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Space Experiment On Tuber Development & Starch Accumulation For CELSS

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Summary

Potato explants (leaf, small stem section, and axillary bud), flown on STS-73, developed tubers of 1.5 cm diameter and 1.7 g mass during the 16 day period of spaceflight. The experiment was undertaken in the ASTROCULTURE™ experiment package under controlled temperature, humidity, lighting, and carbon dioxide concentrations. The tubers formed in the explant system under microgravity had the same gross morphology, the same anatomical configuration of cells and tissues, and the same sizes, shapes, and surface character of starch granules as tubers formed in a 1 g environment. The total accumulation of starch and other energy containing compounds was similar in space flight and ground control tubers. Enzyme activity of starch synthase, starch phosphorylase, and total hydrolase was similar in spaceflight and ground controls but activity of ADP-glucose pyrophosphorylase was reduced in the spaceflight tuber tissue. This experiment documented that potatoes will metabolize and accumulate starch as effectively in spaceflight as on the ground and thus this data provides the potential for effective utilization of potatoes in life support systems of space bases.

Introduction

The ASTROCULTURE™-05 plant experiment was undertaken to establish the effectiveness of potato explants to form tubers and to metabolize and accumulate starch during spaceflight. This process is of critical importance to utilizing plants for providing energy-rich foods in life support systems in future space bases. This study also was of particular importance to plant physiologists because previous experiments with other plant species in space, and some ground-based studies, have documented reduced starch accumulation when plants are subjected to real or simulated microgravity.

Each potato explant used in this study consisted of a leaf, its axillary bud, and a small stem segment harvested from mother plants which had grown in controlled environments on the ground. Tubers formed from the axillary bud in the days immediately after harvest from the mother plants. These explants provided a model system for the study of the physiological and developmental processes that occur during tuber formation in the short period of a shuttle flight .

Methodology

Potato (*Solanum tuberosum* cv. Norland) mother plants were grown from in-vitro propagated plantlets, beginning 6 weeks before launch. Plants were grown at 21 C, $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) for a 12-h photoperiod, 80% RH, and approximately 350 ppm CO_2 in a walk-in plant growth chamber at the Kennedy Space Center. Five explants for flight were harvested from the 7th and 8th leaves of the mother plants, counting basipetally from the youngest leaf that was 1 cm in length. Each explant consisted of a leaf, axillary bud, and small section of stem. Since more leaf tissue was present than could be accommodated in the plant chamber, the lamina was trimmed to fit by cutting off leaflet tips. The stem and lower petiole region of each explant was placed into a tray filled with moistened arcillite (calcined clay particles). Light-tight flexible gaskets were placed around the petiole of each explant and opaque acrylic plates were secured over the base of the explants and arcillite for their containment during space flight.

The group of explants were then secured within the small controlled environment chamber of the ASTROCULTURE™ growth unit detailed in the cooperative hardware evaluation experiment. This flight package was programmed to maintain the plant chamber at 21 C, 80% RH, and a minimum of 500 ppm CO_2 . Above the plant chamber was an array of red and blue LED's to provide $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF for a 12-h light period. Water was delivered to the arcillite-containing plant tray by porous tubes and a negative pressure system.

Six hours after loading the explants, the ASTROCULTURE™ unit was installed into the middeck of the space shuttle Columbia and the unit powered to initiate the programmed environmental conditions. The shuttle launch occurred 22 hours after power up and 28 hours after loading the explants into the package. Details of the precise experimental conditions over the course of the 16-day study are provided in the hardware experimental report prepared by Bula et al. Of particular importance for the study were 1) the downlinking every two days of video images of the explants and the 2) daily downlinking of carbon dioxide concentrations within the chamber, both of which provided information on the physiological vigor and activity of the explants.

The above protocol also was followed for baseline control studies conducted in a controlled environment facility (Biotron) at Madison, Wisconsin, one month following the flight. Care was taken to insure that ground control mother plants, grown at the Kennedy Space Center were the same age from transplanting as the plants used for the flight. The temperature, humidity, and carbon dioxide concentrations of the middeck during the 16 day flight period were duplicated as closely as possible in the controlled environment room of the Biotron. Explants were harvested from mother plants, 12 hours before loading into the ASTROCULTURE™ flight unit and held at $5 \pm 2\text{C}$ for this period in small cold storage units over ice. This provided time to carry the explants from Kennedy Space Center to Madison. (The flight explants were also harvested 12 hours before loading into the ASTROCULTURE™ unit and held in the same storage units in a cold room at $5 \pm 0.2 \text{C}$.)

Results

Inflight-The downlinking of video images demonstrated good plant vitality during the first 12 days of the mission followed by senescence of the leaves. This same pattern of plant vitality was documented by the downlinked carbon dioxide data from the plant chamber, which showed low levels (~ 500 ppm) of carbon dioxide during the light period (due to photosynthetic CO_2 uptake) followed by high levels (~ 3000 ppm) each dark period (due to respiratory CO_2 output) during the

first 12 days (Fig. 1). After 12 days, the carbon dioxide changes over light:dark periods were slower and reduced in magnitude as photosynthesis and respiration slowed.

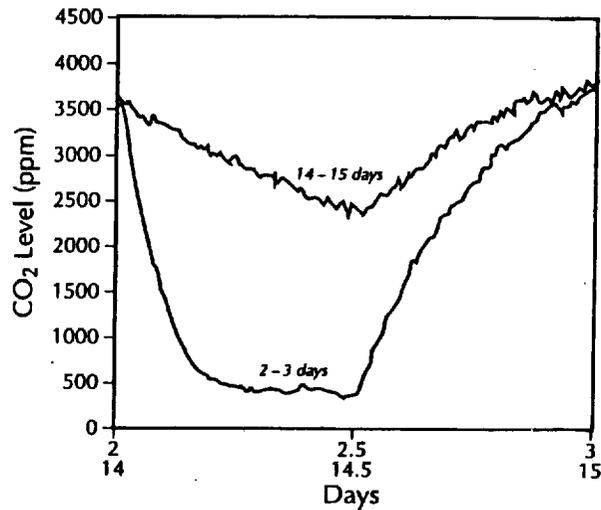


Fig. 1. Concentration of carbon dioxide in the growth unit of the ASTROCULTURE™ unit during 3-5 days and 13-15 days of space flight.

The environmental conditions maintained in the experiment package during the space flight and ground control are shown in Table 1. Similar temperature (22 C) and humidity conditions (80%) were maintained for both the flight and ground controls, some variations in carbon dioxide concentrations did occur between flight and ground controls, however it is felt that this difference was not significant to the maintenance and development of the explants.

Table 1. Environmental conditions in the growth chambers of the spaceflight and of the ground control.

Environmental Parameter	Set Point	Spaceflight			Ground Control		
		Ave*	Max**	Min**	Ave*	Max**	Min**
Temperature (C)	21	22.1	23.9	21.2	22.0	23.9	20.6
Relative Humidity (%)	80	81.3	88.0	76.6	78.2	81.4	75.5
Carbon Dioxide (ppm)	500	2308	4100	350	2102	5758	204

* Average conditions for the 16 day period.

** Maximum and minimum average conditions for 12 hour period.

Post-Flight- The package was recovered from the space shuttle Columbia within 4 hours after touchdown at the Kennedy Space Center and explant harvests completed within 6 hours after touchdown. Explants were photographed, tubers were measured and prepared for anatomical and biochemical analysis. Tissue was placed in a formalin based fixative for anatomical studies and frozen in liquid nitrogen for biochemical analysis.

The size and shape of the space-formed tubers were similar to ground control tubers (Figure 2) Periderm color was similar.

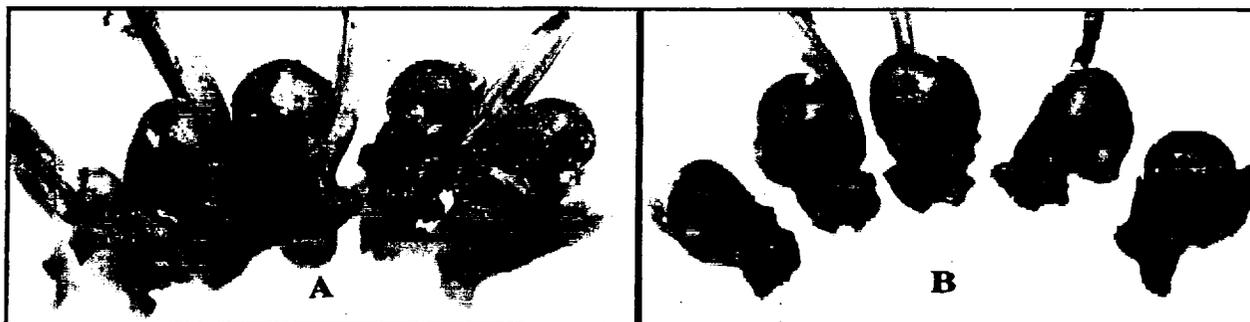


Figure 2. Potato tubers produced by the explants after 16 days of growth in spaceflight (A) and on Earth (B).

All explants showed signs of senescence (chlorosis and /or necrosis) of laminar tissue and the death of the leaf rachis basipetally, but all explants had turgid petioles and stems. The space-grown explant that formed the smallest tuber had green laminar tissue in the terminal leaflet and one of the first pair of leaflets. The entire rachis and petiole of this explant were turgid and dark green in contrast to the other four explants. It had been found in baseline studies that tubers produced from explants of different development age can vary, and this leaf with the small tuber was slightly younger developmentally, based on its leaf position and leaf length. Tubers formed under conditions of microgravity had the same diameter and weight as those formed on earth (Table 2).

Table 2. Tuber growth

<u>Spaceflight</u>		<u>Ground Control</u>	
<u>Diameter</u>	<u>Mass</u>	<u>Diameter</u>	<u>Mass</u>
(cm)	(g)	(cm)	(g)
1.5± 0.8	1.69± 0.34	1.5± 0.1	1.51±0.28

* The small tuber developed on one explant in space flight is not included in this data. For space flight n = 4, for ground control n = 5. Values ± Sd.

Tuber tissues were found in the same geometric arrays, with interior cells arranged in a honeycomb pattern and the tissue at the surface comprised of cell layers with cells of a layer stacked directly on top of those of the previous layer.

Cell wall thickness in space flight tubers varied with some walls one-third as thick as other cell walls. In contrast, the thickness of cell walls in ground grown tubers was uniform.

The number of layers in the periderm was the same as in ground grown tubers, and there was neither starch nor protein in this tissue. Instead, starch and protein were found exclusively in the interior cells in both the space flight and in the ground control tubers.

Starch grains had comparable shapes, similar smooth surfaces, and a size range that was the same as those in ground controls (Figure 3).

The distribution of grains into size categories differed in tubers developed under space flight conditions compared to those formed on the ground. A greater percentage of grains were found in the smaller size classes in space flight than were found in earth grown tubers (Figure 4). The aspect ratio (length/width) of the grains formed in space was not different from that of ground-control tuber grains.

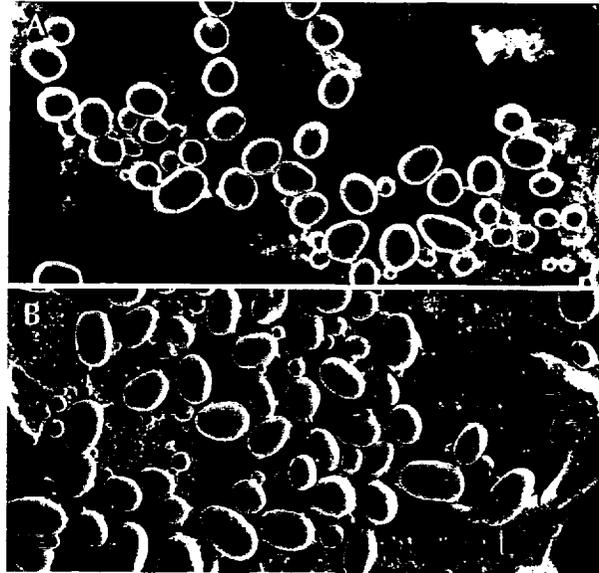


Fig. 3. Starch grains from potato tubers formed in space flight (A) and on the ground (B)

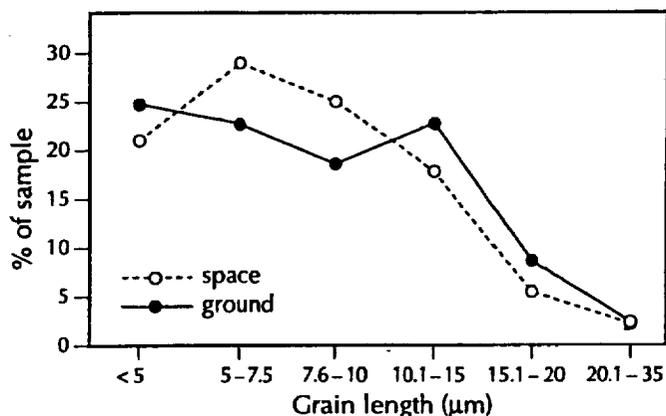


Fig. 4. Percentage of the starch grains by size category formed in potato tubers during space flight and on the ground.

Protein crystals were found in tissues of the tubers developing both in space flight and on the ground. No obvious differences in crystal size, or distribution within the tuber, were evident.

The biochemical analysis of the tuber tissues did not document any large differences in tissues developed in space flight compared to the ground controls. The starch, sucrose, glucose and soluble protein concentrations in the tubers of flight and ground controls were similar (Table 3). Although there were some differences, the standard deviation of values was quite large and thus no significance can be placed on the small differences.

Table 3. Carbohydrates and soluble proteins in potato tubers

Constituent	Spaceflight*	Ground Control
Starch (%)	43.4 ± 13.4	40.1 ± 12.3
Sucrose (%)	0.9 ± 0.1	0.7 ± 0.2
Glucose (%)	0.2 ± 0.1	0.1 ± 0.0
Soluble protein (%)	12.6 ± 1.2	11.5 ± 0.9

*Values represent the means of 4 replicates ± S_d on a dry weight basis.

Activities of enzymes controlling starch formation in plant tissues were similar between flight and ground control tubers with one notable exception (Table 4). ADP-glucose pyrophosphorylase, a rate-limiting enzyme in starch synthesis had significantly reduced activity in the space flight tubers compared to the ground controls. However, another enzyme involved in starch synthesis, starch synthase, showed similar activity in tubers developed in spaceflight compared to the ground. Two enzymes involved in starch degradation, starch phosphorylase and total hydrolase, showed similar activities in tubers from space flight and from the ground (Table 4).

Thus, although the activity of the ADP-glucose pyrophosphorylase was reduced, this had no significant influence upon the total quantity of starch accumulated in the tubers, which was similar between the space flight and ground explants (Table 3). This decrease in activity of ADP-glucose pyrophosphorylase is consistent with research with other plant tissues, which has documented reductions in activity of this enzyme in space flight and in simulated microgravity experiments on the ground.

Table 4. Enzymes controlling starch synthesis and degradation in potato tubers

<u>Enzyme</u>	<u>Spaceflight</u>	<u>Ground Control</u>
	nmol (gfw.min) ⁻¹	
ADP-glucose pyrophosphorylase	8.7 ± 1.9	16.2 ± 2.5
Starch synthase	71.0 ± 10.5	73.0 ± 2.5
Starch phosphorylase	8.3 ± 0.5	9.3 ± 1.2
Total hydrolase	48.1 ± 9.0**	39.0 ± 3.6**

*Values represent the means of 4 replicates ± S_d on a dry weight basis.

**μmol (gfw.min)⁻¹

Conclusions

This study has documented that starch accumulation occurred effectively in potato tubers in the spaceflight environment. The tubers formed in the explant system under microgravity had the same gross morphology, the same anatomical configuration of cells and tissues, and the same sizes, shapes, and surface character of starch granules as tubers formed in a 1 g environment. Space flight did not alter these fundamental features of tuber differentiation. The total accumulation of starch and other energy containing compounds was similar in space flight and ground control tubers. However the reduced activity of the one critical starch synthetic enzyme, ADP-glucose pyrophosphorylase, deserves further study and evaluation with other crop plants that are being considered for food production in life support systems for long term space bases.

Publications

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