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Root-cap columella with movable amyloplasts may cause gravitropism of primary roots of *Brassica rapa*

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Complete absence of amyloplasts in columella cells meant there was no gravitropic response in the primary roots of *Brassica rapa*; and abnormal development of amyloplasts reduced the response. These facts suggest that the amyloplasts need to move freely in the cytoplasm as part of gravisensing. The seedlings were turned upside down and the columella cells with amyloplasts that aligned significantly towards the gravity vector were mapped. It was found that movable amyloplasts are localized in young and central columella cells. It therefore appears that young and central columella cells generate the largest gravisensing signal.

Key words: amyloplast, *Brassica rapa*, columella, gravitropism, root cap.

Introduction

There is much evidence that root caps have developed the structural and mechanical devices needed for gravity perception (reviewed in Wilkins, 1984; Bjorkman, 1988; Masson, 1995). In particular, the amyloplasts in the columella of the root caps are involved Němec 1990; Audus, 1962; Sievers & Volkmann, 1972; Barlow, 1974; Miles, 1981; Sack, 1997). The columella derives from a small group of initials at the apical side of the root meristem, which are transformed into secretory tissue and finally sloughed off the root at the tip (Kordyum & Guikema, 2001). Consequently, the columella cells are polarized along the longitudinal root axis both structurally and physiologically. Gravisensing by the roots necessarily involves gravisusceptors and gravireceptors (Perbal, 1999) gravisusceptors transform gravistimulation into mechanical or physical energy, which then stimulates gravireceptors located on the endoplasmic reticulum or plasma membranes and causes stretch-activated channels on these membranes to open and induce a signal (Sievers & Volkman, 1977; Behrens et al., 1982; Bjorkman & Leopold, 1987; Sievers & Bush, 1992; Sievers et al., 1995). It is therefore possible that the extent of motion of the amyloplasts is related to the strength of the induced gravisensing signal. However, an *Arabidopsis* mutant exists with no amyloplasts in its columella cells, but with a root that shows orthogravitropism (Caspar & Packard, 1989). The coleoptile of a starchless wheat mutant is also sensitive to gravistimula-

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tion (PICKARD & THIMANN, 1966). These results suggest that other cell structures can function as gravisusceptors. The most likely candidate is the whole protoplast. Its weight could act on receptors localized between the plasma membrane and the extracellular matrix (reviewed in ROSEN et al., 1999). The direction of cytoplasmic streaming may be controlled by protoplast-mediated graviperception (WAYNE et al., 1990).

It is generally accepted that the columella cells are the sites of gravity perception and that the amyloplasts within them are gravisusceptors. However, it is not settled whether the amyloplasts of all the columella cells can function as the gravisusceptors. The present paper is concerned with evidence suggesting that amyloplasts are required for gravity perception in the primary roots of *Brassica rapa* L., and with identifying and studying those columella cells with movable amyloplasts under gravistimulation. The results favour the hypothesis that young and central columella cells produce the strongest gravisensing signal.

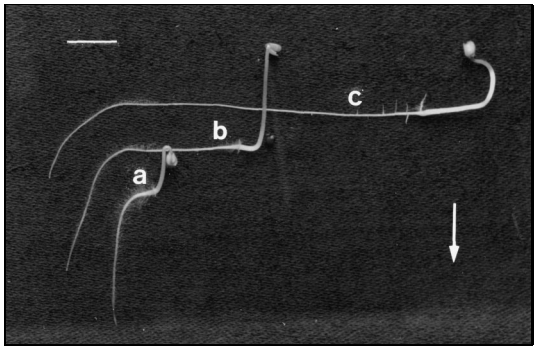


Fig. 1. A representative result showing the gravitropic response of seedlings obtained at 30 h (a), 2 days (b) and 3 days (c) after seeding. The arrow indicates the direction of gravity. Scale bar 1 cm.

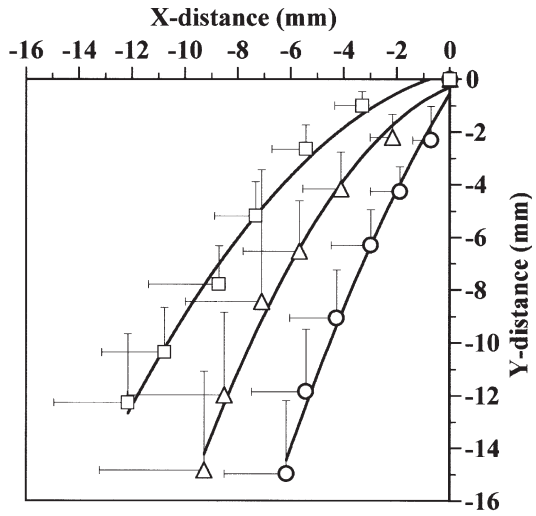


Fig. 2. Profiles of the gravitropic response of 30 h (○), 2 day (△) and 3 day-old seedlings (□). $n = 43$ for each.

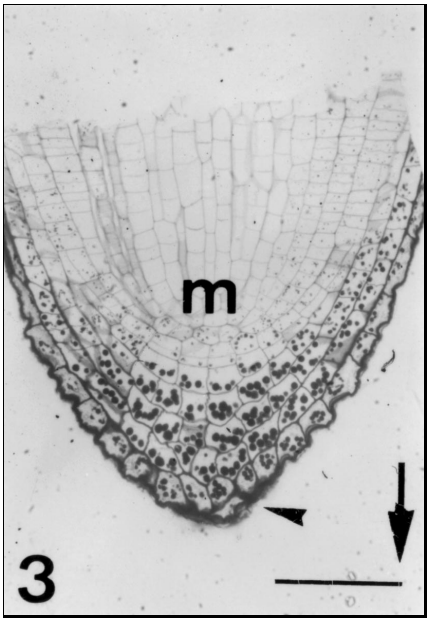
Material and methods

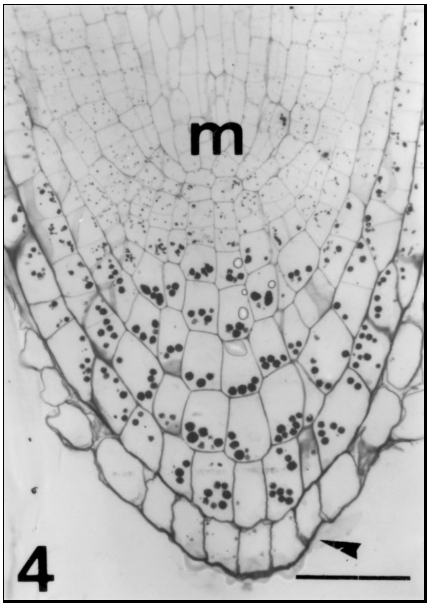
Plant culture and measurement of gravitropic growth
Seeds of *Brassica rapa* L. cv. "Iyo-hikabu" from commercial sources were placed on moistened cotton wool in a plastic vessel, which was then sealed with a transparent plastic cover. The plastic vessel was placed in darkness in a constant-temperature room at 24 ∼ 25 ℃ to encourage germination. The seeds usually germinated within one day.

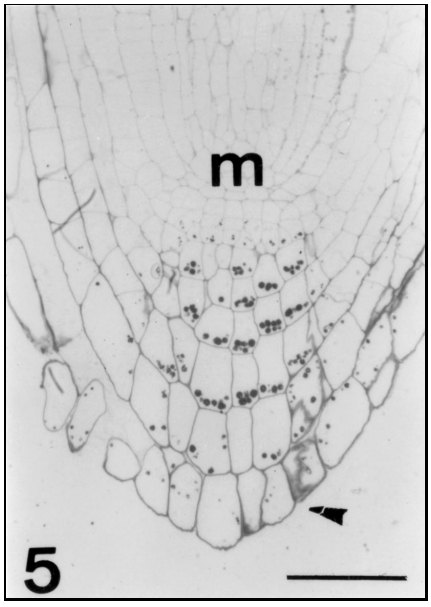
The plastic vessel, containing seedlings grown for 24 h, was turned on its side through 90° to give a gravistimulus to the roots, and was then placed in a large dark box equipped with a charged-coupled device camera (Sony Corporation) under a green safelight. The gravitropic growth was monitored for 24 h using a time-lapse video recorder (NV 8050, National). The positions of the root apices were marked every hour on a transparent sheet placed on the monitor screen. Continuous curves were drawn connecting these positions using a computer and curve fitting. The angle between the horizontal line and the tangent to the fitted curve at any given time is the gravitropic deviation.

Light microscopy
The apical parts (about 2 mm) of the roots were excised and fixed overnight at 4 ℃ in 4% (v/v) glutaraldehyde suspended in 1/15 M phosphate buffer (pH 7.2). They were then dehydrated through a graded alcohol series and embedded as specified by Spurr (1969). Longitudinal sections of roots, 1μm thick, were cut with an LKB ultrotome and transferred onto a droplet of water on a glass slide. Any wrinkles on these sections were removed by exposure to xylene gas followed by drying on a hot plate. The sections were then processed by the periodic acid Schiff (PAS) reaction (McManus and Mowry, 1964), which specifically contrasts cell walls and amyloplasts.

Determination of the movability of amyloplasts
The seedlings were turned upside-down and the root tips were excised after 0, 10, 30 and 60 min, and were promptly fixed in 4% glutaraldehyde in 1/15 M phosphate buffer (pH 7.2). They were embedded in Spurr's resin, then cut into semi-thin sections and stained by the PAS reaction. The positions of the amyloplasts in each cell were evaluated as the ratios of the distance of amyloplasts from the lower cell wall to the longitudinal cell length (relative positions of amyloplasts).







Figs 3–5. Light micrographs showing the development of amyloplasts in root caps of 30 h- (Fig. 3), 2 d (Fig. 4) and 3 d-old seedlings (Fig. 5). m; meristem. Scale bar 100 μm.

Results

Degeneration of amyloplasts and gravisensing
Seedlings obtained at 30 h, 2 and 3 days (d) after seeding were held continuously on their side for 24 h. Figure 1 shows representative growth profiles of these seedlings. When the gravireaction of the three groups of seedlings was statistically analysed, the gravitropic response of the roots was found to decrease significantly as the

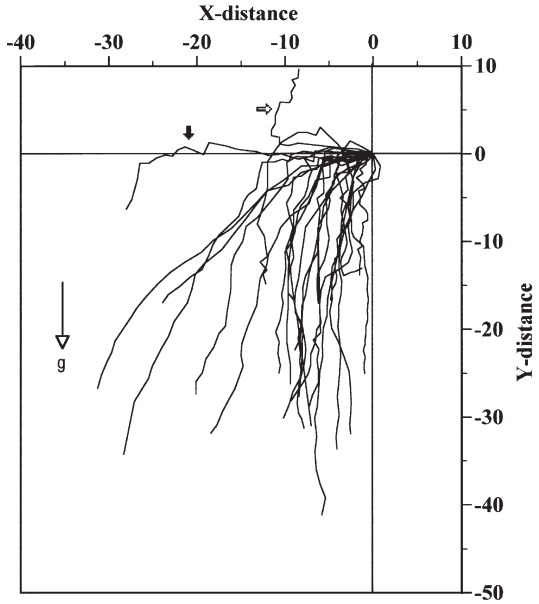


Fig. 6 Profiles of the gravitropic response of the roots of 2 d-old seedlings. Positions of the root apices were plotted every hour on the coordinate system. Open and solid arrows indicate an abnormal gravitropic response.

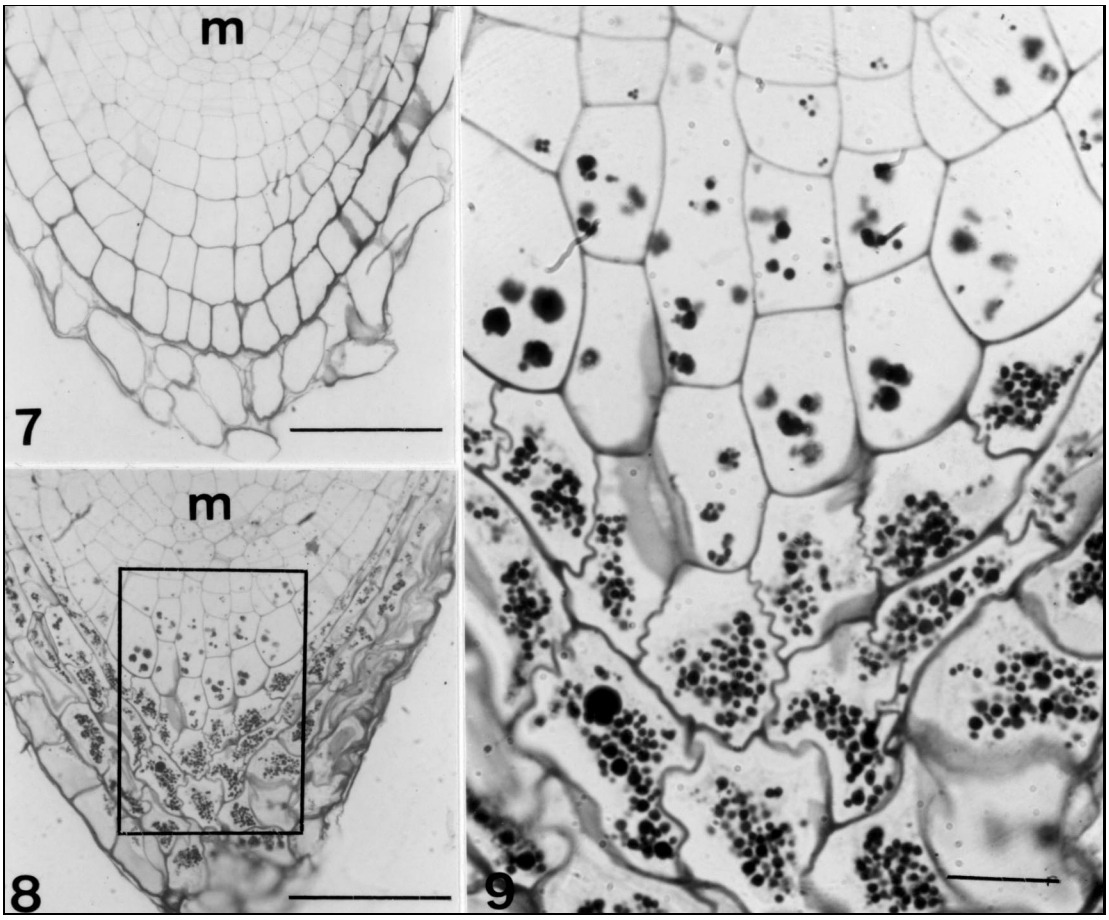


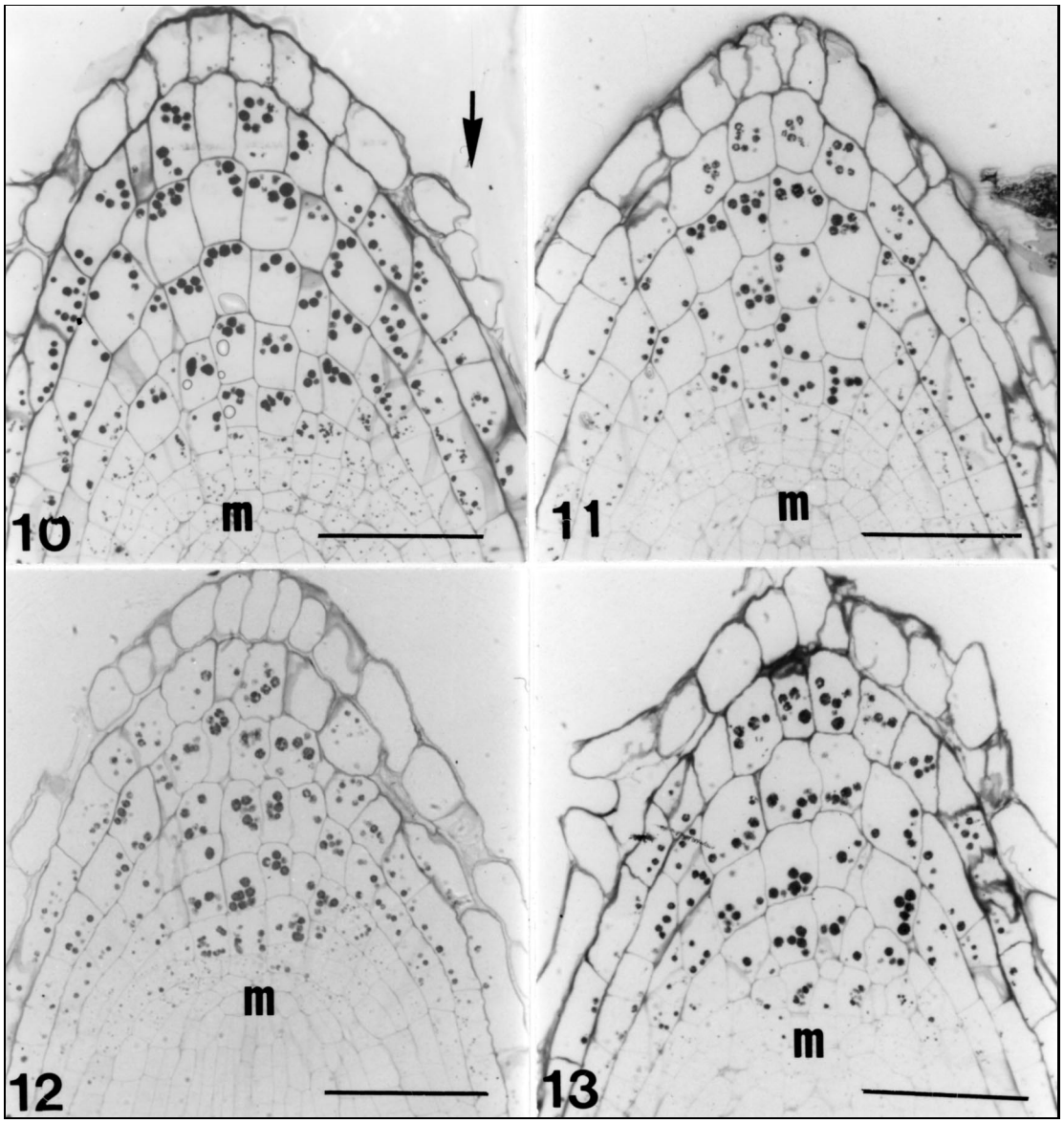
Fig. 7. Light micrographs of the root caps of seedlings showing abnormal gravitropic response. No amyloplast is seen in the root cap of the seedling indicated by an open arrow in Fig. 6. Scale bar 100 μm.
Fig. 8. Light micrographs of the root caps of seedlings showing abnormal gravitropic response. Starch grains almost fully occupied the cytoplasm of the seedling indicated by a solid arrow in Fig. 6. Scale bar 100 μm.
Fig. 9. The square region in Fig. 8 is magnified to show abnormal development of the amyloplasts. Scale bar 20 μm.

seedlings aged (Fig. 2). The gravitropic deviation obtained after 24 h on their sides was 72, 67 and 58, for 30 h-, 2 d- and 3 d-old seedlings respectively.

Sections of the root caps were compared for the three groups of seedlings, looking especially at the presence of amyloplasts. Most columella cells from the 30 h-old seedlings were small and spherical in shape (Fig. 3). This suggests that they are immature, since mature columella cells are large and rectangular. Interestingly, all the root cap cells of the 30 h-old seedlings contained amyloplasts, which were more abundant than those of the older seedlings. Even the outermost cells had abundant amyloplasts, in contrast to the 2 d- and 3d-old seedlings. Amyloplasts were also observed in all root cap cells of the 2 d-old seedlings, but in lower numbers (Fig. 4). In the 3 d-old seedlings the amyloplasts decreased in both number and size, and disappeared almost completely in the peripheral tissue of the root cap (Fig. 5). It is clear that the amyloplasts in the root caps gradually degenerate with aging, and are almost entirely confined to the columella cells by 3 days after germination.

Abnormal gravitropism and development of amyloplasts

When the roots were gravistimulated by being rotated to the horizontal position, they showed quite large variations in their gravitropic response



Figs 10–13. Light micrograph showing amyloplasts in the root cap cells at 0 (Fig. 10), 10 min (Fig. 11), 30 min (Fig. 12) and 1 h (Fig. 13) after the seedlings were turned upside down. The amyloplasts in the central columella cells gradually aligned with the gravity vector (arrow). Scale bar 100 μm.

(Fig. 6). Some roots grew almost vertically after about 12 h, but others still grew at an oblique angle to the gravity vector even after 24 h.

Two seedlings showed abnormal gravitropism, however. One grew against the direction of gravity during the whole time of monitoring, and the other was unresponsive to gravistimulation for a while. The root caps of these two seedlings were sectioned in order to study the correlation between the development of amyloplasts and gravitropism. No starch grains were discerned in the columella cells of the seedling whose root grew against the gravity vector (Fig. 7). In the seedling, which showed delayed root gravitropism, numerous small starch grains almost completely filled the cytoplasm of the outer storeys of the columella cells (Figs 8, 9). The inner two storeys of the columella had normally developed amyloplasts.

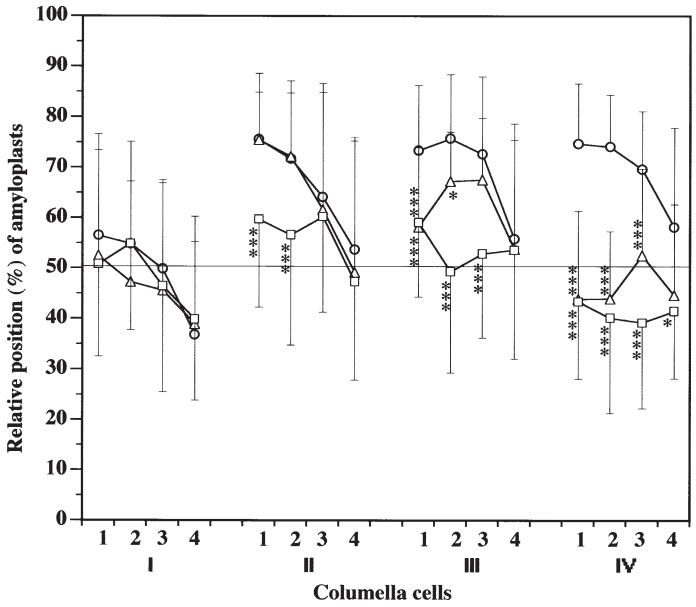


Fig. 14. Relative positions of amyloplasts in the columella cells at 0 (○), 10 (△), and 30 min (□) after the roots were turned upside down. The columella cells specified by the numbers are described in the text. The results show that amyloplasts are readily movable in young and centrally-localized cells. A significant difference against 0 min was determined by the χ^2-test; $^*P < 0.05$, $^{***}P < 0.001$.

Mapping of the columella cells with amyloplasts responsive to gravistimulation

The 2 d-old seedlings were turned upside down and the movability of the amyloplasts in the columella cells was studied (Figs 10–13). The columella cells formed cell files along the longitudinal axis of the root and some discrete storeys in the transverse direction, and to identify them uniquely the cell files were denoted 1 to 4 from the center to the outside, and the storeys except the outermost one as I to IV from the outside to the inside. After 1 h, almost all amyloplasts in storeys II to IV had aligned towards he gravity vector. However, most of those in storey I were localized in the intermediate region of the cells. Thus, the movability of amyloplasts seems to depend on the positions of the columella cells. This observation led us to examine the movability of amyloplasts in each cell; the relative positions of the amyloplasts was statistically analyzed at 0, 10 and 30 min after the plants were turned upside-down (Fig. 14). We found that the amyloplasts did not move significantly in any cells of storey I even after 30 min. In storey II no migration of amyloplasts was discerned after 10 min, but after 30 min they were significantly aligned with the gravity vector, especially in the centrally localized cells. After 10 min, the amyloplasts were aligned with the gravity vector in cells of storey III cells except the outermost ones. Cells in storey IV were the most sensitive to gravistimulation, having moved significantly in files 1–4 after only 10 min. Clearly the movability of amyloplasts under gravistimulation varies according to the position of the columella cells. In particular, amyloplasts are easily movable in cells that are young and are localized in the center of the columella.

Discussion

The 30 h-old seedlings were more responsive to gravistimulation than the 3 d-old seedlings, and the amyloplasts in the former were more abundant than those in the latter. As the plants grew, the number and size of amyloplasts in the columella cells decreased and the gravitropic response decreased. These findings suggest that gravitropic response is influenced by the development of the columella cells or the amyloplasts or both. This conclusion is supported by IVERSON (1969), who removed the amyloplasts from the root caps of *Lepidium sativum* using kinetin and gibberellic acid (GA_3) and found that plants then grew only a little more slowly but were unresponsive to gravity. Decapped roots temporarily lost gravireaction, but recovered it once a new root cap had reformed (JUNIPER et al., 1966). BUSCH & SIEVERS (1990)

later claimed that hormone treatment must affect more than loss of starch because the polar organization of the columella cells was reduced. If the root-tips are surgically removed, the roots become insensitive to gravistimulation for a period (KONINGS, 1968; BARLOW, 1974; KODERA & SATO, 2001). The gravitropic response is significantly correlated with the extent of removal of the root caps (KODERA & SATO, 2001), suggesting that the size of the gravitropic response depends on the total gravisensing signal from all the columella cells.

Interestingly, no amyloplasts were found in any columella cells of the root, which grew against gravity. This finding does not accord with the idea that the whole protoplast can function as the gravisusceptor (PICKARD & THIMANN, 1966; WAYNE et al., 1990) but favours the claim that starch is necessary for full sensitivity to gravistimulation (KISS et al., 1989). Starch grains allow the amyloplasts to sediment readily towards the gravity vector. There is close correlation between amyloplast sedimentation and gravitropism (SACK, 1991; SALISBURY, 1993). Development of amyloplasts with too much starch weakened rather than promoted the gravitropic response, as found here. Starch grains increase the weight, so that amyloplasts with many starch grains could be very effective at gravisensing. However, excessive development of amyloplasts probably reduces their movability within the cell because they almost fully occupy the cytoplasm. Amyloplasts need to be movable in the cytoplasm in order to induce a signal (AUDUS, 1979; SACK, 1997; BLANCAFLOR et al., 1999; KORDYUM & GUIKEMA, 2001). To summarise our results, the amyloplasts are the gravisusceptor and must move freely in the cytoplasm for effective gravisensing in *Brassica rapa*.

The cells with movable amyloplasts were gradually localised in the central region of the columella as they matured. In particular, no columella cells of storey I or the outer cells of storeys II and III showed any significant amyloplast movability. It therefore seems that not all the columella cells participate in generating a physiological signal, but only young or centrally localized cells. This hypothesis is supported by an experiment in which a laser beam was used to ablate the columella cells. Ablation of cells in the inner storeys had the greatest effect on presentation time, but ablating the columella cells in the outer storeys had a smaller effect or none (BLANCAFLOR et al., 1999). A study of the *in vitro* statolith sedimentation rates showed that the amyloplasts of the central columella cells moved more rapidly than those of the flanks. These authors therefore proposed that cells with the most freely mobile amyloplasts generate the largest gravisensing signal. This agrees with our own results presented here.

References

AUDUS, L. J. 1962. The mechanism of the perception of gravity by plants. Symp. Soc. Exp. Biol. **16:** 197–226.

AUDUS, L. J. 1979. Plant geosensors. J. Exp. Bot. **30:** 1051–1073.

BARLOW, P. W. 1974. Recovery of geotropism after removal of the root cap. J. Exp. Bot. **25:** 1137–1146.

BEHERENS, H. M., WEISENSEEL, H. & SIEVERS, A. 1982. Rapid changes in the pattern of electric current around the root tip of *Lepidium sativum* L. following gravistimulation. Plant Physiol. **70:** 1079–1083.

BJORGMAN, T. 1988. Perception of gravity by plants. Adv. Bot. Res. **151:** 41.

BJORGMAN, T. & LEOPOLD, A. C. 1987. An electric current associated with gravity sensing in maize roots. Plant Physiol. **84:** 1–8.

BLANCAFLOR, E. B., FASANO, J. M. & GILROY, S. 1999. Laser ablation of root cap cells: Implications for models of graviperception. Adv. Space Res. **24:** 731–738.

BUSCH, M. B. & SIEVERS, A. 1990. Hormone treatment of roots causes not only a reversible loss of starch but also of structural polarity in statocytes. Planta **181:** 358–364.

CASPER, T. & PICKARD, B. G. 1989. Gravitropism in a starchless mutant of *Arabidopsis*. Implications for the starch-statolith theory of gravity sensing. Planta **117:** 185–197.

IVERSEN, T. H. 1969. Elimination of geotropic responsiveness in roots of cress (*Lepidium sativum*) by removal of statolith starch. Physiol. Plant. **22:** 1251–1262.

JUNIPER, B. E., GROVES, S., LANDAU-SCHACHAR, B. & AUDUS, L. J. 1966. Root cap and the perception of gravity. Nature **226:** 115–116.

KISS, J. Z., HERTEL, R., & SACK, F. D. 1989. Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. Planta **177:** 198–206.

KODERA, Y. & SATO, S. 2001. Recovery of gravitropic response during regeneration of root caps does not require developed columella cells and sedimentation of amyloplasts. Cytobios **104:** 53–65.

KONINGS, H. 1968. The significance of the root cap for geotropism. Acta Bot. Neerl. **17:** 203–211.

KORDYUM, E. & GUIKEMA, J. 2001. An active role of the amyloplasts and nuclei of root statocytes in gravireaction. Adv. Space Res. **27:** 951–956.

MASSON, P. H. 1995. Root gravitropism. BioEssays **17:** 119–127.

McManus, J. F. A. & Mowry, R. W. 1964. Carbohydrate. In Staining Methods. Harper and Row, Tokyo. John Weatherhill, Inc., pp. 124–51.

Miles, D. 1981. The relationship of geotropic response and statolith in a nuclear mutant of maize. Physiol. Plant. **67 Suppl.:** 100.

Němec, B. 1900. Über die Art der Wahrmehmung des Schwerkraftreizes bei den Pflanzen. Der Deutsch. Bot. Ges. **18:** 241–245.

Perbal, G. 1999. Gravisensing in roots. Adv. Space Res. **24:** 723–729.

Pickard, B. G. & Thimann, K. V. 1966. Geotropic response of wheat coleoptiles in absence of amyloplast starch. J. Gen. Physiol. **49:** 1065–1086.

Rosen, E., Chen, R. & Masson, P. H. 1999. Root gravitropism: a complex response to a simple stimulus? Trends Plant Sci. **4:** 407–412.

Sack, F. D. 1991. Plant gravity sensing. Int. Rev. Cytol. **127:** 193–252.

Sack, F. D. 1997. Plastids and gravitropic sensing. Planta **203:** 63–68.

Salisburry, F. B. 1993. Gravitropism: Changing ideas. Hortic Rev. **15:** 233–278.

Sievers, A. & Busch, M. B. 1992. An inhibitor of the Ca^{2+}-ATPases in the sarcoplasmic reticula inhibits transduction of the gravity stimulus in cress roots. Planta **188:** 619–622.

Sievers, A., Sondag, C., Trebacz, K. & Hejnowicz, Z. 1995. Gravity induced changes in intracellular potentials in statocytes of cress roots. Planta **197:** 392–398.

Sievers, A. & Volkmann, D. 1972. Verursacht differentieller Druck der Amyloplasten auf ein komplexes Endomembransystem die Geoperzeption in Wurzeln? Planta **102:** 160–172.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26:** 31–43.

Wayne, R., Staves, M. P. & Leopold, A. C. 1990. Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*. Protoplasma **155:** 43–57.

Wilkins, M. B. 1984. Gravitropism, pp. 163–185. In: Wilkins, M. B. (ed.), Advanced plant physiology, Pitman, London.

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