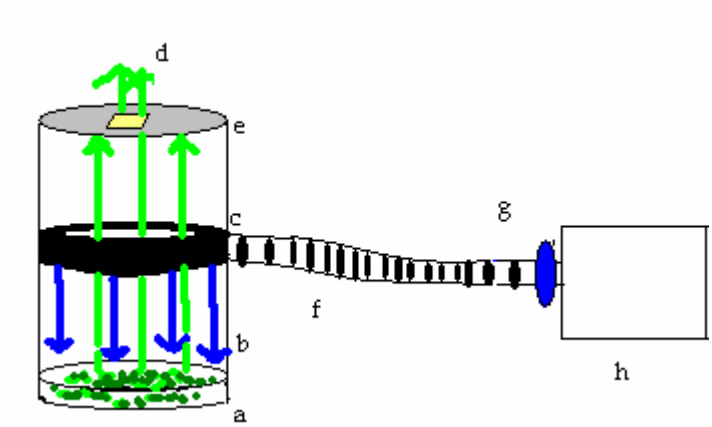

Figure 2 Imaging Equipment - Petri dish (a); 468 nm (blue) light (b) from O-Ring light source (c); fluorescing green from plants observable by viewer (d); window filtering all blue (e) fiber optic cables (f); blue pass filter (g); white light source (h)

3.3 846/GUS exposure to Magnetic Fields

This experiment is exactly the same as the "846/GFP exposure to Magnetic Fields Experiment" as far as growth and magnetic field exposure were concerned, apart from the fact that the samples were of the genetic engineering strain β-glucuronidase (GUS) of the Arabidopsis plant. Once affected by the magnetic field, the plants were histochemically stained with a chemical called X-glucuronidase, or simply X-gluc, which enabled the experimenter to observe the expression of the reporter gene coding protein, GUS. The plants were then rinsed with ethanol twice to remove any chlorophyll that might obstruct view of the Blue GUS expression. Plant samples were harvested from the agar at 0, 24, and 48 hours over two trial runs. Plants were harvested after 48 hours for RNA analysis as performed in the ultrabrite experiment.

3.4 Flooded 846/GFP

Two Petri dishes containing approximately 20 individual GFP Arabidopsis plants were left to grow for 18 days. They were then flooded for two days in zip lock bag (unsealed). The Petri dishes remained in the same vertical position as they had been for growth. They were then removed from the bag, drained, and tested to check for fluorescence. One of the Petri dishes was then placed in a 9 T magnet. The fluorescence was checked at 6, 24, and 48 hours. The control plant was left on the bench and was also tested at these time intervals.

4. Results and Discussion

One prerogative involved our ability to image the plants correctly and precisely. Because the plants were 570 mm deep in a bore where our light sources were diminished by at least 45% by two necessary color light filters, we found that it was much easier and more accurate to do imaging on the lab bench. The bench was also a much more desirable location since the magnetic field would not affect the pixels or electrical functions of the digital camera. However, before we were able to image on the bench, we needed to run the ultrabrite experiment. By running such an experiment, we would know whether or not the magnetic field disrupted the ability of the electrons to vibrationally relax to the lowest singlet state of the lowest excited state [6]. Continuing from this quantum level, would the electrons drop to a vibration level of ground state by radioactive deactivation? This final process results in fluorescence.

4.1 Ultrabrite fluorescence testing

After completing imaging, it was found that the intensity of fluorescence at 0 T and at 9 T remained constant. One unique attribute of the Ultrabrite plant is that its promoter is genetically designed to always be in the "on" position. Thus, we *know* that regardless of any stress or lack of stress, the plants will glow a bright green color around the wavelength of 508 nm due to the reporting GFP [5]. Therefore, if there was any deviation in the intensity of the plants at 0 and 9 T inside the bore, it could be postulated that the magnetic field was disrupting the electrons' ability to shine fluorescent light. However, this magnetic induced disruption was not the case. After comparing 10 separate leaves at 0 and 9 T, it was found that the percentage difference in density of intensity was never more than 1%.

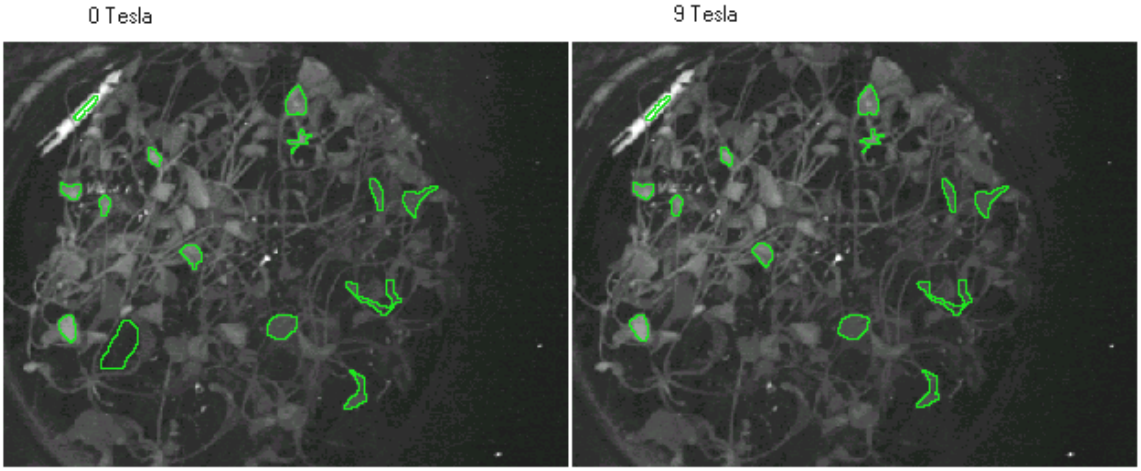

Fig. 3 A comparison of fluorescence at 0 and 9T

A chart with these quantitative results is shown in Figures 4 and 5. The relative precision of these results allows us to deduce that the magnetic field does not affect the ability of electrons to fluoresce.

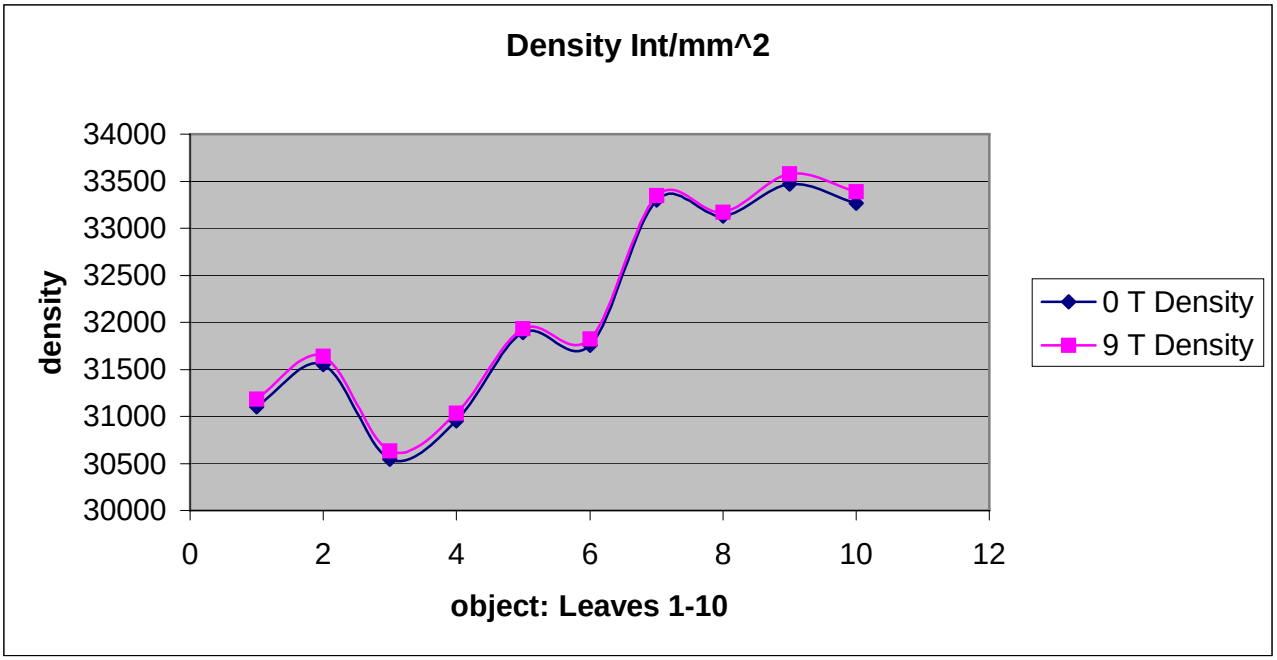

Figure 4 Comparison of intensity density of *Arabidopsis ultrabrites* at 0 and 9 T

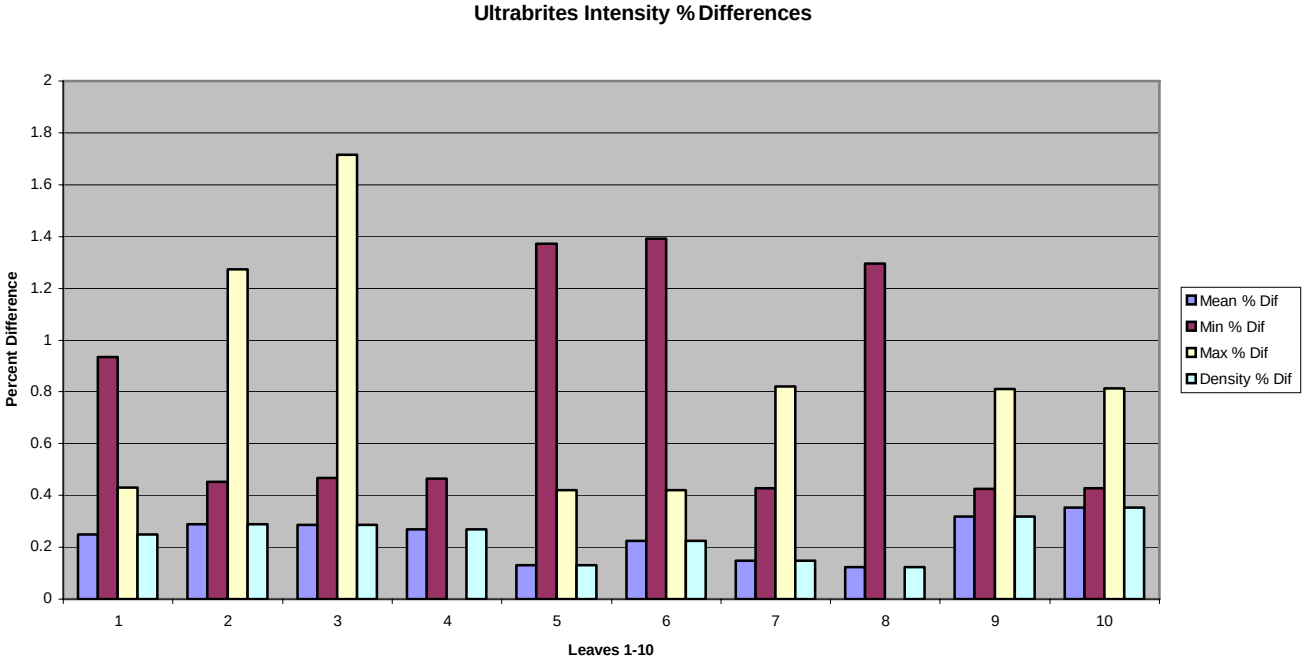

Figure 5 Comparison (using percentage difference) of various intensity measurements at 0 and 9 T for *Arabidopsis* ultrabrites

Knowing that a magnetic field of 9 T does not affect fluorescence, we could now run experiments with plants that did not fluoresce at all times. If changes in intensity occurred, we would know that these alterations were due to transcriptional changes in the plant induced by the magnetic field and were not due to the physical act of fluorescence in a magnetic field. A second accomplishment of this experiment was that it allowed us to begin imaging on the bench. We knew that if the Arabidopsis fluoresced in the bore at 9T, then they would also fluoresce outside of the bore. This assumption is also a derivative of the fact that magnetic fields do not affect electrons' changing states.

4.2 846/GFP exposure to Magnetic Fields

One downfall of Green Fluorescent Protein is that its expression is often inhibited by competing fluorescence. Inherent in the Arabidopsis plant is a fluorescing red color. Therefore, when weak promoter activity is present, the appearance of fluorescing green may be difficult. This red fluorescence competition was the case for this experiment. As can be observed from Figure 6, the emergence of GFP activity does not seem to be observable. Another possible cause of decreasing fluorescence is due to temperature. GFP is a thermo-sensitive protein, and its expression is decreased at temperatures above 30^0C [5]. However, for our lab data, all experimentation took place under 25^oC.

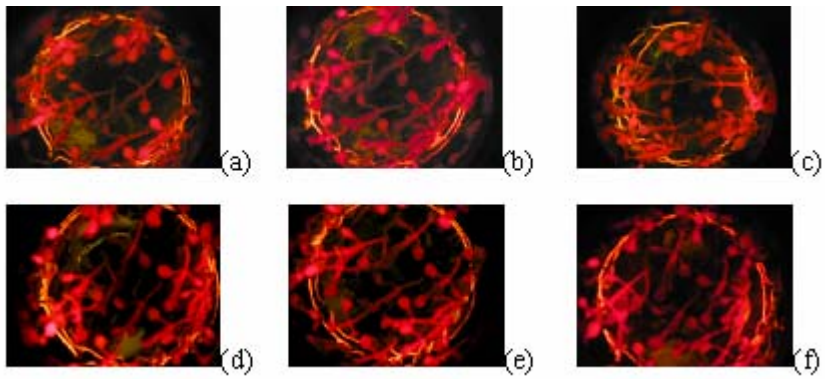

Figure 6 GFP under 9 T for 1 hr (a); 4 hrs (b); 8 hrs(c); 12 hrs (d); 24 hrs (e); 48 hrs (f)

If the GFP is present, its concentration is minimal at all times up to two days. However, these results are consistent with previous findings of GUS expression with relation to magnetic field applied for 2.5 hours. One formula quantifying GUS representation to magnetic field is the log-normal function: $y=A*\exp[-\ln^2(x/x_c)/2w^2]$; where x is the magnetic field and A, x_c and w are variance estimates with values of approximately 11, 19.9 and .12, respectively. Using this model, it appears that GUS activity does not begin to take place until 14 Tesla and reaches a maximum near 17 Tesla. However, this expression only applies to 2.5 hours of exposure to magnetic field. We do not know if these results agree with similar findings after magnetic field application for 48 hours. Because the reporters GUS and GFP are controlled by the same promoter (alcohol dehydrogenase), it can be assumed that expression levels of either reporter should correspond to the other. For all intensive purposes, this seems to be true.

4.3 846/GUS exposure to Magnetic Fields

For all lengths of time, expression of GUS never consistently spread beyond the root-shoot junction area. Occasionally, GUS expression could be found in the roots and the vascular tissue of a small percentage of the leaves (see Figure 7).

Figure 7 GUS after 9T for 0 hrs (a), 24 hrs (b) and 48 hrs (c)

Because the plants were placed in a static uniform magnetic field, these inconsistencies led us to believe that the magnetic field was not the stressor causing the expression of GUS. Again, this conclusion agrees with previous models showing initial GUS response to magnetic fields at 14 T. These results also parallel the findings of our 846/GFP experiment. In an article by Mantis and Tague, it was noted that after 48 hours at higher magnetic fields, GUS was found at the shoot apex and in the petioles of cotyledon leaves, whereas GFP response was only found at the shoot apex. Due to the overshadowing auto-florescence at the root-shoot junction, anther and pollen granules, older roots, and developing seeds and cotyledons in the GFP engineered plants, it now becomes apparent why the use of GUS lends to higher expression levels. For our use, this information adds validity to the agreement between the GFP and GUS experiments in that it explains why GFP was not able to fluoresce at the root-shoot junction.

4.4 Flooded 846/GFP

After the were flooded plants for 48 hours, results show that the Arabidopsis 846/GFP plants did fluoresce. This simply means that the flooding acted successfully by turning the Adh promoter "on." This pre-stressing allowed for a rich fluorescing green to be expressed. Once the expression of GFP was activated, applying a magnetic field for any time under 48 hours did not seem to turn the promoter coding sequence "off"(Figure 8). This experiment indicates that magnetic fields have no bearing on the transcriptional activity of Arabidopsis at magnetic field levels around 9 T.

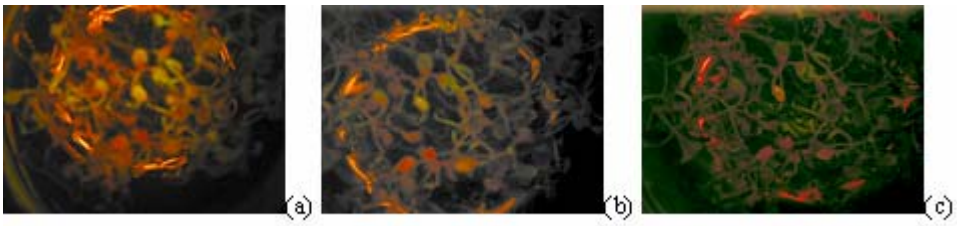

Figure 8 Flooded GFP at 9 T for 0 hrs. (after flooding for 48 hrs)(a), 24 hrs.(b) and 48 hrs.(c)

Discussion Overview

The experiment shows that magnetic fields do not affect the ability of the Adh promoter to turn either "on" or "off." However, it may be possible that the magnetic field *IS* capable of inflicting changes on transcription, but these alterations are counteracted by alteration of the Green Fluorescent Protein itself. Because of red auto-fluorescence of Arabidopsis, we only assume that the GFP and GUS experiments under magnetic fields agree. It may be possible that weak fluorescence is occurring in a number of areas on the plant (i.e., cotyledons, vascular tissue, root-shoot junction, root tissue, and shoot apex) but is being masked by the overpowering red fluorescence. One reason that GFP is used is because it, like Arabidopsis, has been well researched.

GFP is a highly rigid aromatic protein. It consists of 11 strands of sheet amino acids surrounding a helix and short helical segments near the end of what appears to make a three-dimensional cylindrical shape, most often referred to as a beta-can. Fluorophores, which are the main cause of fluorescence, are protected inside the cylinder and have structures consistent with the formation of aromatic systems made up of the amino acid Tyrosine. We know, however, that many amino acids are anisotropic and may be susceptible to magnetic fields.

For macromolecules we know that energy variations arise two ways. One is through anisotropy of the magnetic susceptibility, and the second is through inhomogeneities in the magnetic field resulting in an overall migration of the macromolecules. But, it may be possible that these findings carry over to the micromolecular level. Perhaps the amino acids are lining up with the magnetic field resulting in an unwinding of this beta-can housing the fluorophores. This unwinding could prevent fluorescence. However, we do know that fluorescence occurs in the ultrabrites and the previously stressed GFP plants. Therefore, magnetic fields do not completely disable GFP itself from fluorescing. Nevertheless, there may be an intermediary process between the promotion of Adh and the expression of the reporter GFP that is affected by the magnetic field. The answer to this enigma is beyond the scope of this report.

5. Conclusion

Moderate magnetic fields around 9 T do not seem to affect the promoter, alcohol dehydrogenase, as stresses such as hypoxia, flooding, or coldness might. We also

conclude that magnetic fields at approximately 9T do not affect the process of fluorescence and therefore do not show any alteration in the transcription of Adh. While these conclusions imply a null response to magnetic stimuli around 9T (and may not seem exciting), they do show agreement to previous experiments utilizing middle range magnetic fields. However, nearly all conclusions are based on macroscopic evidence of microscopic processes. Therefore, it does not appear that we know exactly how (or why) magnetic fields are not causing any changes.

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