Mechanotransduction in root gravity sensing cells

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Received 16 May 2003; revised 17 June 2003

The analysis of the dose–response curve of the gravitropic reaction of lentil seedling roots has shown that these organs are more sensitive when they have been grown in microgravity than when they have been grown on a 1g centrifuge in space before gravistimulation. This difference of gravisensitivity is not due to the volume or the density of starch grains of statoliths, which are about the same in both conditions (1g or microgravity). However, the distribution of statoliths within the statocyte may be responsible for this differential sensitivity, since the dispersion of these organelles is greater in microgravity than in 1g. When lentil roots grown in microgravity or in 1g are stimulated at 0.93g for 22 min, the amyloplasts sediment following two different trajectories. They move from the proximal half of the statocytes toward the lower longitudinal wall in the microgravity grown sample and from the distal half toward the longitudinal wall in the 1g grown sample. At the end of the stimulation, they reach a similar position within the statocytes. If the roots of both samples are left in microgravity for 3 h, the amyloplasts move toward the cell centre in a direction that makes an average angle of 40° with respect to the lower longitudinal wall. The actin filaments, which are responsible for this movement, may have an overall orientation of 40° with respect to this wall. Thus, when roots grown in microgravity are stimulated on the minicentrifuge the amyloplasts slide on the actin filaments, whereas they move perpendicular to them in 1g grown roots. Our results suggest that greater sensitivity of seedling roots grown in microgravity should be due to greater dispersion of statoliths, to better contacts between statoliths and the actin network and to greater number of activated mechanoreceptors. One can hypothesize that stretch activated ion channels (SACs) located in the plasma membrane are responsible for the transduction of gravistimulus. These SACs may be connected together by elements of the cytoskeleton lining the plasma membrane and to the actin filaments. They could be stimulated by the action of statoliths on the actin network and/or on these elements of the cytoskeleton which link the mechanoreceptors (SACs).

Introduction

Animal and plant cells are able to detect mechanical stimuli of the environment by the means of receptors which convert forces into ionic currents, which in turn lead to specific responses (Bourque 1996, Ramahaleo et al. 1996). Gravity sensing cells (statocytes) in plants can perceive their orientation with respect to the earth gravitational field and can induce the re-orientation of the extremity of the organs. These cells contain amyloplasts (statoliths) which are denser than the surrounding cytoplasm and therefore sediment under the influence of gravity (Sack 1991). The low potential energy dissipated by the downward movement of statoliths (Björkman 1988) could activate calcium channels (Plieth and Trewavas 2002) which could be located in the plasma membrane. However, the presence of stretch activated ion channels within statocytes has to be demonstrated as it has been done in some other cell types (Pickard and Ping Ding 1992). The results of the space experiment performed in the frame of the Shuttle-Mir Mission (SMM05 1997) and reported here have permitted a better understanding of the role of the cytoskeleton in the transmission of the physical action of statoliths to mechanoreceptors. This research was based on previous experiments, which showed that: –1 for low doses of stimulation, graviresponsiveness was greater in roots of Lepidium sativum seedlings grown in microgravity than in seedling roots grown on a 1-g centrifuge in space (Volkmann and Tewinkel 1996) and that –2 the amyloplasts were preferentially located close to the nucleus in statocytes of seedlings grown in near weightlessness (Perbal et al. 1987, Smith et al. 1997), whereas they are sedimented.
on endoplasmic reticulum at the distal pole of statocytes of seedlings grown on a 1-g centrifuge in space with a root-tip directed accelerated. It has also been suggested that the amyloplasts in statocytes could be connected to actin filaments via the motor protein myosin (Baluska et al. 2000, Driss-Ecole et al. 2003). The transfer from 1 g to microgravity provoked a displacement of the organelles along these filaments (Volkmann et al. 1991, 1999) due to a force which should be 7 times lower than the gravity force (Driss-Ecole et al. 2000a).

The analysis of the role of the ARG1 gene (Altered Response to Gravity) which encodes a DNaJ-like protein in Arabidopsis (Sedbrook et al. 1999) indicated that the ARG1 protein participates in a gravity-signalling process involving the cytoskeleton. It has also been shown that amyloplast sedimentation kinetics in statocytes of maize roots support an eventual involvement of actin filaments in the transduction of gravity stimulus (Yoder et al. 2001) and that in lentil roots, the sedimentation of statoliths was made possible because of the thin actin network (Driss-Ecole et al. 2000b) which can be disrupted. However, the fact that statoliths are in contact with actin filaments and that the cytoskeleton is involved in the perception of gravity does not prove that actin filaments are responsible for the transmission of gravistimulus to mechanoreceptors. There is at least one argument against the possible role of actin filaments: when organs are treated by cytochalasins (B or D) or latrunculin, they are still able to respond to gravitistimulus (Blancaflor and Hasenstein 1997, Nick et al. 1997, Staves et al. 1997, Yamamoto and Kiss 2002, Hou et al. 2003) although these drugs are known to perturb the polymerization of actin filaments. However, these results do not prove that the actin network is not involved in graviperception since it has not been demonstrated that this network is completely destroyed by the treatment. In this study we present arguments for an active role of the cytoskeleton in root gravisensing.

Materials and methods

In the frame of the SMM05 Mission of the Shuttle (1997), lentil seedlings were grown for 26 h (Fig. 1) in minigrowth chambers (minicontainers) on board Spacehab, either in microgravity or on the BiOrack 1 g centrifuge (Brillouet and Brinckmann 1999). The seedlings were then subjected to centrifugal accelerations ranging from 0.39 to 0.93 g on a minicentrifuge (Fig. 2) for 9 or 22 min (Fig. 1). The lowest dose of stimulus was therefore 0.39 g x 9 min = 3.51 g x min and the highest dose was 0.93 g x 22 min = 20.46 g x min. In two samples (Fig. 1), root curvature was followed for 3 h by time lapse photography. The two sides of the 4 minicontainers were observed on the minicentrifuge (Fig. 2) through mirrors orientated at 45° with respect to their transparent cover. Some samples were fixed chemically in space in order to determine the distribution of statoliths within the statocytes after stimulation on the minicentrifuge or after a period of 3 h in microgravity following stimulation. The minicontainers were inserted by the crew in a special device for chemical fixation (termed a fixer) as previously described (Perbal et al. 1987) and 4% glutaraldehyde (in a sodium phosphate buffer 0.1 M, pH7.4) was injected into the minicontainers by means of a spring driven piston. The fixed samples were treated for routine electron microscopy. Dehydration of the specimens was done by alcohol of increasing degrees and epoxy-1,2-propane. The samples were embedded in Durcupan ACM (Fluka, St. Quentin Fallavier, France). The results concerning lentil seedlings grown (for 27 h) in microgravity or in 1 g and not subjected to any stimulation were obtained in the frame of the study of the SMM03 Mission (Driss-Ecole et al. 2000a).

The location of 200 amyloplasts within 20 statocytes in each sample was analysed by considering the relative distance of their gravity centre to the transverse (distal) and longitudinal wall (Driss-Ecole et al. 2000a). Their statistical distribution within the statocyte was analysed by means of a computer program (Statistica, StatSoft, Tulsa, OK, USA). The average optical density, integrated optical density and area of starch grains in the amyloplasts, were analysed by ANOVA. The comparison by pair of the dispersion of the amyloplasts or their average position in the statocyte were analysed with F and t-tests, respectively.

Results

Figure 2 shows a photograph of the minicentrifuge with lentil seedlings, which were first grown in microgravity or on the BiOrack 1 g centrifuge for 26 h and then subjected for 22 min (on the minicentrifuge) to accelerations ranging from 0.39 g to 0.93 g. After stimulation these
seedlings were left in microgravity for a period of 3 h during which time the gravitropic response was followed by time lapse photography (Fig. 1). Figure 2 demonstrates that the curvature of roots grown in microgravity prior stimulation is more pronounced 3 h after stimulation than that of roots grown on the Biorack 1 g centrifuge. Figure 3 shows minicontainers photographed on the minicentrifuge as in Fig. 2, but after 0, 1, 2 and 3 h in microgravity. The analysis of the curvature of roots grown in microgravity demonstrates that in most cases the root extremity overshot after 2 h the direction of the accelerations, which were responsible for the curvature (Fig. 3, 2 h). This overshooting also happened in 1 g grown samples (Fig. 3, 1 g) but only after 3 h and less frequently because the gravitropic response is slower in this case. The overshooting never occurs when lentil roots curve in the gravitational field on earth since curvature is strongly slowed down after 2 h (Perbal and Driss-Ecole 1994). In maize roots (Evans 1991) overshooting can occur but there is a reversal of growth patterns, which leads to a reduction of the bending of the roots. In lentil roots this behaviour is not observed in the microgravity grown sample where roots keep on curving strongly after overshooting (compare in Fig. 3, 2 h and 3 h).

The dose–response curve of the gravitropic reaction is reported in Fig. 4. The experimental data are fitted with the hyperbolic model (Perbal et al. 2002) which allows the estimation of the curvature per unit dose for threshold stimuli of roots grown in microgravity and roots grown on the 1 g centrifuge: $0.21 \times g^{-1} \times min^{-1}$ and $0.13 \times g^{-1} \times min^{-1}$, respectively. Thus, gravisensitivity is increased by 67% in microgravity grown seedlings.
This difference could have various causes such as modification of root growth and cell elongation which depends upon auxin distribution (Rosen et al. 1999) or auxin carriers (Boonsirichai et al. 2002, Friml et al. 2002). Both could have an effect on graviresponsiveness. However, in a former experiment (Leguë et al. 1996), it has been shown that root growth and cell elongation were similar in microgravity and on the 1 g centrifuge after a period of growth of 26 h. So, higher sensitivity observed in microgravity may not be due to the transmission phase (hormonal distribution) of the gravitropic response.

Another cause of increased sensitivity to gravistimulus could be a change in volume or density of statoliths. The area, the mean optical density and the integrated optical density of starch after staining with periodic acid-Schiff were studied on thick-thin sections by image analysis (Table 1), and the results showed that the statoliths were neither denser nor larger in microgravity than on the 1 g centrifuge. The only differences which were observed dealt with the average optical density in microgravity samples (Table 1) where it could be either greater or less than in 1 g grown samples. Moreover, it has been shown that such a small variation in starch content cannot account for the difference in gravisensitivity (Aarrouf and Perbal 1996, Kiss et al. 1996).

Figure 5A, B shows electron micrographs of statocytes of roots grown for 26 h on the 1 g centrifuge or in microgravity. The statistical analysis of the distribution of 200 amyloplasts in more than 20 statocytes has been studied in different conditions (Fig. 6). On the Biorack 1 g centrifuge, the amyloplasts sediment near the endoplasmic reticulum (Fig. 5A) in the distal half of the cell (Figs 5A and 6A). In microgravity, they are mainly located close to the nucleus (Fig. 5B) in the centre of the statocyte and along the longitudinal walls (Fig. 6B). As shown in Table 2, the average position of the amyloplasts with respect to the distal wall is statistically different in the 1 g and μg samples (no stimulation). Their grouping (Table 3, no stimulation) is tighter in 1 g than in microgravity since the variance of the position of the amyloplasts relative to the distal wall is statistically less in the former sample than in the latter. Centrifugation for 9 min at 0.48 g causes sedimentation of the
Amyloplasts in 1g and microgravity grown seedlings (Fig. 6C,D). However, the sedimentation is different in both cases as assessed by the statistical tests of Tables 2 and 3. The average distance of the amyloplasts relative to the longitudinal wall (Table 2) is less in the microgravity grown sample than in the 1g grown sample but their dispersion with respect to the longitudinal wall is the same (Table 3). These results show that the sedimentation of amyloplasts is more pronounced in the microgravity-grown sample. It must be noticed that in both samples the average position and the dispersion of the amyloplasts with respect to the distal wall are different (Tables 2 and 3).

A stronger acceleration for a longer period (22 min at 0.93g) leads to a rather similar distribution of statoliths in both samples (Fig. 6E,F). The average position of the amyloplasts and their dispersion are not statistically different (Tables 2 and 3, respectively).

When lentil seedlings stimulated for 22 min at 0.93g are left in microgravity for 3 h (Fig. 6G,H), the amyloplasts move toward the cell centre and reach the same average position (Table 2) with respect to the distal and longitudinal walls. This movement, which occurs in microgravity, may be due to the actomyosin system as has been previously suggested (Volkmann et al. 1999, Driss-Ecole et al. 2000a). However, the dispersion of the amyloplasts with respect to the distal wall is greater in the microgravity grown sample than in the 1g grown sample (Table 3) which indicates that the architecture of the network could be slightly different in both cases.

The average location of statoliths in the various conditions is reported on Fig. 7, which shows the direction of the various movements of the amyloplasts in roots grown in 1g or in microgravity and stimulated for 22 min or stimulated for 22 min and then left in microgravity for 3h. This analysis permits determining the average direction of the displacements observed on Fig. 6. For roots grown continuously in 1g or in microgravity (Fig. 7A,B, 1g or μg), the gravity centre of the bulk of amyloplasts is placed close to the middle of the distal wall (ordinate axis; average relative distance: 0.48 and 0.50, respectively). However, along the longitudinal wall the average relative distance is 0.39 and 0.52 in 1g and in microgravity, respectively.
In roots stimulated for 22 min at 0.93 g, the average position of statoliths is about the same in both samples because the amyloplasts are stopped by the more viscous cytoplasm lining the longitudinal wall (Perbal 1978). Consequently, the total potential energy dissipated by the sedimentation of statoliths is similar for both samples since the average downward movement of these organelles, as well as their volume and density, are similar. The difference in sensitivity may therefore reside in the way of dissipating this energy.

If the roots are left in microgravity for 3 h after stimulation at 0.93 g for 22 min (Fig. 7A,B) the bulk of statoliths moves in both samples obliquely toward the cell centre. The direction of this movement makes an angle of 40° with respect to the longitudinal wall. This displacement should be due to the actomyosin system and is independent of gravity since it takes place during the 3 h of microgravity. It could indicate the general orientation of the actin filaments, which may be similar in roots grown in microgravity as in roots grown on the 1 g centrifuge. It must be noted that in microgravity grown seedlings the movement of amyloplasts due to the stimulation on the minicentrifuge (0.93 g × 22 min) is roughly parallel (but in the opposite direction) to that due to the actomyosin system (Fig. 7B, arrows) whereas it is almost perpendicular in roots grown in 1 g (Fig. 7A, arrows).

Discussion

Previous space experiments have helped to elucidate the relationship between statoliths and the cytoskeleton in the root statocytes (Volkmann et al. 1991, 1999). They also allowed estimation of the force which is responsible for the movement of these organelles along the actin filaments (Driss-Ecole et al. 2000a) from the distal toward the proximal part of the statocytes when the root growing on a 1-g centrifuge is transferred to microgravity. This movement may be due to the actomyosin system (reviewed by Driss-Ecole et al. 2003) which can be involved in gravity sensing (Sievers et al. 1991, Ingber 1993, Forgacs 1995, Shafrir et al. 2000).

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Fig. 6. Statolith distribution in root statocytes of lentil seedlings grown in 1 g (A, C, E and G) or in microgravity (B, D, F and H) for 26 h. The frame represents the limits of the statocyte protoplast. At the end of the growth period the roots were fixed (A and B) or the seedlings were stimulated at 0.48 g for 9 min and fixed (C and D) or the seedlings were stimulated at 0.93 g for 22 min and fixed (E and F). G and H: as in E and F but the seedlings were fixed after a 3 h period in microgravity; dw, distal wall; lw, longitudinal wall (lower longitudinal wall for stimulated statocytes); pw, proximal wall; arrows, direction of centrifugal accelerations. The colour chart indicates the number of amyloplasts in a given region of the statocyte per unit (relative) area.

Table 2. Average position of the amyloplasts within the statocytes of lentil roots grown in 1 g or in microgravity and subjected to various gravitropic stimulations on the minicentrifuge. The average relative distance of the amyloplasts is measured with respect to the distal wall, dw, or to the lower longitudinal wall, lw (Fig. 6). The average relative distances for roots grown in 1 g or in μg are compared by the Z-test for each stimulation condition on the minicentrifuge. S, significant; NS, not significant (at the 5% level); HS (significant at the 1% level).

<table>
<thead>
<tr>
<th>Relative distance with respect to the</th>
<th>dw</th>
<th>μg</th>
<th>Z-test</th>
<th>lw</th>
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<th>Z-test</th>
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<tr>
<td>No stimulation</td>
<td>0.393</td>
<td>0.521</td>
<td>HS (7.03)</td>
<td>0.522</td>
<td>0.504</td>
<td>NS (0.75)</td>
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<tr>
<td>0.48 g × 9 min</td>
<td>0.434</td>
<td>0.489</td>
<td>HS (3.24)</td>
<td>0.564</td>
<td>0.612</td>
<td>S (2.4)</td>
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<tr>
<td>0.93 g × 22 min</td>
<td>0.435</td>
<td>0.441</td>
<td>NS (0.32)</td>
<td>0.660</td>
<td>0.655</td>
<td>NS (0.29)</td>
</tr>
<tr>
<td>0.93 g × 22 min + 3 h μg</td>
<td>0.515</td>
<td>0.508</td>
<td>NS (0.35)</td>
<td>0.549</td>
<td>0.561</td>
<td>NS (0.60)</td>
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located close to the cell centre. When the roots of both samples are stimulated on the minicentrifuge (Fig. 7A,B, 0.93 g/C2 22 min), the amyloplasts sediment but their trajectories are different. In the 1 g grown sample these organelles move from the distal wall to the lower longitudinal wall, and in the microgravity grown sample they move from the cell centre to the lower longitudinal wall. If the roots are left in microgravity after stimulation, there is an overall movement of the amyloplasts, which is the same in both samples, and its average orientation with respect to the longitudinal wall is about 40°. This could correspond to the orientation of actin filaments on which motor proteins (myosin) pull the amyloplasts. In microgravity grown samples, the sedimentation due to centrifugation occurs roughly parallel to the actin filaments, whereas in 1 g grown samples the sedimentation is almost perpendicular to these filaments. It is clear that the interactions between the amyloplasts and the actin filaments are more numerous in microgravity grown samples because of their initial dispersion and the direction of their movement, which is parallel to the actin filaments. This result suggests that the differences of interactions between the amyloplast and the actin network should be responsible for greater sensitivity of roots grown in microgravity as opposed to roots grown in 1 g before the stimulation on the minicentrifuge. Thus, even if the potential energy dissipated by the amyloplast movement is the same in the microgravity grown sample as in the 1 g grown sample, its utilization by the statocyte is more efficient in the former than in the latter case. These results could not be obtained on earth since the forces due to the actomyosin system are hidden by gravity.

If the overall orientation of actin filaments is actually about 40° with respect to the longitudinal wall this could explain why the optimal angle of stimulation is not 90° (i.e. when the root is placed in the horizontal position) but is about 130° (i.e. when the root is orientated obliquely upward; Iversen and Larsen 1971, 1973). In this peculiar position (Fig. 8) the force exerted by one amyloplast on an actin filament is maximal, which could lead to greater efficiency in the stimulation. This so-called deviation of the sine rule was also observed in hypocotyls (Kiss et al. 1997), but not in the Arabidopsis root (Mullen et al. 2000). This could be due to the peculiar shape of the Arabidopsis statocyte and the number and volume of statoliths. This could also be due to the method of analysing the root curvature using a special device permitting stimulation of the root tip at a constant angle with respect to gravity. Figure 8 shows the orientation of one actin filament in a plane, but it must be kept in mind

Table 3. Dispersion of the amyloplasts within statocytes of lentil roots grown in 1 g or in microgravity and subjected to various gravitropic stimulations on the minicentrifuge. The relative distance of the amyloplasts is measured with respect to the distal wall, dw, or to the lower longitudinal wall, lw (Fig. 6). The variance of the relative distance of the amyloplasts for roots grown in 1 g or in μg is compared by the F-test for each stimulation condition on the minicentrifuge. HS, significant at the 1% level, S, significant; NS, not significant at the 5% level.

<table>
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<tr>
<th>Variance of the relative distance from the</th>
<th>dw</th>
<th>lw</th>
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<tr>
<td></td>
<td>1 g</td>
<td>μg</td>
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<tr>
<td>No stimulation</td>
<td></td>
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<tr>
<td>0.48 g × 9 min</td>
<td>0.0209</td>
<td>0.0328</td>
</tr>
<tr>
<td>0.93 g × 22 min</td>
<td>0.0290</td>
<td>0.0278</td>
</tr>
<tr>
<td>0.93 g × 22 min + 3 h μg</td>
<td>0.0299</td>
<td>0.0432</td>
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Fig. 7. Average position of statoliths in gravity sensing cells for roots grown on the Biorack 1 g centrifuge in space (A, 1 g) or in microgravity (B, μg). In abscissa: relative distance with respect to the distal wall (dw). In ordinate: relative distance with respect to the lower longitudinal wall (lw). Two samples grown first in 1 g (A) or microgravity (B) were subjected to a stimulation of 0.93 g × 22 min. Two samples grown either in 1 g (A) or in microgravity (B) and stimulated at 0.93 g × 22 min were kept in microgravity for 3 h (0.93 g × 22 min + 3 h). Bars represent confidence intervals of the mean (at the 5% level). Arrows indicate the movements of statoliths.
that the statocyte could be visualized in 3 dimensions. In this case the projection in one plane of all actin filaments makes them appear to be orientated in various directions. This is what is observed when the actin network is analysed by fluorescence and confocal microscopy (Collings et al. 2001), or electron microscopy (Yoder et al. 2001) or immunolocalization (Driss-Ecole et al. 2000b). Moreover, the actin network is probably composed of interconnected short filaments linked by bridging proteins (Driss-Ecole et al. 2003).

It must also be noticed that the sedimentation per se of statoliths is not necessary since their action on the actin filaments needs only a slight displacement of these organelles. For instance, in starch depleted mutants (Caspar and Pickard 1989, Kiss et al. 1989), the movement of plastids does not occur during stimulation (MacCleery and Kiss 1999), although these mutants are able to sense gravity. The bulk of plastids can exert tensions on the actin network, even if these tensions are less than in the wild type. Other organelles (in particular the nucleus) can also play the role of statoliths (Barlow 1995), but they are surely less efficient. The opening of calcium channels (Plieth and Trewavas 2002) could be the first step of a chain of events leading to lateral transport of auxin from the cap toward the zone of reaction (Muday and DeLong 2001, Boonsirichai et al. 2002). One can hypothesize that actin filaments are attached to mechanoreceptors (stretch-activated ion channels; Bourque 1996). Thus, the mechanical action of statoliths can also be exerted on these bridging filaments. These results support the statolith theory since the protoplasm theory apparently cannot explain the difference of gravitaxis observed between the roots grown in 1 g or in microgravity.

Acknowledgements – This work was supported by CNES (Centre National des Etudes Spatiales). The authors are grateful to the Direction of Programs of CNES, to the Biorack team, to the GBMS (Groupe de Biologie et Medecine Spatiales, Toulouse), to COMAT (Conception Mecanique-Assistance Technique, Toulouse), to the Bio- netics team (Cape Canaveral), to ESA (European Space Agency) and to NASA (National Aeronautics and Space Administration). We thank M. Prouteau and P. Julianus for technical assistance.

References


Fig. 8. Orientation of an actin filaments (MF) with respect to the lower longitudinal wall (lw) of a statocyte. The root extremity (RE) is orientated at 130° with respect to gravity (g). In this particular orientation the tension exerted by the weight (W) of one amyloplast (a) on the actin filament (MF) is optimal since W is perpendicular to this filament. dw, distal wall; pw, proximal wall; W, weight of the amyloplast.

filaments parallel to the plasma membrane connecting the stretch-activated ion channels (Bourque 1996). Thus, the mechanical action of statoliths can also be exerted on these bridging filaments. These results support the statolith theory since the protoplasm theory apparently cannot explain the difference of gravitaxis observed between the roots grown in 1 g or in microgravity.
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Edited by D. Van Der Straeten

Physiol. Plant. 120, 2004 311