Hypobaria and hypoxia affects phytochemical production, gas exchange, and growth of lettuce

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Abstract

Hypobaria (low total atmospheric pressure) is essential in sustainable, energy-efficient plant production systems for long-term space exploration and human habitation on the Moon and Mars. There are also critical engineering, safety, and materials handling advantages of growing plants under hypobaria, including reduced atmospheric leakage from extraterrestrial base environments. The potential for producing crops under hypobaria and manipulating hypoxia (low oxygen stress) to increase health-promoting bioactive compounds is not well characterized. Here we showed that hypobaric-grown lettuce plants (25 kPa ≈ 25% of normal pressure) exposed to hypoxia (6 kPa pO_2 ≈ 29% of normal pO_2) during the final 3 d of the production cycle had enhanced antioxidant activity, increased synthesis of anthocyananins, phenolics, and carotenoids without reduction of photosynthesis or plant biomass. Net photosynthetic rate (P_N) was not affected by total pressure. However, 10 d of hypoxia reduced P_N, dark respiration rate (R_D), P_N/R_D ratio, and plant biomass. Growing plants under hypobaria and manipulating hypoxia during crop production to enhance health-promoting bioactive compounds is important for the health and well-being of astronauts exposed to space radiation and other stresses during long-term habitation.

Additional key words: bioprotectants; carbon assimilation; chlorophyll content; dark respiration rate; *Lactuca sativa*; low pressure; oxygen radical absorbance capacity; phytochemicals.

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Introduction

The new Christopher Columbuses of the 21st century are the astronauts and cosmonauts who will push the envelope of long-term space exploration and human habitation. Like the early explorers or the military campaigns of Napoleon, a reliable food supply is needed for survival and sustainable advancement. It is well documented that green plants supply food, oxygen, help scrub the atmosphere of CO_2 and other volatiles, phytoremediate water, and have a significant impact on the psychological well-being of people (Paul and Ferl 2006). The development of safe, sustainable crop production systems is essential for augmenting the food supply and the gaseous environment of the crew. Besides nutritional benefits (carbohydrates, proteins, lipids, and vitamins), plants can also produce functional secondary metabolites, including protective phytochemicals (bioprotectants), particularly important for astronauts to prevent space radiation-induced chronic diseases developed after short- and long-term duration missions.

Hypobaria (low pressure) is an important component in developing sustainable, energy-efficient plant production systems. There are also major engineering and safety

Received 9 August 2012, *accepted* 11 February 2013.
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Abbreviations: C_A – CO_2 assimilation; Car – carotenoids; Chl – chlorophyll; DPR – dark-period respiration; FL – fluorescein; LPPG – low pressure plant growth system; ORAC – antioxidant activity; P_N – net photosynthetic rate; P_N/R_D ratio – net photosynthesis/dark respiration rate ratio; pO_2 – partial pressure of O_2; R_D – dark respiration rate; ROS – reactive oxygen species; TP – total soluble phenolics.
Acknowledgements: We thank Tom Boutton, Ray Wheeler, and Mark Tjoelker for critical review of the manuscript. Supported in part by the National Aeronautics and Space Administration (NASA) through grants No. NAG-9-1067 (Plant Growth and Metabolism at Sub-Ambient Atmospheric Pressures), and No. NAJ04HF31G (Plant Growth at Sub-Ambient Atmospheric Pressures with Control of the Partial Pressures of Constituent Gases), and the Texas AgriLife Research.

advantages of growing plants under subambient conditions for extraterrestrial base environments (Paul and Ferl 2006, He *et al.* 2009b). For a lunar mission, the ambient pressure is near 0 kPa, whereas the Martian ambient pressure varies from 0.2 to 0.9 kPa (< 1% Earth's ambient). Pressure differences between the hypobaric lunar and Martian environment, and maintaining a higher, Earth-ambient total pressure, would result in greater leakage. The reduced pressure differential between a hypobaric plant growth facility and the external environment would reduce structural requirements, permit lighter materials to be used in its construction, and improve safety issues by reducing external and internal pressure differentials (Bucklin *et al.* 2004). This would further reduce the payload volume and mass required for deployment. Furthermore, hypobaric conditions in the plant growth facility would require less buffer gas, typically N_2, to be transported or obtained *in situ* to supplement the physiologically active gases (CO_2 and O_2). Current extra vehicular activity (EVA) suit pressure is 26–30 kPa, so a reduced cabin pressure would minimize prebreathing exercises required for suiting up in the event of an emergency. This is the primary reason for considering reduced pressure by mission planners. NASA has targeted an operation pressure of ≈ 54 kPa for future space exploration for many of these reasons (Wheeler 2009).

Hypobaric environments are typically associated with hypoxia, particularly, when total gas pressure is reduced below 50 kPa. Hence there is a need to supply sufficient partial pressures of O_2 to avoid hypoxia in plants under hypobaric conditions. Earlier studies have demonstrated that seed germination and seedling growth are possible at hypobaric conditions (Musgrave *et al.* 1988, Schwartzkopf and Mancinelli 1991, He *et al.* 2003). It is also a common observation that plants can be grown at high altitude, where pressures are well below 70 kPa (Gale 1972, Davies *et al.* 2005), although the invariable association between increasing altitude, decreasing temperature, and change in light quality confounds the issue of the effect of pressure alone. The question here is whether the rates of plant growth and morphogenesis compare closely with those at ambient pressure. A major potential limitation to plant growth under hypobaria is that oxidative phosphorylation can become limited if the partial pressure of O_2 (pO_2) is reduced (Drew 1997). To test limits of hypobaria, seedlings germinated and grew during a week-long study at 6 kPa total gas pressure, provided the atmosphere that was composed predominately of oxygen (pO_2 = 5 kPa; ≈ 83% O_2); but at lower total pressures and therefore less O_2, seeds failed to germinate (Schwartzkopf and Mancinelli 1991). He *et al.* (2007) reported that biomass accumulation of 'Buttercrunch' lettuce was not affected by hypobaria (25 kPa), but hypoxia (6 kPa pO_2) reduced plant gas exchange (photosynthesis, dark-period respiration) and biomass regardless of the total atmospheric pressure. Plant production could be done in separate hypobaric facilities with the crew using supplementary oxygen supply while tending the space crops. Hence, there is the rationale for growing plants at 25 kPa total pressure.

Plants produce functional phytochemicals that act as antioxidants by neutralizing harmful free radicals, thus protecting cell DNA, proteins, and lipids from oxidative damage (Wargovich 2000). A diet abundant in natural antioxidants and anticarcinogenic compounds is highly desirable for astronauts living in oxidative environments and exposed to ionizing cosmic radiation. Production of functional phytochemicals is influenced by both genetic and environmental factors that induce stress in plants. Light, temperature, salt, and water stress have been shown to enhance functionally important phytochemicals in various crops (Cisneros-Zevallo 2003, Dumas *et al.* 2003, Beckwith *et al.* 2004, Kubota *et al.* 2006).

Stresses caused by hypobaria and hypoxia could affect functional chemicals in a similar manner. There are differing reports on how hypobaria influences the production of functionally important chemicals. Levine *et al.* (2008) reported that hypobaria (33 kPa) at normal oxygen (21 kPa pO_2) had no effect on total antioxidant activity of radish. Stutte (personal communication) found that hypobaria (33 kPa) at normal oxygen level enhanced anthocyanin concentration, but reduced biomass of two lettuce cultivars from 27–41%. Rajapakse *et al.* (2009) reported that hypoxia (6 kPa pO_2) under both ambient pressure and hypobaric (25 kPa) conditions increased the production of protective phytochemicals, including phenolic compounds such as leaf anthocyanins, but there was a 25% reduction in biomass.

While it is important to increase bioprotectants, it is equally critical to maintain photosynthesis and plant biomass production under hypobaria for crop production. We hypothesized that hypobaric-produced lettuce plants could be subjected to hypoxic stress during the final stages of production to enhance bioprotectants without loss of biomass.

Materials and methods

Low pressure plant growth system (LPPG) is a fully automated system, capable of controlling pressure and gas concentrations in ambient or reduced pressure growth chambers (He *et al.* 2006, 2007). The LPPG system consisted of six growth chambers designed to operate at pressures as low as 5 kPa (Fig. 1). The six chambers were housed in an environmentally controlled growth room. Total pressures, and partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) were controlled and monitored during experiments. The LPPG was a semiclosed system, since O_2, CO_2, and N_2 were added and controlled. Temperature was recorded, although not controlled directly by