Available online at www.sciencedirect.com

ScienceDirect

Advances in Space Research 53 (2014) 818–827

ADVANCES IN SPACE RESEARCH (a COSPAR publication)

www.elsevier.com/locate/asr

# Changes in operational procedures to improve spaceflight experiments in plant biology in the European Modular Cultivation System

John Z. Kiss [a,*], Gjert Aanes [b], Mona Schiefloe [b], Liz H.F. Coelho [b], Katherine D.L. Millar [a], Richard E. Edelmann [c]

[a] Department of Biology & The Graduate School, University of Mississippi, University, MS 38677, USA
[b] Centre for Interdisciplinary Research in Space (CIRiS), NTNU Samfunnsforskning AS, Dragvoll Allé 38B, NO-7491 Trondheim, Norway
[c] Department of Botany, Miami University, Oxford, OH 45056, USA

Received 23 October 2013; received in revised form 14 December 2013; accepted 17 December 2013
Available online 31 December 2013

**Abstract**

The microgravity environment aboard orbiting spacecraft has provided a unique laboratory to explore topics in basic plant biology as well as applied research on the use of plants in bioregenerative life support systems. Our group has utilized the European Modular Cultivation System (EMCS) aboard the International Space Station (ISS) to study plant growth, development, tropisms, and gene expression in a series of spaceflight experiments. The most current project performed on the ISS was termed Seedling Growth-1 (SG-1) which builds on the previous TROPI (for tropisms) experiments performed in 2006 and 2010. Major technical and operational changes in SG-1 (launched in March 2013) compared to the TROPI experiments include: (1) improvements in lighting conditions within the EMCS to optimize the environment for phototropism studies, (2) the use of infrared illumination to provide high-quality images of the seedlings, (3) modifications in procedures used in flight to improve the focus and overall quality of the images, and (4) changes in the atmospheric conditions in the EMCS incubator. In SG-1, a novel red-light-based phototropism in roots and hypocotyls of seedlings that was noted in TROPI was confirmed and now can be more precisely characterized based on the improvements in procedures. The lessons learned from sequential experiments in the TROPI hardware provide insights to other researchers developing space experiments in plant biology.


*Keywords:* European Modular Cultivation System (EMCS); Gravitational biology; International Space Station (ISS); Microgravity; Phototropism; Space biology

## 1. Introduction

Research on plants in space has been performed since the dawn of the spaceflight era in the 1960s when seeds were sent on the earliest spacecraft by both the Soviet Union and the United States (Halstead and Dutcher, 1987; Ferl et al., 2002). The use of the microgravity environment of spacecraft in low Earth orbit to study plant biology is significant for two reasons. *First*, microgravity provides a unique opportunity for a better understanding of the fundamental biological processes in all organisms including plants (Roe and Uri, 2003; Robinson et al., 2007; Correll and Kiss, 2008; Wolverton and Kiss, 2009). Gravity has supplied a constant input throughout the evolution of life on Earth, and removing the effective gravity vector allows for novel observations on physiological processes in plants such as cell wall development (Levine and Krikorian, 1992; De Micco et al., 2008), photosynthesis (Jiao et al., 2004), tropisms (Molas and Kiss, 2009; Kiss et al., 1999, 2012), and circumnutation (Solheim et al., 2006; Johnsson et al., 2009). *Second*, since plants are likely to be important in bioregenerative life support systems in

* Corresponding author. Tel.: +1 662 915 7474; fax: +1 662 915 6557.
*E-mail addresses:* jzkiss@olemiss.edu (J.Z. Kiss), gjert.aanes@gmail.com (G. Aanes), mona.schiefloe@ciris.no (M. Schiefloe), liz.coelho@ciris.no (L.H.F. Coelho), kdmillar@olemiss.edu (K.D.L. Millar), edelmare@muohio.edu (R.E. Edelmann).

0273-1177/$36.00 © 2013 COSPAR. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.asr.2013.12.024

space, particularly once humans leave low Earth orbit, it is important to address questions related to the growth, development, and cultivation of plants in space (Massa et al., 2006; Massa and Mitchell, 2012). This latter emphasis includes understanding plants throughout an entire life cycle in microgravity, which would result in the production of viable seed so that plants can be used as a food source for the crew as well as for the continued cycling of the crop through multiple generations (Kuang et al., 2005; Popova et al., 2009).

Major challenges facing space biologists include hardware problems and availability, lack of control of the experimental environment, and limited flight opportunities (Correll and Kiss, 2008). While the International Space Station (ISS) has allowed for longer duration experiments, there appear to be fewer spaceflight opportunities compared to the Space Shuttle era, particularly when there were many investigations when the Spacelab and Spacehab modules flew on the shuttle (Paul et al., 2013). Due to the dearth of spaceflight opportunities, it is often difficult to replicate, develop, and refine experiments as would be possible in a typical laboratory setting on Earth.

However, a few investigators in plant space biology have been able to maintain a progression of experiments with improvements in design that resulted in more refined analysis and conclusions. For example, the CROMEX project on plant developmental and reproductive biology involved a series of six spaceflight experiments that flew on the Space Shuttle between 1989 and 1995 (Musgrave and Kuang, 2001; Kuang et al. 2005). These experiments utilized the Plant Growth Unit (PGU), which was continually improved in terms of its design as a research facility (Levine and Krikorian, 1992; Porterfield et al., 1997). In fact, the PGU evolved to an even more sophisticated laboratory facility termed the Advanced Biological Research System (ABRS) which has been used in several spaceflight studies of plant molecular biology (Paul and Ferl, 2011; Paul et al., 2012).

Our group has been fortunate in having an opportunity to study plant development, tropisms, and gene expression in a series of experiments in the European Modular Cultivation System (EMCS) on the ISS (Millar et al., 2010; Kiss et al., 2012; Correll et al., 2013). The EMCS is an incubator system that was developed by the European Space Agency (ESA) to study plants and small organisms in space (Brinckmann, 2005; Kamada et al., 2007). This facility has atmospheric control, a lighting system, and two centrifuge palettes so that samples can be investigated from microgravity to fractional gravity to a 1*g* control (and up to 2*g*).

Our first experiment in this series, termed TROPI-1 (for tropisms), was performed as the inaugural EMCS experiment in 2006. We were able to identify a novel red-light phototropism in microgravity (Millar et al., 2010) despite having a number of technical and operational difficulties which led to relatively low seed germination (Kiss et al., 2009). This discovery was very exciting since flowering plants were generally thought to lack red light phototropism. However, our studies during TROPI-1 suggested that flowering plants may have retained a red light sensory system for phototropism. Thus, these results from our spaceflight project potentially had important implications for understanding the evolution of light sensory systems in plants and needed to be better characterized in later space studies.

In 2010, we successfully performed TROPI-2, in which seed germination was improved, and we extended our investigations to fractional gravity (less than the nominal 1*g* on Earth) in addition to microgravity (Kiss et al., 2012; Correll et al., 2013). These fractional or reduced gravity studies showed an attenuation of red-light-based phototropism in both roots and hypocotyls of seedlings occurring due to gravitational accelerations ranging from 0.1*g* to 0.3*g*. In addition, to our knowledge, TROPI-1 and TROPI-2 included the first studies of the transcriptome of plants in a spaceflight experiment that also utilized an on-board 1*g* centrifuge as a control (Correll et al., 2013).

In this paper, we characterize further refinements in the TROPI series of experiments in a new project termed Seedling Growth (SG), which considers both tropisms and the plant cell cycle (Matía et al., 2010). SG-1 was launched on the SpaceX-2 vehicle and performed during ISS Increments 35–36 in 2013. We report on improvements in light conditions for phototropism studies and imaging of the seedlings as well as other factors that were changed in order to optimize our spaceflight experiments.

The lessons learned from our series of experiments using the TROPI hardware (spanning from TROPI to SG) are important to plant space biologists for two reasons. First, the EMCS has been one of the more successful facilities on the ISS that has been used by multiple investigators (e.g., Kamada et al., 2007; Johnsson et al., 2009; Millar et al., 2010) and is still being featured in the international call for future experiments by ESA, NASA, and other space agencies. In addition, the problems that we have faced regarding imaging, lighting, and atmospheric control have been issues relevant to many other plant experiments performed by space biologists over the course of the past several decades (Halstead and Dutcher, 1987; Wolverton and Kiss, 2009; Paul et al., 2013).

## 2. Materials and methods

### 2.1. Plant material, spaceflight mission, and ground controls

Seeds of the wild type of *Arabidopsis thaliana* (Landsberg ecotype; Ler), and phytochrome mutants *phyA* and *phyB* (Kiss et al., 2003) were used in these spaceflight and ground studies. In this paper, we compare the results of two spaceflight experiments, TROPI-2 and Seedling Growth-1 (SG-1), as well as consider additional ground-based experiments.

In terms of TROPI-2, experimental containers (ECs) with TROPI experiment unique equipment (EUE) loaded