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Advances in Space Research 52 (2013) 760–772

ADVANCES IN SPACE RESEARCH (a COSPAR publication)

www.elsevier.com/locate/asr

Differential gene expression patterns in white spruce newly formed tissue on board the International Space Station ☆

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Received 12 February 2013; received in revised form 30 April 2013; accepted 5 May 2013
Available online 16 May 2013

Abstract

White spruce (*Picea glauca* [Moench] Voss) seedlings produced by somatic embryogenesis were grown both at the Kennedy Space Center and in weightlessness in the ISS for 30 days. Plants were placed in closed environment incubators (Advanced Biological Research System) under controlled light, temperature, humidity and CO_2 conditions. At the end of the experiment, the leading shoot from three plantlets of each of the three lines tested were sampled and pooled in Kennedy Space Center Fixation Tubes (KFT) containing a RNA stabilization solution. Transcript levels were determined by quantitative real-time polymerase chain reaction (RT-qPCR) for 27 candidate genes and three reference genes on the nine seedlings grown in each environment. About two-thirds of the 27 genes produced a larger number of transcript molecules in microgravity conditions. However, only three genes showed significant differences between the two environments, and all of them were up-regulated in microgravity. These genes appear to be involved in important processes such as cell propagation, plant development and response to stress, and their up-regulation has likely contributed to influencing seedling growth patterns.

Keywords: *Picea glauca*; Glutathione S-transferase; C-terminal-like; Elongation factor EF1B/ribosomal protein S6; Hsp70/Hsp90 organizing protein (Hop); ZPR1 zinc-finger domain protein

1. Introduction

During their development, plants are influenced by diverse exogenous and endogenous signals that can either vary over time or be constant. Gravity is one of the few that do not vary during a plant's lifetime (Valster and Blancaflor, 2008). Plants need to orient their various organs in order to access resources that allow them to survive and ultimately reproduce. Hence, roots will generally grow in the direction of the gravity vector, i.e. downwards, where they find access to water and nutrients, whereas the leader shoot will be negatively gravitropic to have access to the light, moisture, oxygen and carbon dioxide required for photosynthesis.

The gravitational response proceeds through four steps: (1) sensing the direction of gravity, (2) converting this biophysical stimulus into a biochemical signal, (3) transmitting the latter signal to the right tissues, and (4) the organ changing direction if needed (Tasaka et al., 1999). In both roots and stems that change their orientation relative to the gravity vector, the growth regulator auxin accumulates to higher levels towards the gravity vector on the lower side of the affected organ (Friml and Palme, 2002; Perrin et al., 2005). In roots, auxin accumulation results in the localized inhibition of cell expansion and the reorientation of growth towards the gravity vector. In stems, auxin accumulation has the opposite effect and stimulates cell expansion, which causes the growth direction to reorient away from the growth vector.

☆ This paper has been recommended for acceptance by Peter Lindstrom and Scott Scaefer.

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0273-1177/$36.00
http://dx.doi.org/10.1016/j.asr.2013.05.004

Two hypotheses have been proposed to explain how plants perceive the direction of gravity. These are (1) the gravitational-pressure model and (2) the starch-statolith hypothesis (Morita and Tasaka, 2004). The latter is now the most widely accepted as the major gravity-sensing mechanism and states that starch-accumulating amyloplasts move along the gravity vector within gravity-sensing cells (statocytes). This amyloplast movement would be an intricate process involving vacuolar membrane structures and the actin cytoskeleton (Morita, 2010).

When one wants to study the effect of gravity on plant growth, it is very difficult to design an experiment in which the effects of gravity will be largely eliminated while controlling any other factors that might also influence growth. The most effective way to reduce gravity effects on objects like plants is through the use of free fall (Correll and Kiss, 2008). Several devices can create simulated microgravity environments, but only a space infrastructure such as the International Space Station (ISS) can make it possible to study the effects of gravitropism on plants over a long period of time. While there are scientific interests and potential economic benefits to better understanding how plants react to gravitational forces, any knowledge on adaptation of plants to the microgravity environment could improve our capacity to colonize space. Man's ambitions to colonize space have included the idea that a closed ecological system is a necessity for long term life support at great distances from Earth (Dempster, 1999). In such closed ecosystem (Dempster et al., 2004), plants, and especially trees, would help capture CO_2, produce oxygen and provide a continuous supply of moisture in the air through evapotranspiration. Presence of plants would also provide a positive psychological impact on humans living for a long period of time in artificial environments.

Plant response to gravity does not only take place through structural changes in cells and morphological alteration of organs, but can also be seen at the gene expression level. Ground-based studies have, for instance, made it possible to observe transient changes in relative abundance of some transcripts in gravitationally stimulated *Arabidopsis* roots, and gravity induced genes have been identified in root apexes (Kimbrough et al., 2004). *Arabidopsis* transgenic plants were also transported to space on the Space Shuttle for a 5-day mission and a comparison of the number of transcripts of plants exposed to a spaceflight environment compared with that of others grown on the ground showed that a few hundred genes were differentially expressed, including several related to heat shock (Paul et al., 2005). However, Stutte et al. (2006) could not find any differences in gene expression in wheat plants grown for 23 days in the ISS in comparison with others that were grown on the ground.

Experiments conducted on spaceflights to assess the response of trees to gravitational forces are extremely rare. Only one is reported in a peer-reviewed journal for loblolly pine (*Pinus taeda* L.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). The experiment was carried out on the Space Shuttle Columbia during its STS-78 mission with the aim of verifying whether compression wood formation response occurred under conditions of induced mechanical stress in a microgravity environment (Kwon et al., 2001). It is still unclear how the space environment and particularly the lack of gravitational pull affect genetic regulation in trees.

The objective of the present study was to assess the effects of gravity and spaceflight environment on the gene expression profile of white spruce (*Picea glauca* (Moench) Voss) using RNA isolated in shoots recovered from clones that grew for 30 days on the ground or in the ISS transported by the Space Shuttle Discovery for the STS-131 mission (http://www.nasa.gov). We specifically assayed the expression of 27 different candidate genes that had previously been shown to be differentially expressed in angiosperm plants in response to reduced gravity either during spaceflight or in simulations on Earth. Targeted analyses of transcript accumulation levels carried by RT-qPCR were carried out following comparative sequence analysis to identify the closest homologous genes in white spruce.

2. Material and methods

2.1. Plant material

Plants used for the experiment were 1-year-old white spruce seedlings produced by somatic embryogenesis (SE) obtained from J.D. Irving Limited, New Brunswick, Canada. White spruce zygotic embryos were put on culture medium to produce varieties (somatic embryogenesis lines) at Natural Resources Canada facilities in early fall 2008. The dehydrated somatic embryos produced were later transferred to Petri dishes filled with a culture medium favoring the development of shoots and root systems. In early winter 2009, SE plants were transplanted into Jiffy plugs (Jiffy Products NB Ltd, Shippegan, NB, Canada) and raised in a greenhouse in Sussex, New Brunswick, Canada. At the end of the growing season, the plants slowly entered dormancy and when hardy enough, they were stored at temperatures below 0 °C. One-year-old frozen dormant plants of three SE lines (designated as A, B, C) were shipped in early March 2010 to Natural Resources Canada facilities in Quebec City, Quebec, Canada (Fig. 1). Upon arrival, the plants were left in the plastic bags used for shipping and stored in a greenhouse at 10 °C to allow them to slowly thaw.

After one day in the greenhouse, 16 ramets of each of the three SE lines were transplanted into 15-mL Simport P/N T406-2A polypropylene tubes (Simport Scientific Inc, St-Mathieu-de-Beloeil, QC, Canada) filled with Smithers Oasis foam 5200 plugs (Smithers-Oasis Company, Cuyahoga, OH, USA). Before transplanting the SE plants into root tubes, they were cleaned with tap water. The root system was also thoroughly washed with distilled water and laid in the longitudinally split Oasis plug. The tubes were prepared following the flight protocol developed for