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Variation in stem morphology and movement of amyloplasts in white spruce grown in the weightless environment of the International Space Station

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ABSTRACT

One-year-old white spruce (*Picea glauca*) seedlings were studied in microgravity conditions in the International Space Station (ISS) and compared with seedlings grown on Earth. Leaf growth was clearly stimulated in space whereas data suggest a similar trend for the shoots. Needles on the current shoots of ground-based seedlings were more inclined towards the stem base than those of seedlings grown in the ISS. Amyloplasts sedimented in specialized cells of shoots and roots in seedlings grown on Earth while they were distributed at random in similar cells of seedlings tested in the ISS. In shoots, such amyloplasts were found in starch sheath cells located between leaf traces and cortical cells whereas in roots they were constituents of columella cells of the cap. Nuclei were regularly observed just above the sedimented amyloplasts in both organs. It was also frequent to detect vacuoles with phenolic compounds and endoplasmic reticulum (ER) close to the sedimented amyloplasts. The ER was mainly observed just under these amyloplasts. Thus, when amyloplasts sediment, the pressure exerted on the ER, the organelle that can for instance secrete proteins destined for the plasma membrane, might influence their functioning and play a role in signaling pathways involved in gravity-sensing white spruce cells.

1. Introduction

Environmental stimuli such as water, temperature and light constantly vary in nature and they have a great influence on plant growth and development. Although less conspicuous to us, probably in part because we are used to it and it is considered constant over time, gravity is also a natural phenomenon that affects plant behavior (see e.g. reviews by Morita, 2010 and Hashiguchi et al., 2013). Gravity is often described as an attraction between physical bodies that is proportional to their masses and in inverse proportion to the square of the distance between them. By convention, gravity on Earth is expressed as 1*g*, an acceleration vector that is roughly equal to 9.8 m/s^2.

Plant responses involving movement toward or away from such stimuli are called tropisms. Papers on tropisms are numerous and one of the first was published at the beginning of the 19th century (Knight, 1806). In the latter, gravitropism was studied using a complex of wheels moving at different velocities onto which seeds of bean or horse chestnut were attached. Coupled with observations on other plant species growing naturally, Knight was able to draw some insightful conclusions, among which that shoots turn upward if placed horizontally because nutrients present in the sap tend to accumulate on their lower side due to gravity and that is where they stimulate cell extension and re-erection of the shoot apex. It was later shown that some of these so-called nutrients were actually growth regulators such as auxin (reviewed by Morita and Tasaka, 2004). Numerous review papers have been published on tropisms, for instance recent ones in a special issue of a scientific journal (see the introduction paper by Wyatt and Kiss, 2013).

As with several plant stimuli, gravitropism triggers a cascade of events that are often referred to as phases. For instance, Sack (1991) suggested three phases: sensing gravity, transmitting the message to the appropriate cells or tissues, and the ultimate response such as bending of the plant part. Gravity sensing

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would first come through physical information, with one of the most plausible hypotheses being that sedimentation of plastids, in particular amyloplasts, is critical in this respect (Sack, 1997; Hashiguchi et al., 2013). Gravity-sensing cells are generally called *statocytes* whereas sedimenting organelles or other entities within them are referred to as *statoliths* (Morita, 2010).

Research on gravitropism was greatly revitalized by the advent of spaceflight vehicles and the establishment of the International Space Station (ISS) where controlled experiments could be devised under true microgravity conditions. In addition to gaining new insights into fundamental plant biology, these experiments help us understand how plants would survive in a microgravity environment where they could be helpful for recycling water and air, and for providing nutrients for the human crew on long missions (Chebli and Geitmann, 2011; Paul et al., 2013). In addition, plants can also improve the confined environment of space vehicles by having positive psychological effects on astronauts, which for instance may help them fight depression and loneliness (Zimmermann, 2003). It is generally agreed that the view or the touching of plants have positive effects on human health (Koga and Iwasaki, 2013; Ulrich, 1986).

In 2009, the Canadian Forest Service was offered the opportunity to conduct an experiment within the ISS. Considering that very few studies had been carried out with conifer species in a weightless environment, that such a study might help us better understand the effect of gravity on tree growth and wood quality, and that these species are of tremendous importance for Canada's economy, representing for instance 76% of the 230 million cubic meters of wood harvested in 2011 (Natural Resources Canada, 2013), three lines of white spruce seedlings were obtained with the original aim to study more specifically wood formation in a microgravity environment in comparison with controls grown on Earth. The first results of this experiment on the differential gene expression patterns were recently published by Beaulieu et al. (2013). The current study is the second part of this experiment that reports measurements of morphological traits of the stem and their leaves (also called needles in conifers) as well as microscopic observations of features mostly related to amyloplast sedimentation.

2. Material and methods

Details about white spruce seedlings, their preparation for the assays, the growing conditions at every stage and the way they were transported to the Kennedy Space Center (KSC) and to the ISS have been extensively described in Beaulieu et al. (2013). In this section, a summary of the main protocols is provided as well as all the details about the manner in which the morphological data were collected and the samples were processed for microscopic examinations.

2.1. Plant material and its transportation

Three lines (designated as A, B, C) of white spruce (*Picea glauca* (Moench) Voss) were used in the study. These plants, kindly provided by J.D. Irving Limited, New Brunswick, Canada, had been produced by somatic embryogenesis (SE). Upon their arrival at the Natural Resources Canada facilities at the Laurentian Forestry Centre (LFC) in Quebec City in a state of dormancy, the spruces were 1 year old, ranging in height from 4.5 to 8.5 cm. Sixteen 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). The tubes had been cut in 5 cm lengths and four slits were made at their bottom, according to the protocol developed for *Arabidopsis* culture in space (Levine, 2008). To induce bud break, plants were grown in a growth chamber for about 25 days at 15 °C, 70% relative humidity (RH) and photoperiods of 16 hours. On March 30, 2010, the Oasis foam blocks containing the 48 SE plants were placed into Ziploc plastic bags with enough water to avoid desiccation and shipped to the Space Life Sciences Laboratory (SLSL) of the KSC in Florida, USA. Plants were maintained at temperatures between 2 and 8 °C during transportation and they were stored in a refrigerator at 4 °C upon arrival.

On April 1, the plants of each of the three lines were divided into two groups of eight plants and placed in Ziploc plastic bags. Water (100 mL) was added before sealing them. Two replant kits consisting of small aluminum boxes with holes into which the bags were inserted were prepared for flight and ground-control experiments. These kits were stored in a refrigerator at 4 °C.

Both the flight and ground-control experiments were conducted using the Advanced Biological Research System (ABRS) developed by CSS-Dynamac (Fairfax, VA, USA) for NASA. The ABRS has two growth chambers, each one being a closed system capable of independently controlling temperature, illumination, RH, CO_2, ethylene and volatile organic compounds. Two root trays were prepared and preconditioned for the experiment under the hood of a laminar flow. First, the ABRS tray Oasis foam was autoclaved and inserted into the root tray, and three rows of six holes were bored into the foam to hold the tubes with the plants. Autoclaved distilled water was added on the Oasis foam and the root tray was weighed. In order to saturate the Oasis foam, the trays had to be filled with and drained of distilled water several times. Once saturated, the trays were weighed again. A plastic film with small holes to facilitate root oxygen exchange and covering the Oasis foam surface was then fixed to the root tray using Kapton tape strips, and the trays were put in a plastic bag and kept in the laminar flow.

On April 2, the replant kit and the root tray needed for the flight experiment were transported from the SLSL to the KSC Cold Stowage facilities. Replant kits were stored at 4 °C on the ground and in the Space Shuttle until its transfer to the ISS. On April 5, the Space Shuttle Discovery transporting the SE plants was launched under the mission name Space Transportation System-131. Discovery docked with the ISS on April 8.

2.2. Growth experiment conditions

On April 9, the SE trees were extracted from the refrigerator by Flight Engineer T.J. Creamer. The astronaut then planted the more vigorous six plants of each of the three lines in the root tray that had been prepared on the ground. The root tray was inserted into the ABRS labeled as Experiment Rack #2, in the US laboratory known as Destiny. Environmental conditions in the ISS ABRS were obtained by telemetry. The experiment with the ground-control plants began one day later in a second ABRS placed in a controlled environment chamber located at the SLSL. The replant kit was removed from the SLSL refrigerator and the best 18 of the 24 SE plants were planted by Bionetics Corporation personnel in the second root tray. The root tray was then inserted into Experiment Rack #1 of the ABRS. Environmental conditions in the ground ABRS placed in the environment chamber were set to mimic those observed in the ISS ABRS, except for gravity ($1g$ on Earth vs $1 \times 10^{-6} g$ in the ISS). The seedlings grew for 30 days in both incubators. The temperature was set at 24 °C, with 80% RH, CO_2 concentration adjusted at 500 ppm and light intensity at 70–75 µmols on average. As expected, buds burst after 4–5 days and the shoots began growing normally. Due to problems encountered with some samples (see Sections 3.3 and 3.4 for details), roots of seedlings (line B) that were grown in a greenhouse (tem-