

Autotropism under Microgravity Conditions Simulated
by a Three-Dimensional Clinostat

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Gravity strongly influences growth and developmental processes throughout plant life cycle on earth. In order to understand the role of gravity in regulation of growth and development, it is required to examine the changes in each process under a microgravity environment. Microgravity conditions can be produced on earth by a free fall using drop towers or balloons and by a parabolic flight of airplanes and sounding rockets. However, the duration of microgravity conditions produced by these simulations is generally too short for plants to exhibit apparent changes in their growth processes.

The clinostat with a horizontal axis has been routinely used for simulating microgravity conditions in plant science (Sachs 1882, Larsen 1953). The instrument has been useful for analysis of the processes of gravitropism. However, the clinostat rotation in the constant direction often causes undesired side effects. Recently, we developed a new clinostat equipped with two rotation axes (Hoson et al. 1988). On the clinostat, plant materials are rotated three-dimensionally at random rate, thus microgravity conditions are simulated more precisely. We have found that the direction of growth of plant organs was strongly influenced under the simulated microgravity conditions, although the growth rate was not always affected (Hoson et al. 1992). Especially, shoots of plants grown on the clinostat exhibited the spontaneous curvature as well as the altered growth direction. In the present article, we will describe the details and the mechanism of such a spontaneous curvature (autotropism) of shoots grown on the 3-D clinostat.

3-D Clinostat

The 3-D clinostat was designed and constructed as reported previously (Hoson et al. 1992). The main power to rotate plant materials was provided by two geared stepping motors (UPD534-MHG-2B, Oriental Motor Co.). Sample stage, 660 x 340 mm, and illumination apparatus were attached to the opposite sides of a supporting frame at a distance of 220 mm from a rotation axis, which was rotated by one motor (Fig. 1). The frame was attached to the other frame which was further rotated perpendicularly about an axis parallel to the ground by the second motor (Fig. 1). The cylindrical culture container was fixed to the sample stage. The rotation rate of two motors was changed at random from 2 to -2 rpm at every 1 min. The rotation of motors was observed with encoders, an angle sensor, attached to them. Electrical connection to motors, lamps, encoders, and a video terminal was made via six- and twelve-way slip-rings. The onset, the rate, and the duration of rotation of motors were controlled and observed with a personal computer.

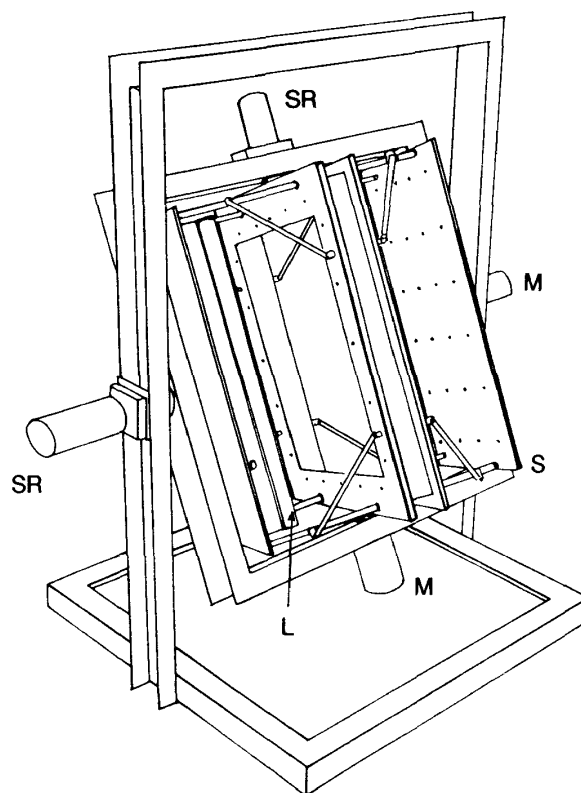


Fig. 1. The clinostat apparatus. L, light; M, motor with encoder; S, sample stage; SR, slip-ring.

Growth Direction

Seeds of cress (Lepidium sativum L.), pea (Pisum sativum L. cv. Alaska), and azuki bean (Vigna angularis Ohwi et Ohashi cv. Takara), and caryopses of maize (Zea mays L. cv. Golden Cross Bantam T-51) and rice (Oryza sativa L. cv. Sasanishiki) were sterilized in 2% sodium hypochlorite solution for 10 min, and then thoroughly washed with water. Cress seeds were immediately attached to a polyethylene sample holder fitted in a culture container. Seeds and caryopses of other species were soaked in water for 24 h, and then germinated ones were used for the experiment. In most of experiments, seeds were positioned in such a way that shoot primordia and root primordia are oriented upward and downward, respectively. The culture container was stood (control) or rotated three-dimensionally on the sample stage of the clinostat.

Cress seeds placed on a wet paper germinated about 16 h after water imbibition. On the surface of the earth, radicles of cress emerged in the direction of the tip of root primordia, but they soon began to bend downward by the positive gravitropism and grew parallel to the gravity vector. On the 3-D clinostat, germinated radicles curved spontaneously toward the seeds and maintained the arc shape for 2 to 3 h. The arc was then opened gradually during the following growth period. At about 30 h after water imbibition, the roots formed a straight line in the direction of root primordia (Fig. 2). Roots of maize, rice, pea, and azuki bean also grew downward on the surface of the earth. On the 3-D clinostat, roots of maize grew in random directions both in a front view and in a side view of embryos (Fig. 2). In a front view, the angle of rice roots on the clinostat shows a bimodal distribution with the peak at 90° and 270° . In a side view, rice roots mainly elongated at 90° (Fig. 2). The data indicate that rice roots rotated on the 3-D clinostat grew parallel to the direction of the tip of root primordia. On the 3-D clinostat, roots of pea and azuki bean grew parallel to the direction of the tip of primordia in the early stage of growth and then grew in random directions both in a front view and in a side view of embryos (Fig. 2).

On the surface of the earth, coleoptiles of maize and rice or epicotyls of pea and azuki bean grew upward, except for a slight abaxial (away from the caryopsis or seed) curvature of maize coleoptiles and epicotyls of pea and azuki bean in a side view of embryos. Growth direction of shoots was more controlled even on the 3-D clinostat. In a front view of embryos, coleoptiles and epicotyls of plants examined grew mostly along the direction of the tip of

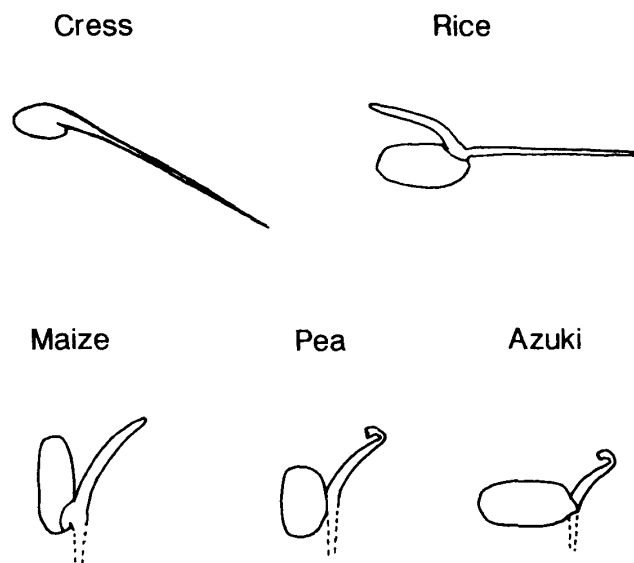


Fig. 2. Seedlings grown on the 3-D clinostat. Roots of maize, pea and azuki bean grew in random directions.

primordia. In a side view, maize coleoptiles exhibited an abaxial curvature (Fig. 2). In contrast, rice coleoptiles showed an adaxial (toward the caryopsis) curvature in a side view (Fig. 2). Epicotyls of pea and azuki bean exhibited an abaxial curvature, like maize coleoptiles (Fig. 2). Thus, the plant species can be divided into two groups according to the behavior on the clinostat. In cress and rice, roots elongated toward the direction of the tip of root primordia and rice coleoptiles bent adaxially. On the contrary, roots of maize, pea, and azuki bean elongated in random directions, and their coleoptiles or epicotyls showed an abaxial curvature.

Autotropism in Maize Shoots

The change in growth direction under simulated microgravity conditions is a complicated phenomenon. The curvature of shoots shown in Fig. 2 is the total of bending at different portions of shoots. The details and the mechanism of such a curvature were further analyzed in maize seedlings grown on the 3-D clinostat.

The curvature of maize shoots was found to consist of three components; 1) the bending in the transition zone connecting roots and mesocotyls, 2) the bending at coleoptile node between mesocotyls and coleoptiles, and 3) the cur-

vature of coleoptiles. Sometimes, the curvature of mesocotyls was also observed. However, in the experiments up to 3 days, such a curvature can be ignored. Even control seedlings showed an abaxial curvature in the transition zone which connects roots and shoots (Fig. 3). On the clinostat, such an abaxial curvature was larger. When seedlings had been transferred to the clinostat after the cultivation on the surface of the earth for 1 day, the angle was in between. In contrast, shoots bent adaxially toward the caryopses at coleoptile node between mesocotyls and coleoptiles, irrespective of experimental conditions (Fig. 3). The angle was small in the control, while it was larger on the clinostat. When seedlings had been transferred to the clinostat after 1 day, the angle was intermediate between the control and the clinostated seedlings from day 0. Control coleoptiles elongated almost straight (Fig. 3). On the clinostat, most of coleoptiles bent abaxially. On the contrary, when seedlings had been transferred to the clinostat after 1 day, coleoptile showed an adaxial or an abaxial curvature, but the percentage of adaxially curved ones was higher.

The rate of plant cell extension is most directly controlled by two factors, the osmotic potential of the cell sap and the mechanical properties of the cell wall (Taiz 1984, Masuda 1990). In order to understand the mechanism by which autotropism of maize coleoptiles is induced, we measured these two

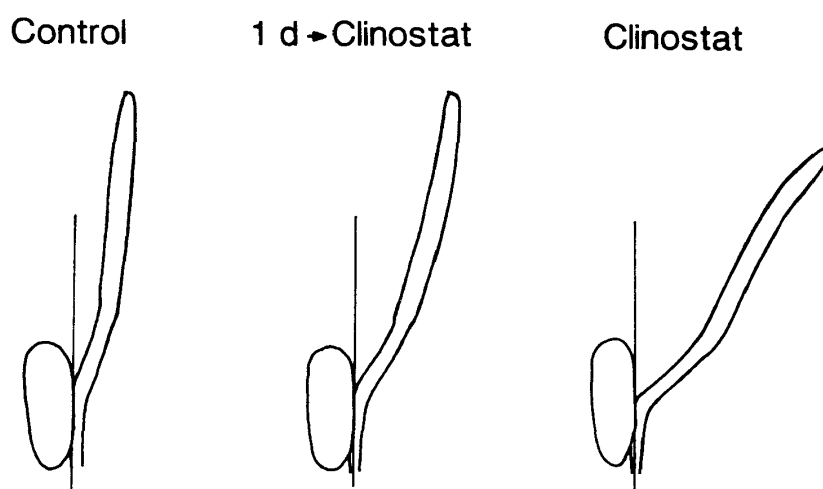


Fig. 3. Maize shoots grown on the surface of the earth (left) or on the 3-D clinostat from day 1 (middle) or day 0 (right).

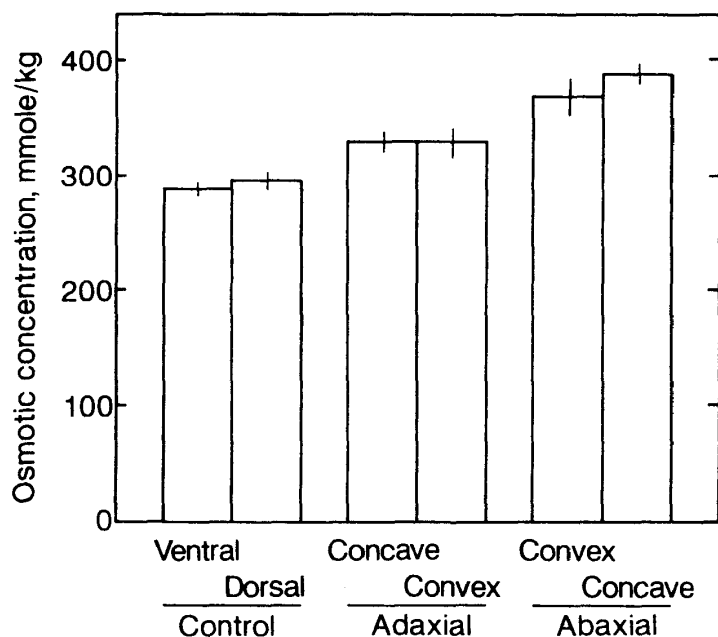


Fig. 4. The osmotic concentration of the cell sap of maize coleoptiles grown on the surface of the earth (left) or on the 3-D clinostat from day 1 (middle) or day 0 (right).

parameters. The osmotic concentration of the cell sap of maize coleoptiles grown on the surface of the earth or on the clinostat using a vapor pressure osmometer (Fig. 4). The osmotic concentration was slightly increased by the clinostat rotation. However, there was no difference in the concentration between the concave and the convex half of either the adaxially or the abaxially curved coleoptiles.

The mechanical properties of the cell wall of each coleoptile half was measured with the stress-relaxation method (Yamamoto et al. 1970). The minimum stress-relaxation time, T_0 and the relaxation rate, R were employed as the parameters exhibiting the physiological extensibility of the cell wall. No difference was found in these parameters between the ventral and the dorsal sides of the control coleoptile (Fig. 5). In the adaxially and the abaxially curved coleoptiles, the convex, rapidly-expanding side of coleoptiles exhibited a smaller T_0 and R (Fig. 5). These results suggest that autotropism of maize coleoptiles under simulated microgravity conditions is induced by cell wall loosening in either side of the coleoptiles.

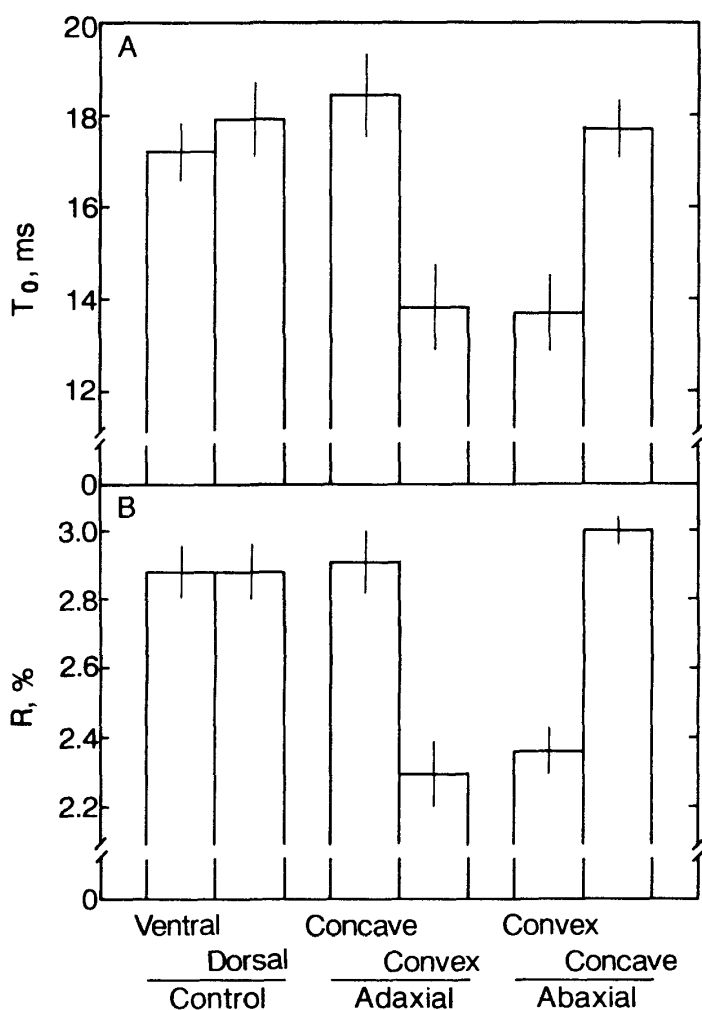


Fig. 5. Effect of the clinostat rotation on the mechanical properties of the cell wall of maize coleoptiles. T_0 and R were calculated by the method of Yamamoto et al. (1970).

Discussion

Under microgravity conditions simulated by the 3-D clinostat, shoots of different plant species exhibit a spontaneous curvature. Such type of asymmetric growth of plant organ is called autotropism (strictly, this phenomenon is not a kind of tropism, because it occurs without any gravity stimulus and the response is not related to the direction of the stimulus). Autotropism of shoots on the 3-D clinostat consists of several components. The details of the curvature were analyzed in maize shoots. Fundamentally, maize shoots showed an abaxial bending in the transition zone which connects roots and

mesocotyls and an adaxial (recovery) bending at coleoptile node between mesocotyls and epicotyls (Fig. 3). Clinostat rotation amplified both types of bending (Fig. 3). In addition to the bending in these two regions, coleoptile itself was found to exhibit a spontaneous curvature.

Autotropism has been observed on a horizontal clinostat in coleoptiles of maize, rice, and oat. Most of these results were obtained in a short-term rotation of seedlings that had been grown vertically on earth. In maize coleoptiles, the curvature consisted of an early abaxial phase followed by a long-lasting adaxial one (Nick and Schafer 1989). Also on the 3-D clinostat, maize coleoptiles mostly bent adaxially when seedling had been transferred to the clinostat after the cultivation on the surface of the earth for 1 day (Fig. 3). In contrast, maize coleoptiles exhibited an abaxial curvature, when the germinated seedlings were rotated on the clinostat from day 0 (Fig. 3). It remains to be clarified which factor determines the direction of bending under stimulus-free conditions. In any case, coleoptiles curved in direction explained by the presence of dorsoventrality.

In order to understand the mechanism by which autotropism of maize coleoptiles is induced, we compared two parameters that control plant cell expansion most directly between the convex and the concave sides of coleoptiles. The results shown in Figs 4 and 5 indicate that autotropism of maize coleoptiles on the clinostat is not induced by the change in the osmotic concentration of the cell sap, but by cell wall loosening in one side of the coleoptiles. There was no difference in the mechanism for induction of curvature between the abaxially and the adaxially curved coleoptiles. The orientation of cortical microtubules was not associated with autotropism of maize coleoptiles on a horizontal clinostat (Nick et al. 1991). Changes in the chemical nature of certain cell wall polymers may be a principal factor for induction of autotropism under microgravity conditions.

In tropistic responses of plant organs to various environmental factors, perceived stimuli or signals are processed and transduced into the asymmetric distribution of hormones, such as auxin or ions, such as Ca^{2+} . The redistribution of these hormones or ions should in turn induce certain changes in the osmotic potential of the cell sap or the mechanical properties of the cell wall. The information about changes in these parameters during tropism has not always been accumulated. In gravitropism of oat coleoptiles (Shen-Miller and Masuda 1973) and cucumber hypocotyls (Iwami and Masuda 1974), the cell wall extensibility of the convex side was higher than that of the concave

side. However, such results have not been reported in gravitropism of other species or in phototropism. Autotropism of maize coleoptiles under simulated microgravity conditions appears to offer an important clue for understanding the mechanism by which the differential growth response is induced in tropism of plant organs.

In conclusion, maize coleoptiles exhibited a complicated autotropism under microgravity conditions simulated by the 3-D clinostat. Coleoptiles of maize bent abaxially or adaxially depending on the timing when they were subjected to the clinostat rotation. Furthermore, it was found that the changes in the mechanical properties of the cell wall were the direct cause of differential growth of maize coleoptiles on the clinostat. Under 1 g condition on earth, the cell wall plays a principal role in supporting plant body as the bone and the muscle in animals. The nature of the cell wall appears to be strongly influenced under a microgravity environment in space. It should be important to clarify the changes in the cell wall under microgravity conditions for understanding the mechanism of regulation of plant life cycle on earth as well as in space.

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