Dynamics of Amyloplast Sedimentation in Growing Yam Tubers and Its Possible Role in Graviperception

Michio Kawasaki, Mitsutaka Taniguchi and Hiroshi Miyake

(Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan)

Abstract: The mechanism of gravitropism in yam tubers was investigated using two cultivars of Chinese yam, cv. Nagaimo which elongates to form a long tuber, and cv. Genkotsujirou which elongates in the initial stage and then spherically thickens. In both cultivars, many amyloplasts were locally formed and settled down vertically in each cell at the part beneath the stele of the elongating tuber. In contrast, amyloplasts in the stele in the apical part of the elongating tuber in these cultivars were smaller in number and size than those in the part beneath the stele and did not settle down vertically. In the thickening tuber of Genkotsujirou, the number of amyloplasts decreased and they did not settle down vertically in the part beneath the stele. In Nagaimo tuber tilted on an inclined plate, amyloplasts in the part beneath the stele in apical part of tuber also settled in the direction of gravity. Tubers elongated vertically as soon as they passed through the inclined plate. In such tubers, amyloplasts in the part beneath the stele were vertically settled down. These results indicated that amyloplasts in the part beneath the stele would play a role as statoliths for the perception of gravity. Crystal cells and tannin cells dispersed in the part beneath the stele of tuber as in the cortex surrounding the stele. The result indicated that the part beneath the stele is a part of cortex. Therefore, the cortex in the apical part of tuber was presumably important as the site of gravity perception.

Key words: Amyloplast, Chinese yam, Geotropism, Gravity, Starch, Tuber, Yam.
then at 20°C for 2 hr. The samples were washed with 0.1 M sodium phosphate buffer, and post-fixed in 0.15 M sodium phosphate buffer containing 1% osmium tetroxide at 4°C for 8 hr. Fixed samples were dehydrated through a graded series of ethyl alcohol and permeated by propylene oxide. The samples were then embedded in Spurr’s resin and polymerized at 70°C for 24 hr. Semiultrathin sections (0.7 μm in thickness) were cut with a glass knife on an ultramicrotome (Ultracut N, Reichert). Sections were stained with toluidine blue O to observe the inner microstructure of tubers. They were observed with an optical microscope (Optiphot-2, Nikon).

3. Transmission electron microscopy

Fixation and embedding in Spurr’s resin was the same as the above process for optical microscopy. Ultrathin sections (80 to 90 nm in thickness) were cut with a diamond knife on an ultramicrotome (Ultracut N, Reichert) and placed on 300 mesh copper grids. Sections were stained with uranyl acetate followed by lead citrate to observe the inner ultrastructure of tubers. Then the sections were examined at 100 kV with a transmission electron microscope (H-600, Hitachi).

Results

In both cultivars, stele containing many storage parenchyma cells and vascular bundles comprised a large percentage of the inside of tubers, and cortex thinly surrounded the stele (Fig. 5). Storage starch accumulated in storage parenchyma cells of stele, but scarcely in the cortex (Fig. 5). In elongating tuber of Nagaimo, however, many amylloplasts accumulating starch were locally formed and vertically settled down in each cell in the part beneath the stele (Fig. 2c). In Nagaimo, amylloplasts in the stele the apical part of elongating tuber were smaller in number and size than those in the part beneath the stele (Fig. 2b and c). Additionally, many amylloplasts in the stele in elongating tuber of Nagaimo were scattered to the peripheral part of each cells and were not vertically settled down in each cell (Fig. 2b).

Many amylloplasts also localized and settled down vertically in each cell at the part beneath the stele in the elongating tuber of Genkotsujirou (Fig. 3c). In Genkotsujirou, amylloplasts in the stele in the apical part of elongating tuber were smaller in number and size than those in the part beneath the stele of elongating tuber, and they did not vertically settle down in each cell (Fig. 3b and c). In the part beneath the stele of the older thickening tuber of Genkotsujirou, amylloplasts were small in number, and scattering in the periphery of each cell (Fig. 4b).

Calcium oxalate crystal idioblasts (crystal cells) and tannin cells dispersed in cortex in the lateral part of tubers (Fig. 5). Those cells were also dispersed in the part beneath the stele (Fig. 2c, 4b, 7b and d). Starch was accumulated as a single grain in each amylloplast in the stele of both cultivars (Fig. 6a and b). In contrast, a compound grain consisting of plural starch granules was formed in each amylloplast in the part beneath the stele of tuber (Fig. 6c and d).

Amylloplasts were vertically settled down in each cell in the part beneath the stele in apical part of the tuber of Nagaimo elongating along the inclined plate (Fig. 7a and b). In the tubers vertically elongating after passing the inclined plate, many amylloplasts were settled down vertically in the part beneath the stele (Fig. 7c and d).

Discussion

Previous studies have indicated that root cap is the site of gravity sensing in roots. Amylloplasts have been reported to be localized in the cap cells and act as gravisensors (Audus, 1962; Barlow, 1974; Sack, 1997; Blancaflor et al., 1998; Perbal, 1999). Perception is confined to the columella cells within the root cap, where amylloplasts sediment in the direction of gravity (Sack, 1991). It was demonstrated with laser ablation of selected columella cells that the tiers of columella cells in Arabidopsis roots are the sites of gravitropism (Blancaflor et al., 1998). Starchless or starch-deficient mutants of Arabidopsis thaliana show substantially reduced gravitropism (Kiss et al., 1989, 1996; Sack, 1997). In elongating tuber of Chinese yam, many amylloplasts were localized in the part beneath the stele, and they settled down vertically in cells. In contrast, many amylloplasts in the stele in the apical part of elongating tuber were scattered in the peripheral part of each cell and did not settle down vertically. In Nagaimo, amylloplasts in the part beneath the stele in apical part of tuber elongating along the inclined plate also settled in the lower part of the cytoplasm. In Nagaimo tubers elongating vertically after passing through the tilted plate, many amylloplasts in the part beneath the stele of tuber slightly changed position in the cells and settled down vertically. Namely, many amylloplasts in the part beneath the stele always settled in the direction of gravity. These results indicated that amylloplasts in the part beneath the stele of tuber would play a role as statoliths for perception of gravity direction in Chinese yam tubers.

In the part beneath the stele in apical part of Genkotsujirou tubers in the thickening growth stage, the number of amylloplasts decreased and they scattered in each cell. This result indicated that amylloplasts in the part beneath the stele were probably essential for growing straight down in the direction of gravity of tuber.

Starch was accumulated as a single grain in each amylloplast in the stele of both cultivars. In Japanese yam, storage starch accumulated as a single grain in each amylloplast in the stele of tuber (Kawasaki et al., 1997). In contrast, a compound grain consisting of
plural starch granules was formed in each amyloplast in the part beneath the stele of tuber in Nagaimo and Genkotsujirou. Transmission electron microscopy revealed that amyloplasts in the root cap of *Vigna angularis* and *Lens culinaris* also contained compound
grain consisting of plural starch granules (Driss-Ecole et al., 2000; Kuya et al., 2006).

The part beneath the stele of Chinese yam tuber had some characteristics similar to the root cap. However, it has not been reported that independent tissue like root caps is formed at the tips of the tubers in Dioscorea. In five species of Dioscorea, the first indication of tuberization was the formation of perivascular cambium in the hypocotyl region, and the shape of the tuber depended on the local persistence of meristematic zone (Lawton and Lawton, 1969). In Dioscorea cayenensis-Dioscorea rotundata complex, the subapical meristematic zone is formed at the apex of tuber (Trouslot et al., 1993). The tuber of Dioscorea glabra is originated from the primary thickening meristem located in the perivascular region of the hypocotyls (Sharma, 1974).

Fig. 5. Inner structure of the lateral part of tubers (cross section). (a) Nagaimo. (b) Genkotsujirou. Arrowheads indicate amyloplasts. Bars=250 μm. Cc, crystal cell; Co, cortex; Cr, cork; St, stele; Tc, tannin cell; Vb, vascular bundle.

Fig. 6. Microstructure of amyloplasts in tubers of Nagaimo (a, c) and Genkotsujirou (b, d). (a, b) Amyloplasts in the apical part of the stele. (c, d) Amyloplasts in the part beneath the stele of tuber. Asterisks indicate starch granules. Bars=1.7 μm. A, amyloplast; G, the direction of gravity.

Fig. 7. Amyloplast distribution in the part beneath the stele in apical part of tilted tuber of Nagaimo and of the vertically elongating tuber of Nagaimo after passing through the inclined plate. (a) Diagram of tilted tuber. (b) Longitudinal sections from the part lying beneath the stele in apical part of tilted tuber. (c) Diagram of the vertically elongating tuber after passing through the inclined plate. (d) Longitudinal sections of the part beneath the stele of the vertically elongating tuber after passing through the inclined plate. Arrowheads indicate amyloplasts. Bars=100 μm. Cc, crystal cell; G, the direction of gravity.
Primary thickening meristem occurs in a pericyclic region in monocot stems between the outer cortex and the stele. This meristem produces cells inward, which results in the thickening of the stele (Rudall, 1991). In Dioscorea glabra, the stele was surrounded by the primary thickening meristem and the tuber elongated due to the increased activity of a deep-seated portion of this meristem (Sharma, 1974). In other words, the growth of stele via primary thickening meristem activity is responsible for the elongation of tuber, implying that the part beneath the stele of tuber in Dioscorea is cortex and not tissue like root cap.

In our observation, crystal cells and tannin cells dispersed in the part beneath the stele of tuber as in the cortex surrounding the stele of tuber. In Japanese yam (Dioscorea japonica Thunb.), crystal cells were also observed in the cortex of the tuber (Kawasaki et al., 2004). These results support the compartimentalization of the inner structure of tuber postulated by Sharma (1974) and indicate that the part beneath the stele of tuber is a part of cortex. Therefore, the cortex in the apical part of tuber was presumably important as the site of gravity perception in tubers. Further molecular evidence will be useful to understand the mechanism of gravity perception in this tissue.

Shoots of higher plants exhibit negative gravitropism. The endodermis has been shown to be essential for shoot gravitropism in Arabidopsis thaliana (Fukaki et al., 1998). In shoots, sedimented amyloplasts are found in the innermost layer of the cortex (Sack, 1987; 1991). The layer is called the starch sheath or endodermis. In this study, some amyloplasts were observed in the cortex of the tuber (Kawasaki et al., 1997). Amyloplasts are necessary for full gravitropic sensitivity in roots of Arabidopsis thaliana. Planta 177 : 198-206.

Kiss, J.Z., Wright, J.B. and Casper, T. 1996. Gravitropism in the roots of intermediate-starch mutants of Arabidopsis. Physiol. Plant. 97 : 237-244.

Kiss, J.Z., Miller, K.M., Ogden, L.A. and Roth, K.K. 2002. Phototropism and gravitropism in lateral roots of Arabidopsis. Plant Cell Physiol. 43 : 35-43.

Kuya, N., Kato, M., Sato, Y., Kaneta, T. and Sato, S. 2006. Comparative study of cellular structures implicated in gravisensing in statocytes of primary and lateral roots of Vigna angularis. Protoplasma 229 : 83-91.

Lawton, J.R. and Lawton, J.R.S. 1969. The development of the tuber in seedlings of five species of Dioscorea from Nigeria. Bot. J. Linn. Soc. 62 : 23-237.

Morita, S. and Abe, J. 1999. Perspective of root research. Jpn. J. Crop Sci. 68 : 453-462.

Onwueme, I.C. 1973. The sprouting process in yam (Dioscorea spp.) tuber pieces. J. Agric. Sci. (Cambridge) 81 : 375-379.

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

Rudall, P. 1991. Lateral meristems and stem thickening growth in monocotyledons. Bot. Rev. 17 : 150-163.

Sack, F.D. 1987. The structure of the stem endodermis in etiolated pea seedling. Can. J. Bot. 65 : 1514-1519.

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

Sack, F.D. 1997. Plastids and gravitropic sensing. Planta 203 : 563-568.

Sharma, O.P. 1974. Anatomy, origin and development of tuber of Dioscorea glabra. Phytomorphology 24 : 297-305.

Trouslot, M.F., Champagnat, M., Tort, M., Loiseau, M. and Eraud, C. 1993. Development morphology of seedlings of Dioscorea cayenensis-D.rudundata complex. Phytomorphology 43 : 49-57.

* In Japanese with English abstract.

Acknowledgements

Thanks are due to Yasuki Tahara, Technical Center of Nagoya University, for his technical supports in the plant cultivation at the farm of Nagoya University.

References

Audus, L.J. 1962. The mechanism of the perception of gravity by plants. Sympos. Soc. Exp. Biol. 16 : 197-226.

Barlow, P.W. 1974. Recovery of geotropism after removal of the plant cap. J. Exp. Bot. 25 : 1137-1146.

Blancaflor, E.B., Fasano, J.M. and Gilroy, S. 1998. Mapping the functional roles of cap cells in the response of Arabidopsis primary roots to gravity. Plant Physiol. 116 : 213-222.

Burkill, I.H. 1960. The organography and the evolution of Dioscoreaceae, the family of the yams. J. Linn. Soc. Bot. 56 : 319-412.