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Evaluation of the three-dimensional clinostat as a simulator of weightlessness

Takayuki Hoson[1], Seiichiro Kamisaka[1], Yoshio Masuda[2], Masamichi Yamashita[3], Brigitte Buchen[4]

[1]Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan
[2]Tezukayama College, Nara 631, Japan
[3]Institute of Space and Astronautical Science, Yoshinodai, Sagamihara 229, Japan
[4]Botanisches Institut, Rheinische Friedrich-Wilhelms-Universität Bonn, Venusbergweg 22, D-53115 Bonn, Germany

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Abstract. Concerns regarding the reliability of slow- and fast-rotating uni-axial clinostats in simulating weightlessness have induced the construction of devices considered to simulate weightlessness more adequately. A new three-dimensional (3-D) clinostat equipped with two rotation axes placed at right angles has been constructed. In the clinostat, the rotation achieved with two motors is computer-controlled and monitored with encoders attached to the motors. By rotating plants three-dimensionally at random rates on the clinostat, their dynamic stimulation by gravity in every direction can be eliminated. Some of the vegetative growth phases of plants dependent on the gravity vector, such as morphogenesis, are shown to be influenced by rotation on the 3-D clinostat. The validity of 3-D clinostatting has been evaluated by comparing structural parameters of cress roots and *Chara* rhizoids obtained under real microgravity with those obtained after 3-D clinostatting. The parameters analyzed up to now (organization of the root cap, integrity and polarity of statocytes, dislocation of statoliths, amount of starch and ER) demonstrate that the 3-D clinostat is a valuable device for simulating weightlessness.

Key words: Automorphosis (root, shoot) – *Chara* (rhizoid) – Clinostat (three-dimensional, random rotation) – *Lepidium* – Gravitropism – Weightlessness (simulated)

Introduction

Plants have evolved in the presence of the gravitational force. Gravity as a stimulus acts permanently on organisms as either static or dynamic stimulation (Sievers et al. 1991). Both gravisensitivity and -response have been studied intensively in fungi (see Kern et al. 1997, this issue) and in lower and higher plants (see Braun 1997, Perbal et al. 1997, Sack 1997, all this issue). A step forward in the understanding of the cellular mechanisms involved in gravitropism has been achieved by using microgravity conditions in space. Competent overviews with references on experiments in space covering a wide range of plant and animal cells have recently been presented (Claasen and Spooner 1994; Brown et al. 1996; Moore and Cogoli 1996; Kordyum 1997).

On Earth, real microgravity conditions (ca. 10^{-4} *g*; no thermal convection; no hydrostatic pressure etc.) can be produced by a free fall from a drop tower, a drop shaft or a balloon or by parabolic flights of airplanes and sounding rockets. However, the duration of microgravity obtained by these methods is generally too short for plants to exhibit obvious changes in growth and development. It is evident that gravity-specific alterations in cells and organisms can be proven beyond doubt only by experiments done in orbit and, in special cases, during parabolic flights of rockets. However, access to spaceflights is limited so that alternatives to simulate weightlessness on Earth have been sought for more than a century: clinostats (slow- and fast-rotating, with one or two axes) were considered as the instruments of choice.

Slow-rotating (e.g. 2–4 rpm) clinostats with one axis (mostly horizontal) have been used to compensate for the unilateral influence of gravity (Sachs 1882; Pfeffer 1904; Fitting 1905). Due to the omnilateral stimulation on a clinostat (instead of a unidirectional stimulation for a given time) the response, i.e. bending upwards or downwards of a plant organ, is eliminated. The physical background and limitations on the use of clinostats have been considered theoretically (Dedolph and Dipert

This review is dedicated to Professor Andreas Sievers for his outstanding work on plant gravitropism as well as his initiative and engagement in the German-Japanese joint research

Abbreviations: 3-D = three-dimensional clinostat; rpm = rotations per minute

Correspondence to: T. Hoson, Fax: 81 (6) 6052577;
E-mail: hoson@sci.osaka-cu.ac.jp; B. Buchen,
Fax: 49 (228) 732677; E-mail: unb10e@ibm.rhrz.uni-bonn.de

1971; Brown et al. 1976a; reviews by Albrecht-Buehler 1992, Briegleb 1992, Kessler 1992; Moore and Cogoli 1996 and references therein). In addition, the effects of slow-rotating (e.g. Gordon and Shen-Miller 1971; Brown et al. 1976b; Salisbury and Wheeler 1981; Sievers and Hejnowicz 1992; Piastuch and Brown 1995; Brown et al. 1996; see also Moore and Cogoli 1996 for further references) and fast-rotating uni-axial clinostats (review by Cogoli 1992; Hilaire et al. 1995; Cai et al. 1997) on the morphological, structural and physiological responses of plant organs have been studied and compared with 1-*g* controls. It has become evident that clinostat rotation in a constant direction often causes undesired side-effects. For example, if the rotation is not fast enough, a chronic dynamic stimulation, instead of no stimulation, may be brought about (Larsen 1957; Hensel and Iversen 1980; Hensel and Sievers 1980, 1981). The fast-rotating clinostat should be designed not to produce such a situation and is useful under certain conditions (Sievers and Volkmann 1977; Sobick and Sievers 1979). On the other hand, if the rotation is too fast or the distance from the rotation axis to plant materials is too large, the centrifugal force produced by the rotation causes a bending of organs (Sobick and Sievers 1979; Hoson et al. 1996).

Experiments in space crafts first allowed results obtained under authentic microgravity to be compared with those under 1 *g* or after simulation of weightlessness by clinostats on the ground. The microgravity data thus serve as the real and valuable controls for gravity-dependent changes in growth behaviour, organization and structure of statocytes (references in Sievers and Hejnowicz 1992; Claasen and Spooner 1994; Moore and Cogoli 1996; Kordyum 1997; see also Moore 1990a, 1990b; Brown et al. 1995, 1996; Hilaire et al. 1995; Laurinavičius et al. 1996; Tripathy et al. 1996).

However, when data from microgravity conditions are compared with corresponding data from clinostatting, divergent results are reported (reviews by Cogoli 1992; Sievers and Hejnowicz 1992; Claasen and Spooner 1994; Moore and Cogoli 1996; see also Brown et al. 1996). This might be based on the fact that the effect of simulating weightlessness by clinostats depends on the rotational frequency, the size, mass and density of gravity-susceptible organelles, and the viscosity and density of the medium surrounding those organelles (see Moore and Cogoli 1996). The following data act as a reminder of how sensitive plants are to gravity and how efficiently clinostats mimic weightlessness. (i) The thresholds for gravity perception are ca. 10^{-3} and 10^{-4} *g* for shoots and roots, respectively. (ii) The perception time, the minimum time the stimulus must be provided is <1 s (Fitting 1905; review Volkmann and Sievers 1979). (iii) Clinostatting at 2 rpm has a rotation period T = 30 s; the plant therefore rotates half a turn in 15 s, and the direction of gravitational force changes by 180° during this period (Sievers and Hejnowicz 1992). (iv) Clinostatting at 60 rpm provides a centrifugal force at the periphery of a circle with a radius of 1 mm of about 4.2×10^{-3} *g* (Briegleb 1992). (v) The higher the rotational frequency the higher is the centrifugal force even at small deviations from the clinostat axis (Albrecht Buehler 1992). Thus, depending on the orientation and size of the object in the rotation axis, and on the speed of rotation, i.e. the alteration of the stimulus angle with time, uni-axial clinostatting might suppress a graviresponse, but not necessarily the perception of the stimulus.

Based on these considerations, facilities have been developed to simulate weightlessness more efficiently. A centrifuge-clinostat with two perpendicular axes has been developed (Shen-Miller et al. 1968; Gordon and Shen-Miller 1971). With this clinostat, plants are rotated on a horizontal axis by a motor whose end is attached to a vertical central hub. Horizontal and vertical rotations are independently driven by two motors and the vertical rotation is solely used to produce a different magnitude of the outward acceleration. Thus, the influence of the gravity vector is in principle compensated by the horizontal rotation as is done with conventional uni-axial clinostats (not by the combined action of horizontal and vertical rotations, as achieved on the 3-D clinostat).

A similar but more sophisticated device has just been applied by Laurinavičius et al. (1997) to estimate the threshold value for the perception time in *Lepidium* roots and shoots and to design a protocol for experiments in space. Moore (1990b) used a fluid-filled "slow-turning lateral vessel" (STLV, rotating at 50 rpm; thus, "slow" is different from the meaning in "slow-rotating" clinostats) for the analysis of *Brassica* statocytes. Whereas statocytes grown on a slow-rotating clinostat differ in ultrastructure from those grown under microgravity, the statocytes grown on the STLV were nearly the same as the microgravity samples, indicating a better simulation of weightlessness by the STLV (Moore 1990b). Recently, Mesland (1996) introduced a "Free Fall Machine" (FFM) as a new device to achieve weightless conditions. The FFM produces weightlessness during the free fall which is interrupted every second by a maximal value of 20 *g* in one direction for 50 ms. These short peaks are considered to be below the threshold of perception time. However, it is necessary to prove that the treatment does not involve gravity stimulation since organisms are able to add up stimuli and plant species might differ in their gravisensitivity and -responsiveness.

By rotating materials three-dimensionally, the above-mentioned problems should be at least partly solved. In general, the dimension of amyloplasts corresponds to 10–30% of the length of statocytes in primary roots (Iversen and Larsen 1973; Volkmann et al. 1986b; Buchen et al. 1993). If we consider the cell to be a cube, the slow rotation on the uni-axial clinostat would appear to give a chronic gravity stimulation on 10–30% of the area of four lateral planes of the cell. On the other hand, with 3-D rotation, such a stimulation, if any, would be shared by all of six planes and would be diminished by 80–93%. In the present article, we describe the machinery of the newly developed 3-D clinostat equipped with two rotation axes placed at right angles (Hoson et al. 1992) and discuss its usefulness as a simulator of weightlessness.

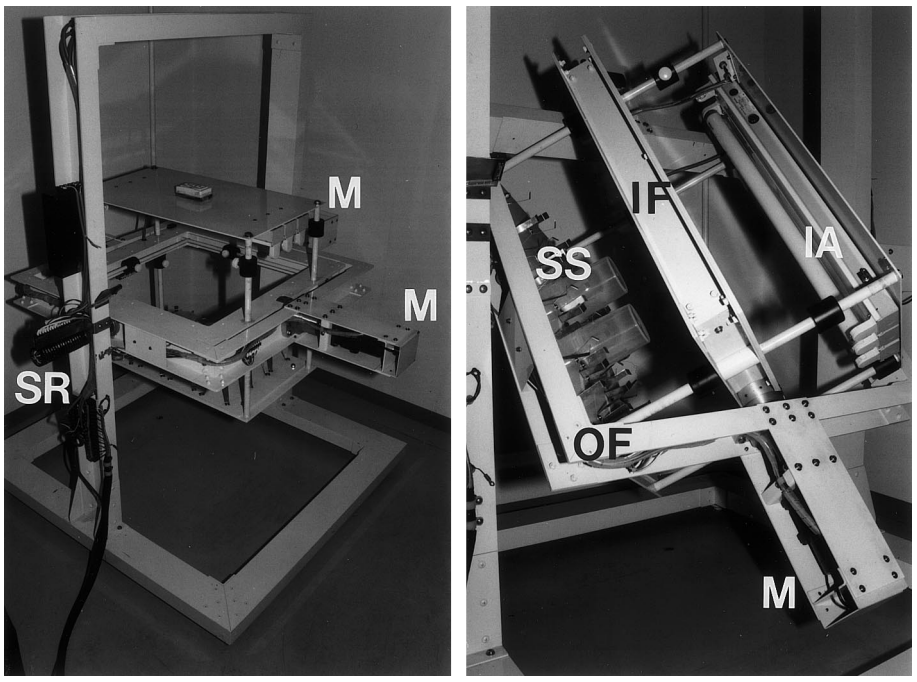

Fig. 1. The 3-D clinostat apparatus. *IA*, illumination apparatus; *IF*, inner frame; *M*, motor with encoder; *OF*, outer frame; *SR*, slip ring; *SS*, sample stage. Actual height of the outer supporting frame (*left*): 1.40 m

The 3-D clinostat

Machinery of the 3-D clinostat. In the 3-D clinostat (Fig. 1), plants are moved along a sphere by the cooperative rotation of two motors (Hoson et al. 1992). The sample stage, 660 mm × 340 mm in area, and an illumination apparatus are attached to the opposite sides of a supporting frame at a distance of 220 mm from the center of rotation. The outer frame is rotated around a horizontal axis. The inner supporting frame is mounted on this outer frame. The rotational axis of the assembly of the inner frame and the sample stage are perpendicular to the first horizontal axis. Rotational motions of the frame and the sample stage are driven by two stepping motors. Onset, rate, and duration of rotation of the motors are controlled with a personal computer. The rotation of the motors is monitored with encoders. Electrical connections to motors, illumination, encoders, and a video terminal are made via slip rings.

Operation of the 3-D clinostat. In order to operate the 3-D clinostat properly, some procedures must be observed. Figure 2 shows the motion of plants placed on the sample stage when they are subjected to two types of rotation. When two motors are operated at the same constant rate, plants rice up to the perpendicular position, but they then return to their original position and the cycle is repeated (Fig. 2a). Under these conditions, the unilateral influence of gravity is never compensated. When two motors are rotated at different constant rates, the positions of plant organs are repeatedly reversed with respect to the gravity vector and the effect of gravity is in essence eliminated. However, samples move only along a fixed path and their motion is not randomized even by this type of rotation (date not shown). True compensation for the effect of the gravity vector can be achieved when the rotation rates of the two motors are changed at random according to the table of random numbers at regular intervals (Fig. 2b). Therefore, in our experiments, the rotation rate of the motors is usually changed at random from 2 to −2 (reverse direction) rpm every 30 or 60 s. These conditions of operation enable a large steric angle to be swept out in a period shorter than the characteristic response time of plants under investigation.

The effectiveness of random rotation in simulating weightlessness can be validated by measuring the components of gravity vectors perpendicular and parallel to the sample stage. Signals from two gravity sensors mounted on the sample stage are transmitted through

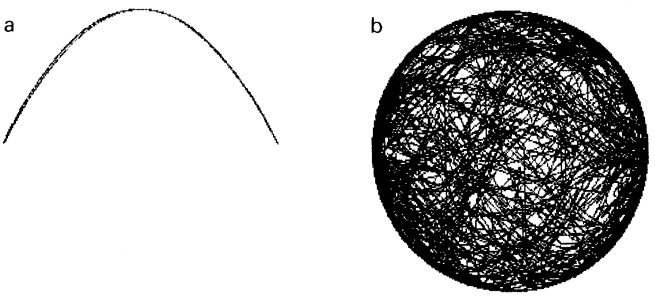

Fig. 2a,b. Motion of plant materials on the 3-D clinostat. The spatial positions of the sample were continuously observed from one direction for 3 h. The rotation started at the top of an arc or a circle where the plants were in the horizontal position. On the equator and at the bottom, the plants were in the vertical and the reversed positions, respectively. The two motors were rotated at the same constant rate (**a**) or at random rates (**b**). The trajectory recorded from the perpendicular direction gives similar patterns

slip rings and analyzed. With random motion, the frequency spectrum of the gravity profile is distributed over a wide range (data not shown). Contrary to this result, sharp peaks are found for motions with constant rates.

Because plant materials are rotated at random on the 3-D clinostat, the gravitational force happens to be unevenly distributed in short-term rotations. Furthermore, because the farthest distance from plant materials to the rotation center is 220 mm, the maximum centrifugal acceleration that can be applied to the materials is 9.8×10^{-4} *g*, which is larger than the smallest threshold value of graviperception reported in oat roots (Shen-Miller et al. 1968) even if the plants are placed closer to the axis in practice and such a situation lasts at most only for 60 s. To discriminate the events occurring under a stimulus-free environment from those induced by gravitational or centrifugal force it is necessary to attach the plant material in different directions within the same culture container and find the induced response irrespective of direction.

Another problem of random rotation originates from the stepwise change in rotation rate. The large angular acceleration which occurs when the rate changes generates an incompatible torque for the motors and causes damage to slip rings through structural distortion of the machinery. At the same time, the mechanical vibration and a stress are also applied to the plants. This problem can be solved by inserting a short intermittent phase of motion between two successive rates. The rotation rate is gradually changed at this phase to suppress the angular acceleration below a permissible level. Since vibration is also generated by the motor, the level of disturbance is reduced by the use of a five-phase stepping motor with a harmonic gear with step angle of 0.0072°. As a measure of the magnitude of the applied stress, the level of ethylene evolved has been determined. So far, no substantial increase in ethylene concentration has been observed either in the gas phase within a culture container or in the intercellular space of plant materials (data not shown).

Evaluation of 3-D clinostatting: organ development

Effect of clinostat rotation on growth and development of plants. Various plant species have been grown on the 3-D clinostat and the effect of clinostat rotation on

Table 1. Effect of 3-D clinostat rotation on various growth processes

Event	Plant material	Reference
Not influenced		
Rate of germination	*Lepidium*, *Pisum*, *Vigna*, *Zea*, *Oryza* *Adiantum*	Hoson et al. (1992), Yamada et al. (1993), Kasahara et al. (1994)
Development of root statocyte	*Lepidium*	Buchen et al. (1993)
Graviresponse of root	*Lepidium*	Hoson et al. (1996)
Rate of organ growth	*Lepidium*,[b] *Pisum*, *Zea*, *Oryza*	Hoson et al. (1992)
Extensibility of the cell wall[a]	*Vigna*, *Zea*	Masuda et al. (1994)
Sugar composition of the cell wall[a]	*Vigna*, *Zea*	Hoson et al. (1995)
Osmotic potential of the cell sap	*Pisum*	Hoson et al. (1993)
Translocation of sugar	*Pisum*	Hoson et al. (1993)
Polar transport of auxin	*Arabidopsis*	Oka et al. (1995)
Growth correlation among organs	*Lepidium*, *Pisum*, *Vigna*, *Zea*, *Oryza*	Hoson et al. (1993)
Influenced		
Rate of organ growth	*Vigna*, *Lepidium*,[b] *Adiantum*	Hoson et al. (1992), Yamada et al. (1993), Kasahara et al. (1994)
Direction of organ growth	*Lepidium*, *Pisum*, *Vigna*, *Zea*, *Oryza*	Hoson et al. (1992, 1995), Hoson (1994)
Straightness of organ growth	*Lepidium*, *Pisum*, *Vigna*, *Zea*, *Oryza*	Hoson et al. (1992, 1995), Hoson (1994)
Axiality of seedling	*Lepidium*, *Pisum*, *Vigna*, *Zea*, *Oryza*	Hoson et al. (1993)
Peg formation	*Cucumis*	Takahashi et al. (1995)
Senescence of leaf	*Avena*	Miyamoto et al. (1995)

[a]Differences were detected between the convex and the concave sides
[b]Growth of *Lepidium* hypocotyls was either promoted (Yamada et al. 1993) or not influenced (Hoson et al. 1992; Yamada et al. 1993) dependent on the condition of clinostat rotation

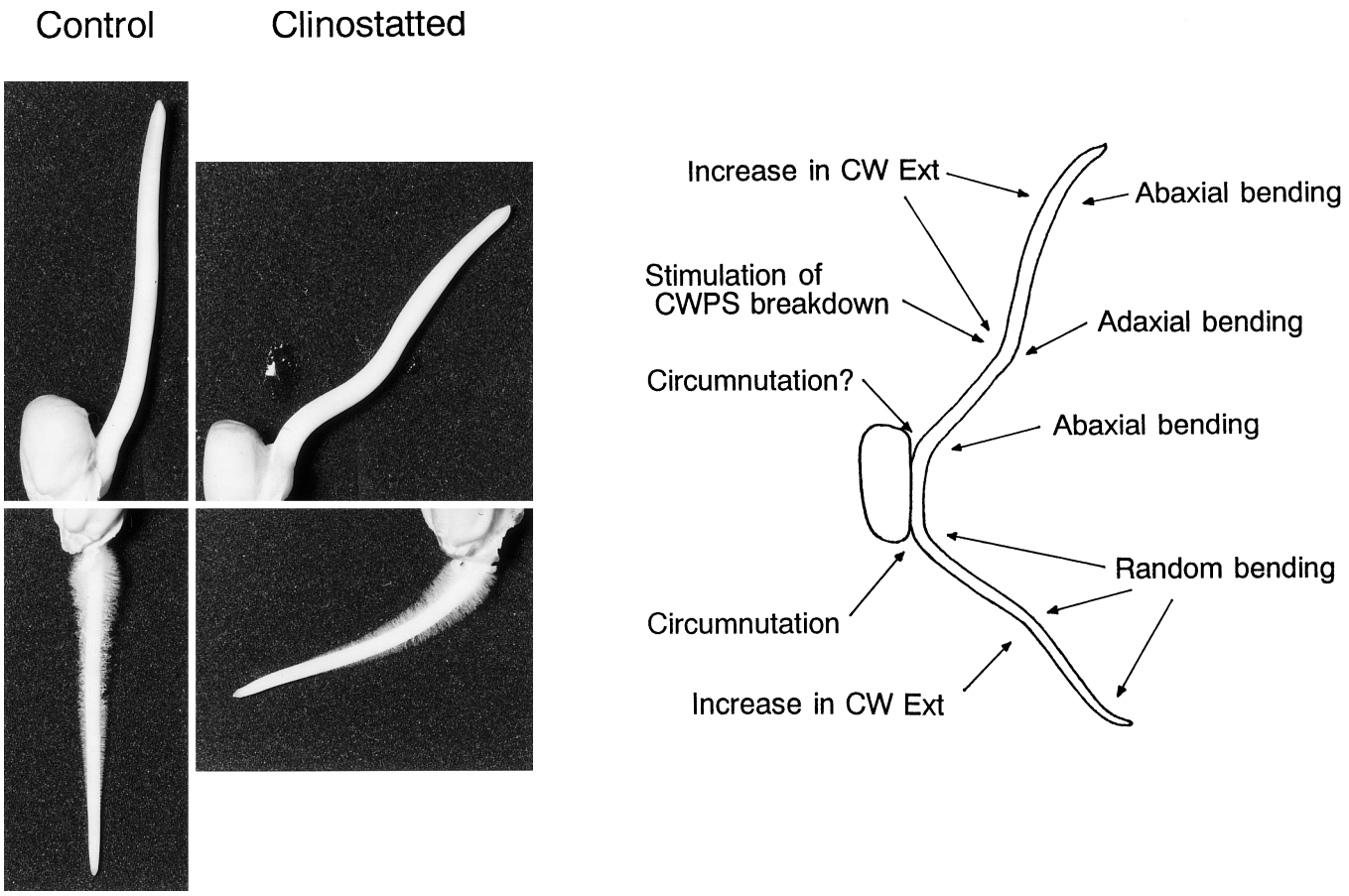

Fig. 3. Maize seedlings grown in the normal gravitational field or on the 3-D clinostat. The type of spontaneous curvature and possible mechanisms involved in the bending on the clinostat are shown in the diagram. *CW Ext*, cell wall extensibility; *CWPS*, cell wall polysaccharides

growth processes has been analyzed and compared with 1-*g* controls (Table 1). The germination rate of seeds of higher plants or spores of ferns is not influenced by clinostat rotation (Hoson et al. 1992; Kasahara et al. 1994), as has also been observed in space experiments (see Halstead and Dutcher 1987). The graviresponse of clinostatted cress roots was also found to be the same as that of control roots, when they were placed horizontally and exposed to the gravity vector (Hoson et al. 1996). Thus, cress roots rotated on the 3-D clinostat develop the gravity-sensing mechanism and respond to the gravity vector.

The growth rate of various organs is usually not affected by clinostat rotation (Hoson et al. 1992), although it can be promoted (Hoson et al. 1992; Yamada et al. 1993) or suppressed (Kasahara et al. 1994) in some species under certain conditions. Clinostat rotation does not influence the osmotic potential of the cell sap (Hoson et at. 1993) or the mechanical properties of the cell wall (Masuda et al. 1994), the major factors controlling the rate of cell elongation. No differences have been detected in growth correlation among different organs between control and clinostatted seedlings (Hoson et al. 1993). In addition, clinostat rotation does not influence either the translocation of sugars from the cotyledons to elongating internodes (Hoson et al. 1993) or the polar transport of IAA in inflorescence stalks (Oka et al. 1995).

On the other hand, the shape of seedlings is changed by rotation on the 3-D clinostat (Table 1). Roots and shoots of various species exhibit a spontaneous curvature as well as an altered growth direction (Hoson et al. 1992). As a result, the axiality along the gravity vector disappears and seedlings form themselves into a sphere-like shape on the clinostat (Hoson et al. 1993). Peg formation in Cucurbitaceae plants is also disturbed by the clinostat rotation. On the clinostat, the proportion of seedlings without a peg or with two pegs increases (Takahashi et al. 1995; see also Takahashi 1997, this vol.). Thus, growth responses directly dependent on the gravity vector are especially influenced by clinostat rotation. It has also been reported that the senescence of excised leaves is stimulated on the 3-D clinostat (Miyamoto et al. 1995).

Automorphosis. Under a stimulus-free environment, higher plants show spontaneous growth responses called "automorphosis" (Pfeffer 1904). On the 3-D clinostat, in general, shoots show curvature either towards the seed or caryopsis (adaxial) or away from the seed or caryopsis (abaxial) depending on the species and growth conditions, while roots grow in the direction of the tip of primordia in the early stage of growth and later in a

random fashion (Hoson et al. 1992, 1993). Such a curvature of various plant organs, often called autotropic reactions or nastic bending, can also be induced by a uni-axial horizontal rotation (Larsen 1953; Nick and Schäfer 1989; Lorenzi and Perbal 1990). On the uni-axial clinostat, the automorphic bending is often exaggerated or suppressed as compared with on the 3-D clinostat (Hoson et al. 1992, 1996). This difference between the two types of clinostat appears to be caused by the centrifugal acceleration produced by the horizontal rotation (Hoson et al. 1996). Plant organs also show automorphosis in satellite orbit (Volkmann et al. 1986b; Chapman et al. 1994; Heathcote et al. 1995; Johnsson et al. 1996).

The spontaneous bending of plant organs observed under simulated weightlessness is a complex phenomenon. The details and the mechanism of such a spontaneous growth response have been further analyzed in maize seedlings using the 3-D clinostat (Fig. 3).

On the 3-D clinostat, maize shoots exhibit curvatures in three different parts: (i) the basal transition zone connecting roots and mesocotyls, (ii) the coleoptile node located between mesocotyls and coleoptiles, and (iii) the elongating region of the coleoptiles (Hoson et al. 1995a). Even non-clinostatted control shoots show some degree of curvature away from the caryopsis in the transition zone, toward the caryopsis in the coleoptile node, and bending again away from the caryopsis in coleoptiles. Rotation on the 3-D clinostat strongly stimulates these curvatures. On the 3-D clinostat, maize roots also exhibit curvatures in three different parts: (i) the basal region just protruding from the coleorhiza, (ii) the middle region between the mature and the elongation zone, and (iii) the elongation zone, several millimeters from the tip (Hoson 1994). Control roots show some degree of curvature, which occurs at random without any dorsiventrality. Bending is most prominent in the basal regions and appears to be related to circumutations (Fig. 3).

There is no difference in the osmotic concentration of the cell sap between the convex and the concave halves of any bending region of maize seedlings. However, in coleoptile nodes and elongating coleoptiles or in the middle portion of roots, the faster-expanding convex side exhibits a higher extensibility of the cell wall than the opposite side, and this appears to be a cause of the curvature (Hoson 1994; Hoson et al. 1995a). Chemical analysis of the cell wall constituents and the measurement of enzymic activities in the cell walls has revealed that either the breakdown or the accumulation of wall polysaccharides, such as $(1 \rightarrow 3)$ $(1 \rightarrow 4)$-β-glucans, is involved in the curvature of clinostatted coleoptile nodes and coleoptiles (Hoson et al. 1995b). Similarly, the extensibility of the cell wall of the convex side is higher than that of the concave side in gravi-stimulated stems (Shen-Miller and Masuda 1973; Iwami and Masuda 1974; Bagshaw and Cleland 1990; Cosgrove 1990) and pulvini (Gibeaut et al. 1990). The metabolism of β-glucans (Gibeaut et al. 1990) or xyloglucans (Talbott and Pickard 1994) and the synthesis of cell wall polysaccharides (Edelmann and Sievers 1995; Montague 1995) also contribute to gravicurvature. Thus, cell wall changes appear to be the major factor controlling both the spontaneous and the tropistic curvatures of plant stems, even if the rate of the former is less than one-tenth of the latter. These data indicate that the 3-D clinostat is useful for analyzing not only growth responses of plants under weightlessness but also the mechanism of gravitropism.

Table 2. Morphometric analysis in statocytes (storeys III and IV) of *Lepidium* roots. For starch quantification, on at least four median-to-tangential longitudinal sections of control and clinostatted roots ($n = 4$ samples each experiment set), the area of starch grains was measured with a Videoplan (Kontron, München, Germany) and is expressed as percent of the area of amyloplasts (= 100%). The length of the ER and the diameter of lipid bodies (10–15 each experiment set) were measured on three serial sections ($n = 4$; see also Buchen et al. 1993)

	1-*g* control (24 h)	Weightlessness Spacelab D1 (32 h)	3-D clinostatting (24 h)
Starch content	100%	70%	85%
ER content	100%	195%	180%
Lipid bodies (diameter, μm)	0.1–0.3	< 3	2

Evaluation of 3-D clinostatting: Lepidium statocytes and Chara rhizoids as model cells

The reason for choosing these cells is obvious: both cell types have been structurally and physiologically well examined on the ground, on slow- and fast-rotating 2-D clinostats and – most important – under microgravity (Volkmann et al. 1986b, 1991; Volkmann and Sievers 1990; Sievers et al. 1991; Laurinavičius et al. 1994, 1996). Thus, results obtained under microgravity could be used as real controls in this examination.

Lepidium roots. The following criteria have been analyzed in 24-h-old root statocytes of *Lepidium sativum* L. germinated and grown on the 3-D clinostat (Buchen et al. 1993): (i) cellular organization of the root cap; (ii) polarity of statocytes; (iii) dislocation of statoliths; (iv) ER content in statocytes; (v) starch content in amyloplasts (statoliths); and (vi) diameter of the lipid bodies. All criteria are related to structural features of the gravisensing cells and displacements of organelles/particles susceptible to gravity. To evaluate the quality of the 3-D clinostat at the molecular and biochemical level, however, other parameters must be analyzed.

After development and growth for 24 h on the 3-D clinostat, the external shape and internal structure of the root cap and the symmetrical organization of four storeys of statocytes are equal to those of 1-*g*-and microgravity-grown roots (Buchen et al. 1993). The polarity of the statocytes (nucleus proximal, ER complex distal; Fig. 4, 5b) is expressed as in cress roots grown during spaceflights (Volkmann et al. 1986a,b; Laurinavičius et al. 1996) a phenomenon which is in principle also shown for other species (Perbal and

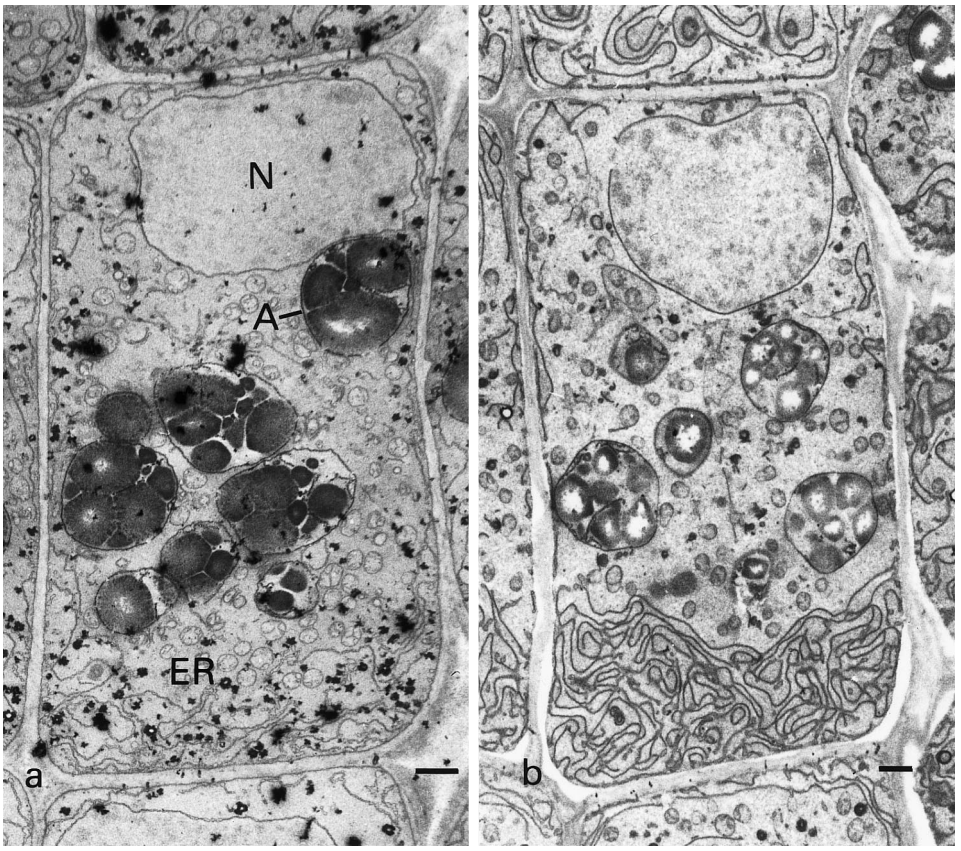

Fig. 4a,b. Statocytes of 24-h-old *Lepidium* roots (storey III) grown on the 3-D clinostat (**a**) and under microgravity conditions during the D1 mission (**b**; from Volkmann et al. 1986a). *A*, amyloplasts; *ER*, endoplasmic reticulum; *N*, nucleus. X 5800 (**a**), 4200 (**b**); bars = 1 μm

Driss-Ecole 1989; Lorenzi and Perbal 1990; Hilaire et al. 1995). Morphometric analyses of the starch content in the statoliths, the distance of the statoliths from the lower cell wall, the ER content and the diameter of the lipid bodies in the statocytes show the general tendency for a decrease in starch content and an increase in the other parameters (Table 2). This is in agreement with findings under microgravity conditions (Volkmann et al. 1986b; Volkmann and Sievers 1990; Laurinavičius et al. 1996) although the values might slightly differ in independent experiments due to natural variations and differences in seedling age. The tendency for reduced starch content also occurs in other species investigated in space (Johnson and Tibbits 1968: *Capsicum*; Alijyev et al. 1987: *Pisum*; Moore et al. 1987, Moore 1990a: *Zea*; Moore 1990b: *Brassica*; Brown and Piastuch 1994: *Glycine*; Laurinavičius et al. 1994: *Arabidopsis*; Tripathy et al. 1996: *Triticum*).

Following the experimental schedule of Hensel and Sievers (1980, 1981), a crucial test for validating the 3-D clinostat has been performed: the cellular integrity of statocytes grown for 24 h under 1 *g* in normal vertical orientation and then for 20 h on the slow-rotating uni-axial clinostat has been compared with that of statocytes rotated on the 3-D clinostat. In contrast to results obtained using the horizontal clinostat (2 rpm; Hensel and Sievers 1980, 1981), rotation on the 3-D clinostat does not induce self-destruction of the statocytes and neither autophagosomes nor destruction of cell organelles and cell walls have been observed (Fig. 5). The conclusion is evident: chronic dynamic overstimulation produced by the slow-rotating uni-axial clinostat is avoided and thus the 3-D clinostat operates in favour of a stimulus-free situation.

Chara rhizoid. The *Chara* rhizoid as model for studies of gravitropism in unicellular systems (Sievers et al. 1996; see Braun 1997, this issue) has been rotated on the 3-D clinostat and cell polarity and dislocation of statoliths have been compared with results obtained under microgravity (Sievers et al. 1991; Volkmann et al. 1991) and on uni-axial clinostats (Cai et al. 1997).

Tip growth and typical polar organization of the rhizoid (see Braun 1997, this issue) is maintained when growth continues on the 3-D clinostat. This corresponds to results from experiments under microgravity (TEXUS: Volkmann et al. 1991; Spacelab IML-2: Braun et al. 1996). After 6 min in both authentic microgravity and under simulated weightlessness on the 3-D clinostat the statoliths are basipetally displaced (Fig. 6). The pattern of statolith distribution is similar to that found after short-term (TEXUS, 6 min: Volkmann et al. 1991) and long-term periods of microgravity (IML-2, 30 h: data not shown) and within the range of natural variation. So far, only the static end points of the basipetal displacement have been analyzed in fixed rhizoids, since no in-vivo videomicroscopic observation has been performed on the 3-D clinostat. Therefore, the kinetics of the basipetal displacement of the statoliths have not yet been followed.

A basipetal dislocation of statoliths has also been described on slow- and fast-rotating uni-axial clinostats

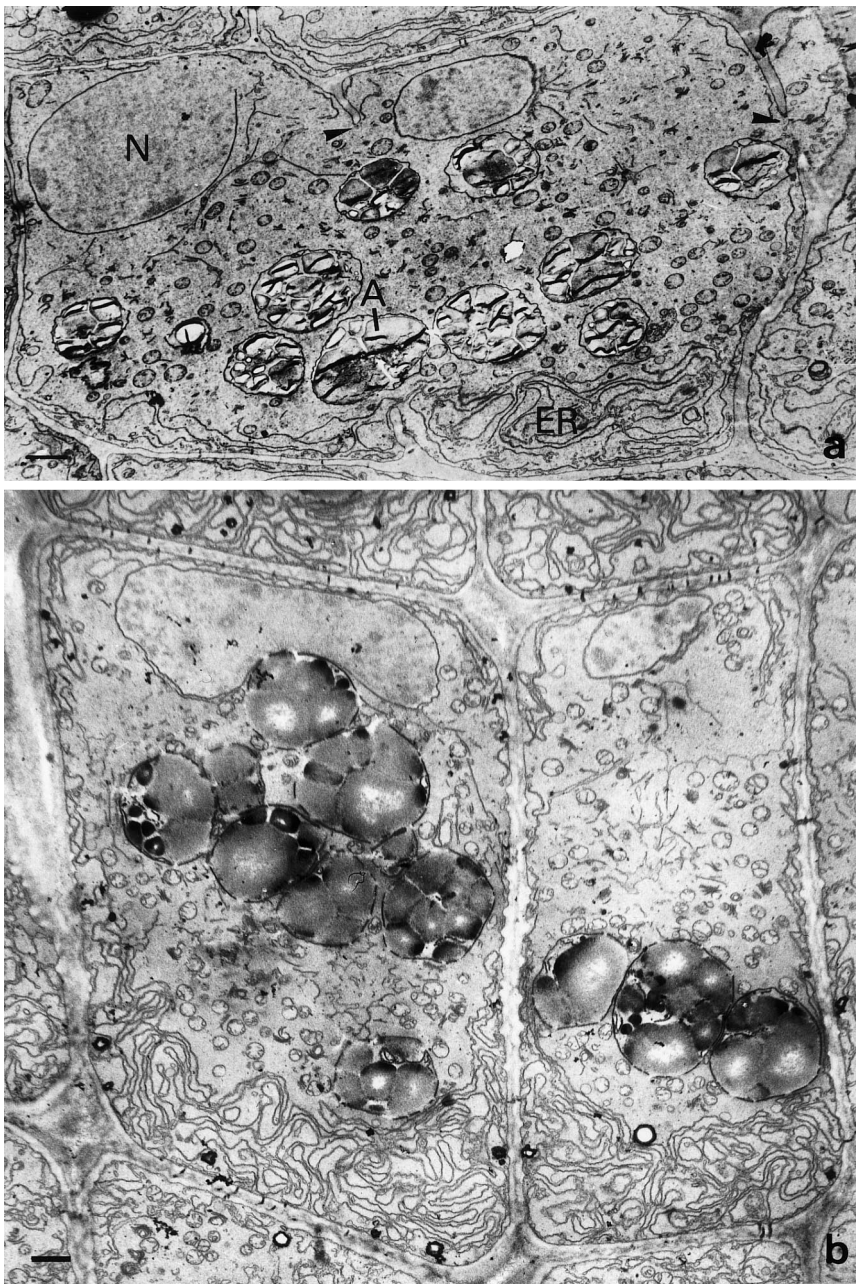

Fig. 5a,b. Statocytes of *Lepidium* roots grown for 24 h in normal vertical orientation and then for 20 h on a slow-rotating clinostat at 2 rpm (**a**, from Hensel and Sievers 1980) and on the 3-D clinostat (**b**). Cell integrity and polarity is maintained after 3-D clinostatting, contrary to 2-D clinostatting at 2 rpm. Symbols as in Fig. 4; *arrowheads*, remains of cell walls. × 5000; bars = 1 μm

(Cai et al. 1997). The movement of statoliths shows a time-dependent rapid adaptation of the cell to changes in gravity conditions and an acropetal retransport of statoliths. Thus, different patterns of statolith distribution are visible, e.g. after 45 or 120 min of rotation on the fast-rotating uni-axial clinostat (Cai et al. 1997).

Basically, it holds true for all conditions – microgravity, simulated weightlessness on slow- and fast-rotating uni-axial as well as 3-D clinostats – that the statoliths are basipetally dislocated within a short time (as fast as 6 min). These first studies of *Chara* rhizoids on the 3-D clinostat also confirm that this clinostat is a valuable device for simulating weightlessness. However, in-vivo videomicroscopy on the 3-D clinostat is especially necessary for more-detailed studies.

Concluding remarks on the usefullness of the 3-D clinostat in simulating weightlessness

Looking back at 30 years of research in plant biology in space, we become aware that the role of space experiments has still been confined to examining and supplementing the results obtained by weightlessness simulation experiments on Earth. Arguments against the use of clinostats – regardless of the type – are numerous. Clinostat experiments clearly demonstrate that care is necessary when reaching conclusions on microgravity effects. However, much valuable and already long-known information, as well as inputs for space experiments, would never have been obtained without clinostat experiments. For example, the question of the perception time was investigated years ago by

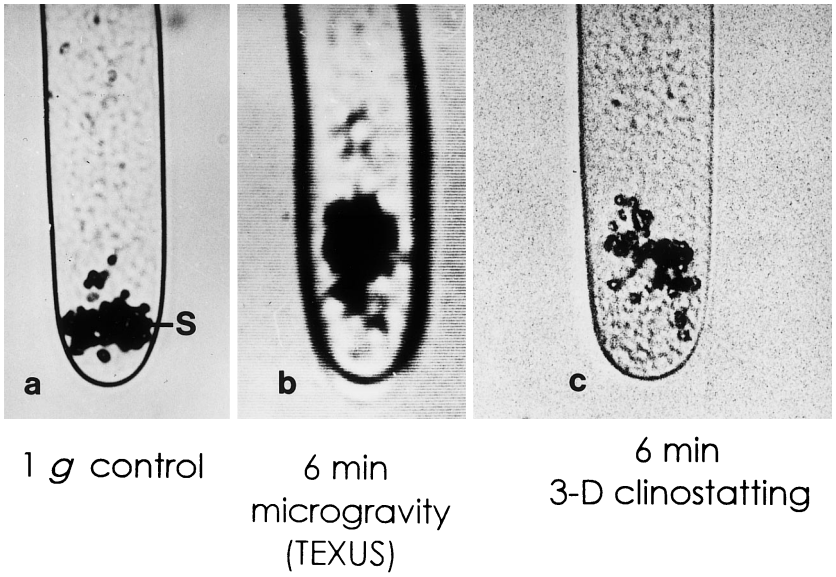

Fig. 6a–c. Statoliths in *Chara* rhizoids **a** under 1 *g* in normal vertical orientation, **b** after 6 min of microgravity (TEXUS, Volkmann et al. 1991), and **c** after 6 min of 3-D clinostatting. The basipetal displacement of statoliths (*S*) under microgravity is similar to that occurring after simulation of weightlessness on the clinostat. Rhizoid diameter is ca. 25 μm

clinostatting of plants. The range of stimulus duration deduced from these experiments has been taken as indicator for experimental schedules performed in space (see Perbal et al. 1997, this issue). It is necessary to choose the weightlessness simulator that is the most suitable for the purpose of the study and for the plant material to be studied. Clinostat experiments will be needed to propose and plan rare but crucial space experiments as long as no space station allows long-term experiments or scientists to work in space.

The 3-D clinostat has been proven as a most useful device to simulate weightlessness and is preferable to slow- or fast-rotating uni-axial clinostats: the size of the object and the orientation within the clinostat axis is not as critical as on the uni-axial clinostats. Some processes in plant growth, such as morphogenesis, have been examined in detail using the 3-D clinostat. The structural parameters so far analyzed and compared with results from microgravity experiments demonstrate the validity of the 3-D clinostat.

On a biochemical and molecular level, however, stress-related effects of clinostatting might nevertheless occur: this has to be critically checked in future. In addition, if objects are big (e.g. whole plants, stems), the 3-D clinostat is unlikely to simulate weightlessness in a realistic way. It is always important and indispensible to have key data from experiments in space: these are the real controls. They are needed because of the divergence in plant sensitivity and responsiveness to gravity reflecting evolutionary and ecological adaptations. Experiments proposed for spaceflights, however, should be first performed using clinostats with emphasis on the use of the 3-D clinostat in order to minimize the physical problems. The data should be used to focus on basic experiments in space. Missing data from spaceflight experiments could be obtained by using clinostats [for example, with the slow-rotating centrifuge microscope (NIZEMI) on spacelab IML-2, only two experiments on the threshold value in *Chara* rhizoids have been performed; to obtain a more accurate value, additional intermediate experiments are necessary]. Moreover, statistical analyses might be done by using clinostat data. Use of the 3-D clinostat might be made more effective by combining the clinostat with other simulators, for instance, by loading it on airplanes for parabolic flight experiments, and by improving the machinery of the clinostat further. Experiments to solve old questions in sensory biology, e.g. on stimulus-summation (“memory”), should be performed first on clinostats before space experiments are done in order to justify the expensive work in microgravity. However, control and key experiments in space are needed in addition to experiments on clinostats.

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