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Gravisensing in hypocotyls and roots of garden cress seedlings

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Gravisensing in negatively and positively gravitropic plant organs was studied by analysing the location and motion of amyloplasts in hypocotyl endodermal and root columella cells of *Lepidium sativum* L. seedlings during a subsequent 6-min period of lateral (reoriented 90°) gravitropic stimulation. The amyloplast positioning has been evaluated taking linear measurements of each plastid position with respect to the longitudinal wall and original bottom of the gravity sensing cells.

After growth for 30 h at 1 *g*, the amyloplasts are distributed symmetrically across the cells and located closer to the bottom cell wall in the endodermis than in root columella (15.2% and 27.7%, respectively). A more rapid plastid displacement (approximately 3 μm/min) towards the gravity and simultaneous sliding along the cells were determined within the first minute of gravitropic stimulation in hypocotyl endodermal cells as compared with that (1 μm/min) in root statocytes. During the second minute, the amyloplast location remained almost unchanged in endodermal cells, while they continued to slide intensively along the columella cells shifting slightly downwards. After a 6-min period, the final relative statolith distances from the lower longitudinal wall and bottom of the statocytes equalled to 31.1% and 28.9% in hypocotyls and to 36.5% and 38.8% in roots. The data show that, in agreement with the gravity force, the elastic forces of the cytoskeleton act actively transporting the amyloplasts along the statocytes into both organs within a 6-min gravitropic stimulation. However, the magnitude relation of these forces changes differently in gravisensing cells of hypocotyls and roots during the first minutes of gravitropic stimulation.

Key words: gravisensing, statocyte, amyloplast, root, hypocotyl, garden cress

INTRODUCTION

A great deal of experimental evidence demonstrates that starch containing amyloplasts in the columella of the root cap and in the endodermal layers of the stem-like organ are significant for gravity sensing [1–4]. Gravity-directed sedimentation of amyloplasts (statoliths) is a characteristic attribute of cells (statocytes) in these highly specific tissues and constitutes one of the initial events in gravity perception of positively and negatively gravitropic organs.

In addition to amyloplasts, the actomyosin system is also considered to play a role in the early phases of gravitropism. Space and ground experiments have helped to clarify the relationship between amyloplast positioning and the cytoskeleton in the root statocytes [3, 5, 6, 7–9]. Although many studies have focused on the importance of actin microfilaments in root gravisensing, there have been few investigations on its role in gravity-related signaling events of stem-like organs [4, 10, 11]. Data on gravitropism mutants as well as actin disrupting drug effects in stems versus roots suggest that the gravisensing mechanisms exhibit differences among these organs. It is supposed that these differences are related to the specific structure and function of the cytoskeleton and vacuoles in gravisensing cells. Experiments employing pharmacological agents often give conflicting results depending on the plant species [3, 7], the organ studied [10, 11], the drug and its dosage [4, 10, 11]. Most of such experiments were performed either on root or on stem-like organs, but sparsely on both axial organs of the same plant.

According to data on the graviresponsiveness of plant axial organs, roots may be more sensitive to gravitropic stimulation [12–14]. On the other hand, the *g*-threshold values have been shown to be about 3.0×10^{-3} *g* for roots as well as hypocotyls of garden cress [15]. It may be suggested that the gravitropic sensitivity of axial organs could be related to the initial events of gravity perception – the process of statolith sedimentation.

Therefore, a detailed analysis of amyloplast movement in gravistimulated seedlings during shorter periods than a reliable latent time of gravitropic response [6] could help to clarify differences in the gravity perception mechanisms of hypocotyls and roots. The objectives of the present work were: 1) to study amyloplast location in gravity sensing cells of hypocotyls and roots; 2) to analyse and compare the gravity-induced amyloplast motion within these cells.

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MATERIALS AND METHODS

Investigations were carried out with seedlings of garden cress (*Lepidium sativum* L.) in the dark, at 23 ± 1 °C. The seeds were germinated vertically at 1 *g* in 10 µl thin glass funnels to retain their respective orientation and to mark the direction of gravitropic stimulation. For uniformity [16], each seed was planted to match the orientation of the seedling with the hook and cotyledons positioned at the upper side during subsequent manipulations. After 30 h of growth, the seedlings were reoriented rightwards by 90° in respect to the gravity for 1, 2, 4 and 6 min. Then the seedlings were fixed for 45 min in 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), preserving at the same gravitational conditions as in the period of stimulation. After ttransferring into a fresh portion of glutaraldehyde solution for 2 h at 4 °C, the samples were postfixed in 1% (w/v) OsO_4, dehydrated and embedded in Epon by standard procedures.

Morphometrical analysis of gravisensing cells was performed employing light microscopy on semi-thin (1 µm) median longitudinal sections of apical hypocotyl segments (2 mm) and root caps. The plane of sections was parallel to the direction of gravistimulation. Toluidine-blue (1% (w/v) in 0.1% (w/v) $Na_2B_4O_7$) stained sections were photographed with a PENTAX*ist D digital camera attached to the SMP 03 microscope photometer (Opton, Germany). The images were analysed using the SigmaScanPro 5 program (Jandel Scientific Software). As a rule, 2–3 sections of both axial organs were examined for each test variant.

In order to characterize the amyloplast positioning, the length and width of the 2nd to 5th columella storey cells in root caps and endodermal cell profiles of hypocotyls were determined. The location of the individual amyloplast was characterized by the horizontal (*x-position*) and vertical (*y-position*) coordinates in the statocyte as a two-coordinate system. The *x-positions* represent plastid distances from the morphological cell bottom, which are expressed in percentage from the cell length. The *y-positions* represent the distances of the plastid centre from the right / lower longitudinal cell walls in the vertical / horizontally-stimulated organ in percentage of the cell width.

Statistical analysis was carried out using the MS EXCEL 7 standard package (Microsoft Corporation). The values are presented as a mean ± standard error (SE). Statistical significance was determined using the Student's t test.

RESULTS

Morphometry of gravisensing cells in axial organs of garden cress seedlings. Hypocotyls of garden cress consist of one epidermal, three to four cortical, one endodermal layer of cells and a stele containing vascuolar systems [17]. In the central longitudinal sections of hypocotyls vertically grown at 1 *g*, endodermal cells are presented as two profiles on either side of the stele. Morphometrical analysis of hypocotyl sections has revealed that the endodermis profile consists of 33–33 cells. The length of endodermal cells increased from 38 µm to 105 µm along the profile (Fig. 1A), while their width changed only from 21 µm to 26 µm (Fig. 1B). The length and width of the average endodermal cell of the hypocotyl segments were 57.8 ± 0.3 µm and 24.1 ± 0.3 µm, respectively. The average number of amyloplasts within the endodermal cells tested equalled to 5.9 ± 0.4. Despite the significant elongation of statocytes, the mean distances of the cell amyloplast complex with respect to the right longitudinal wall and the bottom of the cells increased but slightly – from 10 µm to 13 µm and from 6 µm to 12 µm, respectively.

The root cap columella of garden cress consists of six storeys of compact cells, the 2nd to 5th of which are functional statocytes [6]. The measurements of these cells are presented in Table. As is shown, the statocyte length and width along the columella increased less than along hypocotyl segments (Fig. 1A). In roots, the average location of the amyloplast complex with respect to the distal and longitudinal cell walls changed proportionally the measurements of the hypocotyl statocytes. The length of the average statocyte in the storeys of root columella equaled to 23.9 ± 0.6 µm and the width to 15.47 ± 0.4 µm.

Table. **Morphometric parameters of functional statocytes in root cap columella of garden cress seedlings**

Columella storeys	Statocyte measurements (µm):		Distance of amyloplasts (µm) from:	
	length	width	distal cell wall	right longitudinal cell wall
2	16.9 ± 0.3	13.7 ± 0.2	4.7 ± 0.2	6.8 ± 0.4
3	20.3 ± 0.5	15.2 ± 0.1	5.6 ± 0.2	7.6 ± 0.5
4	24.4 ± 0.7	15.7 ±0.3	6.8 ± 0.2	7.8 ± 0.5
5	30.0 ± 0.9	17.2 ± 0.4	8.3 ± 0.3	8.6 ± 0.4

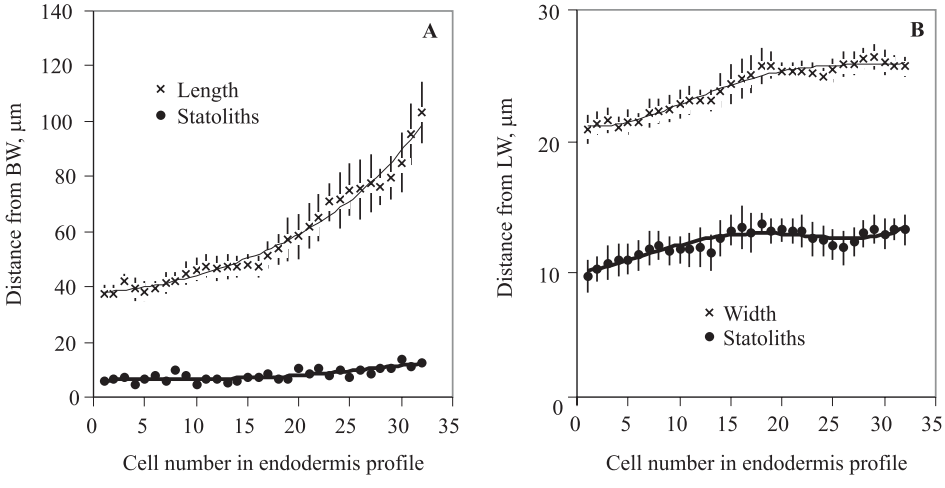

Fig. 1. Intracellular positioning of amyloplasts, statocyte length (A) and width (B) in endodermis profiles of garden cress hypocotyls. BW – basal cell wall, LW – right longitudinal wall

Gravity-induced statolith sedimentation in hypocotyl endodermal cells. In hypocotyls grown at 1 *g*, amyloplasts are dispersed transversely near the basal wall of endodermal cells (Fig. 2). After keeping seedlings horizontally for 6 minutes, the majority of plastids moved to the lower longitudinal cell wall. The statistical analysis of this motion allowed characterizing the statolith sedimentation kinetic. As is seen in Fig. 2, a statistically significant and more pronounced shift of statoliths downwards from the initial *y-position* of 48.5% to 37.2% from the total cell width was determined within the first minute ($p \leq 0.01$). Besides, the plastids changed their position simultaneously along the cells from the initial *x-position* of 15.2% to 20.4% ($p \leq 0.05$). The effect of the second stimulation minute was negligible in either direction. Later, the amyloplasts continued moving in the longitudinal direction and reached the *x-position* of 26.7% after a 4-min stimulation ($p \leq 0.05$). In contrast, the *y-position* was shifted downwards only slightly. After a 6-min period, the plastids were shifted towards the gravity to the *y-position* of 31.1% and in longitudinal direction to the *x-position* of 28.9%.

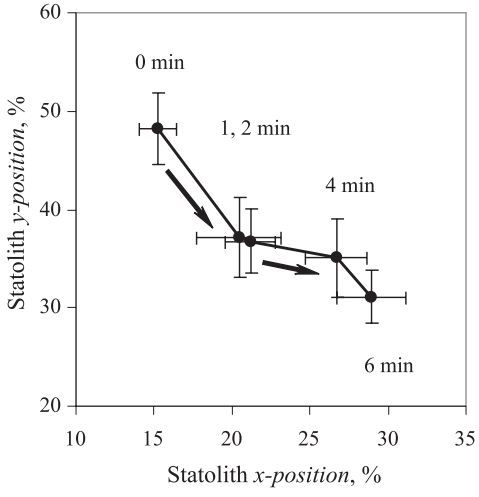

Fig. 2. Gravity-induced sedimentation of amyloplasts in endodermal cells of garden cress hypocotyls within 6-min stimulation

Translating into the comparatively real values of cell measurements and plastid distances from the respective cell wall, the velocities of statolith motion in the gravity direction were found to be 2.7 μm/min and 0.1 μm/min within the first and second minutes of stimulation, while the velocities of simultaneous sliding along the cells were 3.0 and 0.4 μm/min, respectively. Within the following two minutes, the amyloplasts moved slowly (0.2 μm/min) downwards and shifted considerably from the cell bottom in the longitudinal direction at a speed of 1.6 μm/min. During stimulation from the 4th to 6th minute, the plastids continued moving in both directions at approximately the same velocity of 0.5–0.6 μm/min.

Statolith movement in root statocytes during gravistimulation. In vertically grown root statocytes, the amyloplasts are dispersed smoothly across the distal pole of the statocytes (Fig. 3). The reorientation of such roots with respect to gravity had a considerable effect on the distribution of organelles within the statocytes in transverse as well as longitudinal direction. After the first minute, significant changes of statolith positioning in both directions were determined (Fig. 3). The *y-position* decreased from 49.7% to 43.1% ($p \leq 0.01$). Simultaneously, the *x-position* increased from 27.7% to 32.3% ($p \leq 0.05$). During the second minute, the statoliths continued moving in this bottom-to-side direction, the *y-position* diminished to 40.5% and the *x-position* increased up to 42.9%. Later, the trajectory of amyloplast motion changed considerably. A significant reversible movement to the *x-position* of 38.8% ($p \leq 0.05$) and a simultaneous downwards directed shift of statoliths was observed to the *y-position* of 36.4% ($p \leq 0.05$). The velocity of statolith sedimentation within the first and second minutes of gravitropic stimulation was found to be about 1 μm/min and 0.7 μm/min, while the velocity of sliding along the cells equaled to 1 μm/min and 1.7 μm/min, respectively.

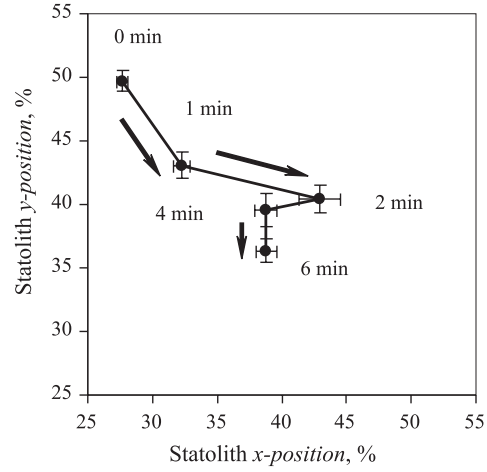

Fig. 3. Movement of amyloplasts in statocytes of garden cress roots during 6-min gravitropic stimulation

DISCUSSION

Measurements of amyloplast location in gravisensing cells of hypocotyls and roots show that before stimulation, the statoliths are located closer to the cell bottom in endodermis than in root columella cells (27.7% and 15.2%, respectively). A more rapid change of amyloplast location was determined within the first minute of gravitropic stimulation in hypocotyl statocytes as compared with that in roots. However, after a 6-min stimulation the final statolith distances from the lower longitudinal and basal cell walls equalled to 28.9% and 31.1% in hypocotyls and to 38.8% and 36.5% in roots.

It is known that the perpendicular and longitudinal components of the gravity force are responsible for plastid sedimentation within the statocyte, and the former component is the most important during gravitropic stimulation [18]. However, experiments with root manipulations [3, 5, 6, 8] as well as our data (Fig. 3) on statolith motion in garden cress root statocytes demonstrate that active amyloplast displacement in the longitudinal direction could also be an essential factor in gravisensing. Supposedly, this displacement in roots is mediated by the elastic forces of the cytoskeleton.

In endodermal cells of hypocotyls, the role of the cytoskeleton in amyloplast redistribution during gravitropic stimulation remains unclear. Endodermal cells of stem-like organs possess very large central vacuoles that are more likely to restrict the statolith

sedimentation. Earlier it has been supposed that actin microfilaments are not directly involved in hypocotyl gravisensing and it occurs when the central vacuole is deformed by amyloplast displacement [17]. According to Palmieri and Kiss [4], F-actin actively participates in amyloplast movement within hypocotyl endodermal cells of *Arabidopsis*. Our data on statolith redistribution in the statocytes of garden cress seedlings are consistent with this opinion. The determined downward shift towards gravity and a simultaneous increase of the relative distance from the original cell bottom within the 6-min gravitropic stimulation (Figs. 2 and 3) testify significantly to a relationship between amyloplast repositioning and the elastic forces of the cytoskeleton, transporting the plastids along the cells. On the other hand, the magnitude relation between these forces changes differently in the gravisensing cells of hypocotyls and roots during the first minutes of gravitropic stimulation.

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GRAVITACIJOS JUTIMO YPATUMAI SĖJAMOSIOS PIPIRNĖS DAIGŲ HIPOKOTILIUOSE IR ŠAKNYSE

Santrauka

Gravitacijos jutimas sėjamosios pipirnės (*Lepidium sativum* L.) ašiniuose organuose tirtas analizuojant amiloplastų lokalizaciją bei judėjimą gravisensorinėse hipokotilių endodermio ir šaknies šalmelio ląstelėse, kai daigai buvo gravitropiškai dirginami 6 min. pavertus juos 90° kampu. Amiloplastų padėtis įvertinta išmatavus kiekvienos plastidės nuotolį nuo išilginės gravisensorinės ląstelės sienelės ir morfologinio jos dugno.

Vertikaliai 30 val. normalaus svarumo sąlygomis augusių daigų amiloplastai abiejų tipų ląstelėse pasiskirstę simetriškai skersine kryptimi, bet endoderminėse ląstelėse jie išsidėstę arčiau ląstelės dugno, lyginant su šaknies šalmelio statocitais (nuotolis 15,2% ir 27,7% atitinkamai). Pirmąją gravitropinio dirginimo minutę plastidės hipokotilių endodermio ląstelėse judėjo kur kas greičiau (apie 3 μm/min) gravitacijos vektoriaus kryptimi, kartu panašiu greičiu slinkdamos išilgine kryptimi nei šaknų statocituose (1 μm/min). Kitą minutę amiloplastų padėtis endodermio ląstelėse beveik nekito, o šalmelio ląstelėse jie ir toliau intensyviai slinko išilgai ląstelės lėtai judėdami žemyn. Po 6 min. galutinis vidutinis statolitų nuotolis nuo apatinės išilginės statocitų sienelės ir jų dugno buvo 31,1% ir 28% hipokotiliuose, o šaknyse – 36,5% ir 38,8% atitinkamai. Duomenys leidžia manyti, kad abiejų tipų ląstelėse kartu su gravitacija veikia citoskeleto generuojamos elastinės jėgos, aktyviai tempiančios amiloplastus išilgine kryptimi; pirmosiomis gravitropinio dirginimo minutėmis nustatyti šių jėgų santykio skirtumai hipokotilių ir šaknų gravisensorinėse ląstelėse.

Raktažodžiai: gravitacijos jutimas, statocitas, amiloplastas, šaknis, hipokotilis, sėjamoji pipirnė