Growth and Development of Seedling and Stem Cell under Microgravity Conditions

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Abstract

The effect of clinostatting and microgravity on plant cells and organs is considered for two types of gravistimulation: static and dynamic. The former is eliminated by both clinostatting and microgravity; the latter is eliminated by microgravity, but is inevitable during clinostatting, and may be perceived by cells if rotation is not fast enough. To test the effect of clinostatting on root cells and development, lettuce seedlings were germinated and grown for two weeks in a spacetron, keeping the centrifugation rate at zero. In the clinorotated plants, amyloplasts were distributed throughout the cells and were not sedimented as in the stationary control. Cells of seedlings grown in a spacetron have significantly different ultrastructures from those grown under control conditions of 1 *g*.

Keywords: Clinostat, Spacetron, Simulated microgravity, CELSS, Space biology

INTRODUCTION

For over one hundred years, biologists have used clinostats to study how organisms might adapt to the microgravity environment, and what effects the force of gravity has on plant and animal development and behavior. Clinostats are the best and only way for Earth-bound scientists to learn about how the upsetting of normal gravitational cues can affect plant growth and development. Clinostat experiments provide valuable insight into future space experiments, as well as bringing us closer to understanding exactly how plants sense and respond to gravity. The clinostat is a simple device that places a plant, small organism, or cell growing in culture on a rotating platform. Rotation causes the biosystem under test to be subjected to gravity vectors from "all" directions. From the system's perspective, the rotation cancels the gravity vector by continuous averaging, thereby approximating the highly reduced vector found in the actual space environment. As "Moore (1990)" stated, clinostats randomize rather than abolish gravity. That is, seedlings rotated on clinostats are constantly exposed to 1 *g* albeit from ever-changing directions. Furthermore, a clinostat stresses plants mechanically, thereby altering the plant's physiological properties "Brown et al. (1976); Brown and Chapman (1985)".

Scientists use space as a unique research laboratory to answer fundamental questions in basic biology and physiology. Space biology researchers investigate how cells, plants, and animals sense and respond to gravity. Plant development has emerged as

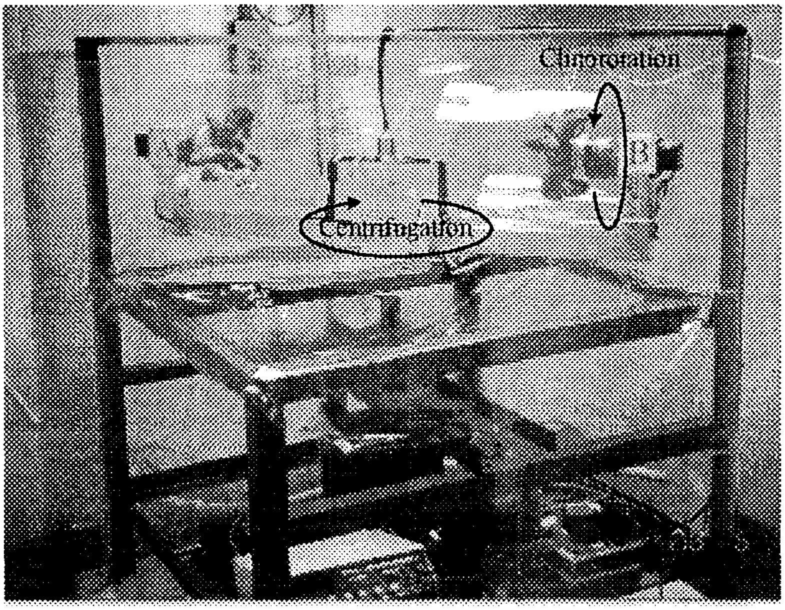

Fig. 1 Spacetron was used to create simulated micro- and hypergravit conditions by controlling the centrifugal force can be used as clinosta and centrifuge.

a particularly challenging aspect of space biology, largely due to a number of intriguing observations that suggest that space flight affects how plants develop and reproduce.

The growth and development of higher plants are strongly influenced by gravity, and cell walls play an important part in supporting the plant body under terrestrial gravity conditions. Therefore, the physical properties of the cell wall will change greatly in the microgravity environment of space. Columella cells of seedlings grown on a clinostat have different structures from those grown at 1 *g* "Moore (1990)".

Researchers have also seen changes in how plant cells divide. By understanding the role of gravity in these changes, scientists hope to develop the knowledge needed to engineer new plant types with potential economic value. This research will also contribute to our general understanding of gravity's effects on plant cells and development.

MATERIAL AND METHOD

Plant material and hardware

Lettuce (*Lactuca sativa* L. cv. Okayama) was used as a model plant for the experiments, because it grows quickly. The seeds were planted at 0.8~1.0-cm depths in a rockwool cube (Gordan Multiblock AO 36/40 hole 10/10). The chamber had a temperature of 24 °C and a relative humidity of 60%. The average photosynthetic photon flux density (PPFD) on the top of the rockwool cube was 100 μmol m^{-2} s^{-1}, with a 24-h photoperiod. The previously developed spacetron (Fig. 1) was used to stimulate

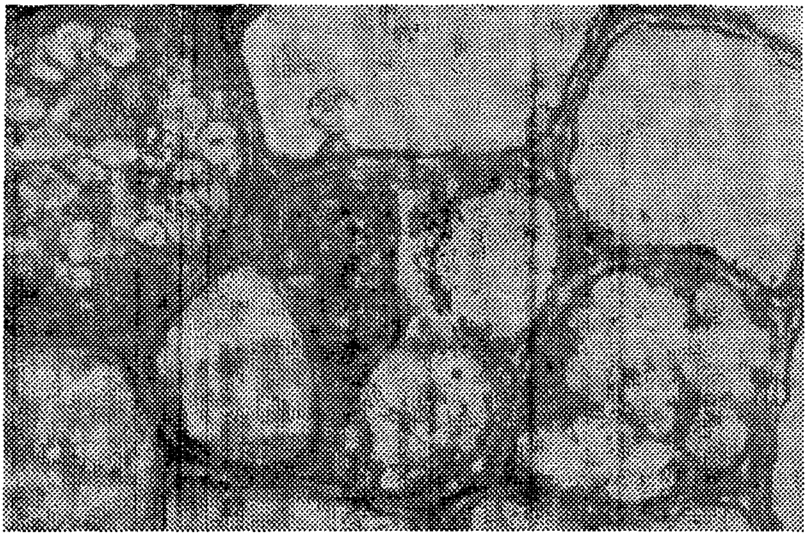

Figure 2 Ultrastructure of root grown on spacetron at 25 rpm clinorotation, shows that the clinorotation induced random

microgravity "Zaidi et al. (1997)". Clinorotation rates of 25 and 50 rpm were used, with the centrifuge rate fixed at zero. On the first day, the cubes were soaked with water. Beginning on the third day, the seedlings were nourished with 100 ml of half-strength Otsuka nutrient solution every day. After spending the first week on the ground, the seedlings were grown in the spacetron for two weeks.

Controls

Controls were grown in the same chamber of the spacetron using identical materials, procedures, and times, except there was no rotation.

Electron microscopy

Both control and spacetron seedling materials were glutaraldehyde-fixed, washed in buffer, left in buffer overnight, and then post-fixed in 2% (w/v) osmium tetroxide for 2-3 hours. Then, the samples were dehydrated through an ethanol series, transferred to BGE (butyl glycidyl ether), and infiltrated into 80% Quetol 653 resin for 2 h.

Ultrathin (100 nm) sections were cut using an ultramicrotome and the sections were transferred to grids and stained with uranyl acetate. These sections were then viewed and photographed with a transmission electron microscope.

RESULTS AND DISCUSSION

Figure 2 shows the ultrastructure of a root germinated in the spacetron and grown at 25 rpm clinorotation. Cell polarity is evident, with the nucleus located proximally. Amyloplasts are not sedimented, but are randomly distributed in the cell. Hilaire et al. (1995) used clinorotation of 2 rpm and concluded that in clinorotated plants, amyloplasts

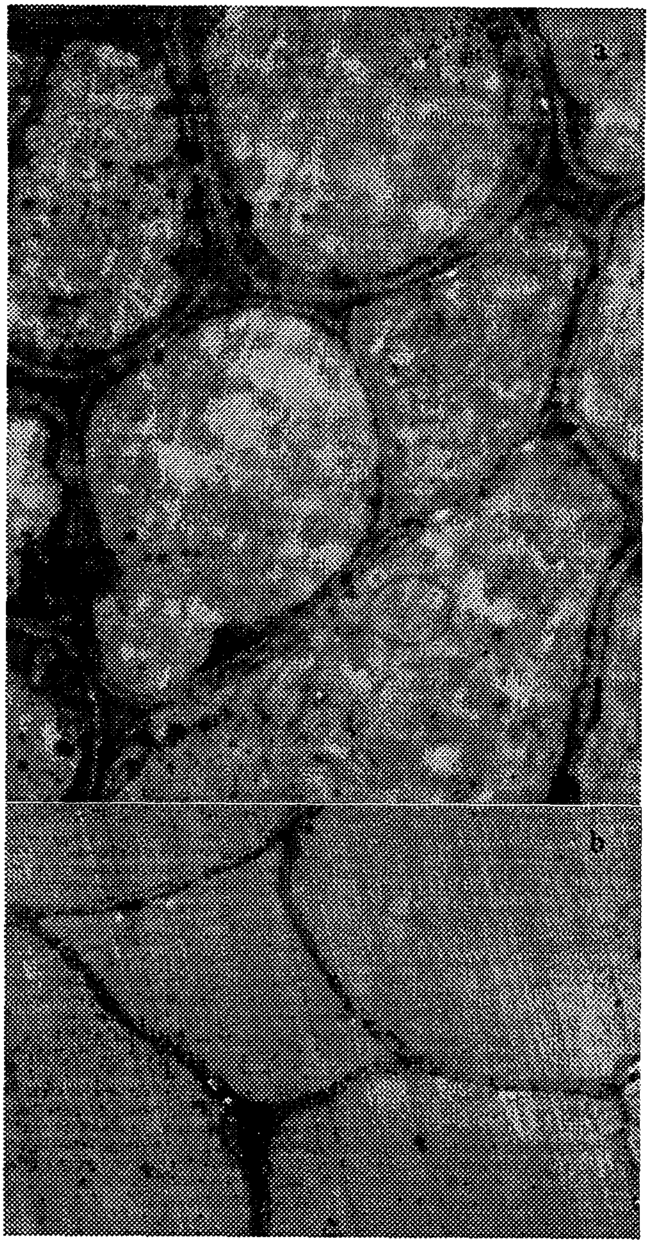

Figure 3 a, Showing the unilateral distribution of dark precipitates along the lateral cell walls, when the cell wall of control "b" is clear.

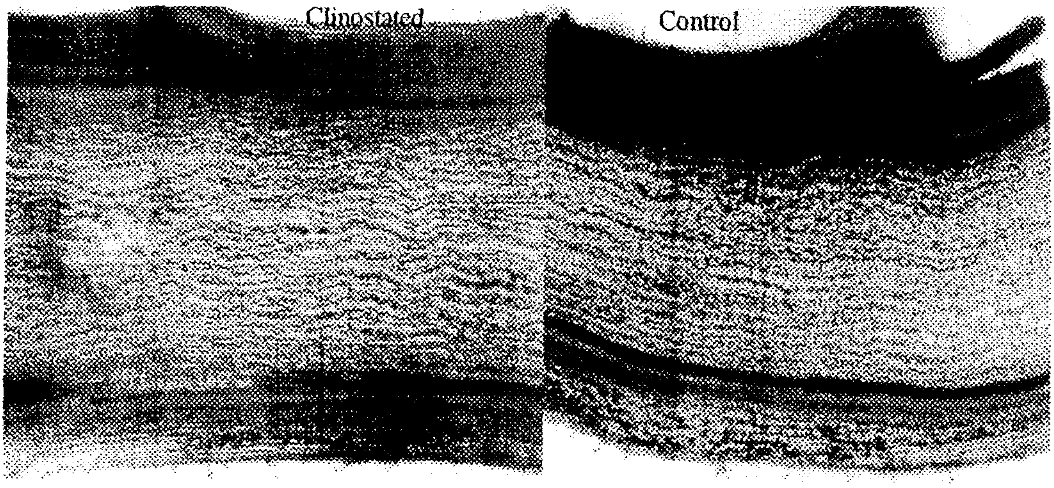

Figure 4 Longitudinal sections of lettuce stem of control and Clinostated plant.

were distributed throughout the cells and not sedimented as in the stationary control. The ultrastructural preconditions for graviperception "Volkmann and Sievers (1979)" develop independently of the direction of the gravity vector and are thus determined genetically "Sievers et al. (1976)".

If changes in plant organs occur after clinorotation, these effects should be due to continuous gravitropic stimulation of cells able to perceive gravity. Clinorotation of roots causes a continuous change in the direction of the gravity vector in relation to the root axis. Thus, the ultrastructure of statocytes is a clear response of sensitive cells to prolonged omnilateral gravistimulation "Hensel and Sievers (1980)". Rotation of roots on a horizontal clinostat leads to continuous movement of the amyloplasts in the statocytes

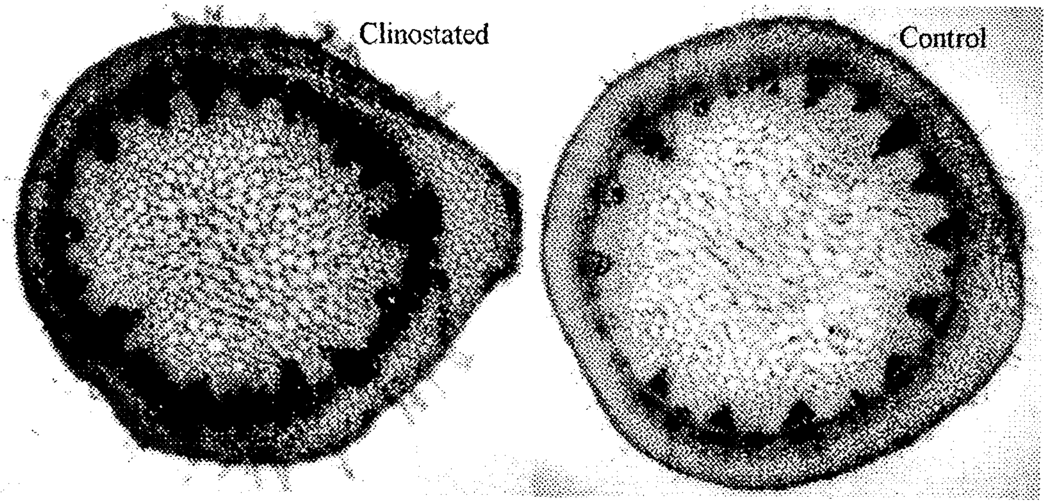

Figure 5 Cross sections of lettuce stem of control and Clinostated plant.

"Huisinga (1968)". The different position of amyloplasts in clinostat-grown as compared to Earth-grown seedlings is consistent with amyloplast movement being due primarily to the organelle's density "Audus (1979)".

In the electron micrographs, the unilateral distribution of chloroplasts along the lateral cell walls was very clear (Fig. 3a) and the same pattern was present in cells throughout the thin section, and was not present in the stationary control (Fig. 3b). The thickness of the cell wall and the intercellular space of clinostated seedling roots were significantly different than in an earth gravity of 1-*g*. The differences between the ultrastructure of 25 and 50 rpm were not identified at the present stage.

Figure 4 shows the picture of stem longitudinal section of lettuce plant grown on spacetron and control, showing the difference between stem cell structure, in clinostated section accumulated irregular masses of tannin then the control, tannin is a chemical, that is bitter and deter insects from eating the tissue.

Figure 5 show the picture of stem by light microscope the cell wall is dark as compare to control. Also shows that the cortex (clinostated) is the narrow band of cells between epidermis and the vascular bundles and the cortex of this lettuce stem is even narrow.

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