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An illuminated growth system for the study of *Arabidopsis thaliana* during diamagnetic levitation by a superconducting magnet

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Abstract

The effect of gravity on plant growth is an interesting topic in its own right, but it is also important because it impacts the possibility of long-term space travel. Plants may be grown in microgravity simulated by diamagnetic levitation within superconducting magnet, but this approach is limited by the size and other objective conditions of the superconducting magnet. Tremendous difficulties exist in evaluating the effects of simulated microgravity on plant seedling growth under lighting conditions. Therefore, we developed a lighting system and culturing system that can meet the demands of growing plant seedlings in a superconducting magnet. This system mainly consists of an illumination system, suitable containers and a method to cultivate *Arabidopsis thaliana* seedlings. In order to prove the suitability of this light-growing system, *A. thaliana* was cultured in a superconducting magnet for four days. The status of seedlings was recorded and total RNA was extracted for gene expression analysis. Our results showed that *Arabidopsis* seedlings could germinate and grow successfully in this light-growing system. In addition, it was observed that under diamagnetic levitation conditions, the seedling bended and gene expression of *PGM* and *MOR1* decreased significantly compared to a control group. Nonetheless, there were no substantial differences between the diamagnetic levitation group and RPM group. Our results suggest that this light-growing system is expedient and beneficial for plants grown in a superconducting magnet. Our experiment also provides a way to utilize diamagnetic levitation in a superconducting magnet that simulates the conditions necessary to study plant physiology and biochemical responses in a microgravity environment.

Keywords: *Arabidopsis*; Light-growing system; Illumination device; Simulated microgravity; Diamagnetic levitation; Superconducting magnet

1. Introduction

Plants growing on Earth use the force of gravity as a guide for their growth and development. Plant physiological processes are affected by changes to the magnitude and direction of gravity. As space exploration increases in frequency and duration, plant cultivation is becoming an essential aspect of space craft life support systems since plants can provide both food and oxygen. Thus, it is necessary to understand how plants respond to micro gravity during space flight.

Limited opportunities and the high cost of space flight results in low repeatability and reliability for experiments in real microgravity environments. Therefore, researchers have utilized a variety of methods to simulate microgravity

Abbreviations: PGM, phosphoglucomutase; MOR1, microtubule organization 1 protein.

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on Earth (Herranz et al., 2013a,b), such as parabolic airplanes (Di et al., 2011; Grosse et al., 2012; Zupanska et al., 2013), drop towers (Anken et al., 2006; Huang and Mao, 2013), 2-D clinostats (Shevchenko, 2012; Xu et al., 2012), and random positioning machines (RPM) (Borst and van Loon, 2009; van Loon, 2007). With the rapid development and wide application of superconducting technology, it is possible to generate a high static magnetic field and in recent years, diamagnetic levitation has been used to simulate real microgravity. When diamagnetic materials (water, cells, proteins, lipids, etc.) are exposed to a large magnetic gradient, they experience a repulsive force. Based on the direction of the magnetic gradient, the magnetic force is in the same direction as gravity or against the force of gravity. Therefore, the materials can be levitated under these conditions. This particular simulation is different from currently existing ground-based techniques since the essence of magnetic force is body force, similar to gravity. In diamagnetic levitation the same magnetic force is applied to each part of the diamagnetic materials and thus it is a stable system and a valuable and suitable simulation technology for long biological experiments. Diamagnetic levitation is a new and potentially transformative method to explore how gravity affects living organisms and many researchers have begun to use it in their studies (Valles et al., 1997; Liu et al., 2010, 2011; Herranz et al., 2012; Tian et al., 2010; Qian et al., 2009, 2013; Gao et al., 2010; Dijkstra et al., 2011; Yan et al., 2012).

In the 1990s (Kuznetsov and Hasenstein, 1996, 1997; Kondrachuk and Hasenstein, 2001; Hasenstein et al., 2013) began to study the influence of magnetic gradients on plant gravitropism and amyloplast settlement. They analyzed bending in the coleoptile and hypocotyl of plant seedlings in detail. In recent years, functional genomics has allowed a growing number of researchers to study changes in gene expression under diamagnetic levitation with high through put sequencing technologies, such as gene chips. Babbick et al. (2007) found that expression of the WRKY, MADS-box and MYB transcription factors, as well as the AP2/EREBP gene family of *Arabidopsis* was significantly different when plants were grown under different levels of gravity. In addition, RPM and magnetic levitation are regarded as more accurate simulation of microgravity than 2-D clinostats. Later, intensive research conducted by Manzano and Herranz found that synergic gravity and magnetic fields had an influence on gene expression (Manzano et al., 2012) and protein expression (Herranz et al., 2013a,b) in *Arabidopsis*, especially on aspects of cell proliferation and ribosome synthesis (Manzono et al., 2013; Herranz and Medina, 2014).

A great deal of work hasbeen done by other researchers to provide a solid foundation for the development of this research area. However, most of the previously published research utilizes callus or suspension cells as experimental materials and very few studies focus on plant seedlings or investigate how the whole organism responds to changes of gravity in a superconducting magnet under light culture conditions. Experiments that use superconducting magnets to study plants have been limited by the need to design illuminated growth devices and culture methods. In this study we developed a system to culture plant seedlings in a superconducting magnet with an illumination auto-control device designed to support plant seedling growth. Additionally, we established a convenient and efficient *Arabidopsis* seedling culture method. This paper aims to provide a reference method for *Arabidopsis* illuminated growth under diamagnetic levitation conditions. We do not present new quantitative data concerning magnetic effects on seed germination, but that study is forthcoming. This new culture system provides the logistical foundation for research on plant seedling growing in diamagnetic levitation.

2. Materials and methods

2.1. Illumination device for diamagnetic levitation in a superconducting magnet

A low temperature superconducting magnet (JMT16T50F) in the Key Laboratory for Bioscience and Biotechnology of NPU was designed and manufactured by Japan Superconductor Technology, Inc. (JASTEC). The diameter of the magnet's inner chamber is approximately 51 mm and the maximum magnetic field intensity of the magnet is 16.1T.The magnet is formed by Nb_3Sn and NbTi superconductors and can provide three different magneto-gravity environments (μg, $1g$, $2g$) and the corresponding magnetic induction and magnetic field gradients are: 12 T, $-1360\ T^2/m$; 16 T, $0\ T^2/m$; 12 T, $1312\ T^2/m$ (Fig. 1). By employing the superconducting magnet, researchers have established a diamagnetic levitation experimental platform that is suitable for biological research.

The diamagnetic levitation platform mainly includes the superconducting magnet, an objective stage, a gas control system and a temperature system etc. The platform can provide the basic requirements for plant and animal cells, microorganisms and protein crystal growth. The objective stage (Fig. 2F) is made of glass fiber reinforced plastics. There are three corresponding apparent gravity positions available for sample placement in the objective stage. In this study the illumination was introduced from the top of the magnet as direct light, and therefore, the samples were placed on top of the objective stage during the experiment, (Fig. 2G).When the gravity environment is replaced, the height of the lamp must be adjusted accordingly to ensure that the light is equally distributed across the sample of plants.

The temperature control system uses a constant temperature electric water bath (Fig. 2C) and a water jacket (Fig. 2E) including a water inlet, water outlet, water pipe, and the wall of the water jacket. The wall of the water jacket is brass to withstand the high magnetic field environment. The water jacket is installed in the bore of the superconducting magnet to maintain homogeneous temperature.