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INVESTIGATION ON GRAVITY SENSING IN GARDEN CRESS SEEDLINGS

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The summary of the doctoral dissertation has been sent on January 14, 2013
The dissertation is available at the libraries of the Nature Research Centre, Institute of Botany and Vilnius University

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SĖJAMOSIOS PIPIRNĖS GRAVITACIJOS JUTIMO TYRIMAS

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INTRODUCTION

The ability of plants to sense gravity and respond by growth movements has been studied for more than 150 years. Much attention has been devoted to clarify the mechanisms of gravity sensing which are not understood fully until now. Space flights were an important factor for the expanded surveys on plant reactions to alterations of gravitational force. In the process of evolution on the Earth, a complex gravity-sensing system has been formed through which the plants can sense and orientate their organs with respect to the gravity vector direction (roots) or in the opposite direction (aboveground part of the plant). It has been shown that during space flight where the magnitude of gravity is reduced to microgravity level plants can undergo a full cycle of ontogenesis (Merkys, Laurinavičius, Švegždienė, 1984; Musgrave et al., 2000). On the other hand a small, but statistically significant morphological and metabolic changes have been recorded in many tests with plants under altered gravity conditions in space and on the ground (Soga et al., 1999; Hoson et al., 2009; Wakabayashi et al., 2009; Matía et al., 2010; Manzano et al., 2012). These findings display the existing mechanisms of interactions between plant cells and gravity. From this viewpoint, the studies on the formation of specialized gravity-sensing tissue cells under altered gravity conditions are especially important, because they help us to understand how the mechanisms of spatial orientation are functioning and how these processes are integrated into the overall program of plant growth and development.

Although gravity acts equally on all plant cells, gravity sensing occurs in specialized cells (statocytes), which are localized in root tip columella and shoot endodermis. Starch containing, mobile amyloplasts are characteristic atribute of gravisensing cells. Most studies to date support the starch-statolith hypothesis of gravity perception associated with the movement of amyloplasts acting as statoliths (Laurinavičius et al., 2001; Kato et al., 2002; Driss-Ecole et al., 2003; Vitha et al., 2007; Kumar et al., 2008). According to starch-statolith hypothesis, gravity-induced sedimentation of amyloplasts (statoliths) constitutes one of the initial events in gravity perception. It is supposed that sedimentation of statoliths along the gravity provides a conversion of gravitational potential energy into sensor-activating kinetic energy and triggers subsequent intracellular signaling processes. The question how the gravity-

induced displacement of amyloplasts triggers these processes remains open. The cytoskeleton system is supposed to be implicated in gravity signal perception in roots through the positioning of amyloplasts and modulation of their movement (Yoder et al., 2001; Hou et al., 2003; Blancaflor, Masson, 2003; Perbal, 2009; Kumar et al., 2008).

In vertically growing root caps, the gravitational and opposite endocellular forces (buoyant, drag, the elastic forces generated by cytoskeleton elements, etc.) have been suggested to determine the equilibrium position of statoliths in the distal region of statocytes (Björkman, 1988; Todd, 1994; Yoder et al., 2001). There are some reports to indicate that after reducing the magnitude of gravity from 1 *g* to microgravity in space or simulated on Earth, the latter forces pull the amyloplasts towards the centre of gravisensing cells of roots (Volkmann et al., 1991; Laurinavičius et al., 2001; Yoder et al., 2001; Driss-Ecole et al., 2008). However, little is known how these forces interact and regulate the statolith positioning and movement during alterations of the magnitude and action direction of gravitational force.

In stem-like organs, the actual relationship between movable statoliths and cytoskeleton is not clearly understood, and data of experiments with gravitropic mutants (Kumar et al., 2008; Nakamura et al., 2011), actomyosin system disrupting drags (Hou et al., 2003; Palmieri et al., 2007) as well as visualization of cytoskeleton elements and their contacts with statoliths (Collings et al., 2001; Palmieri et al., 2007; Zhang et al., 2011) are often contradictory. Comparison of amyloplast positioning and movement in root and shoot statocytes of the same seedling in response to alterations of the magnitude and action direction of gravitational force could help to clarify the role of the cytoskeleton in the early phases of graviperception in roots and above-ground organs. Quantitative studies on statolith location and motion in response to precisely controlled stimulation procedures could help to reveal the rheological properties of cytoplasm in different statocyte regions as well as the direction and magnitude of elastic forces exerted by the cytoskeleton. Furthermore, both above mentioned attempts could provide opportunities to ascertain the similarities and differences of gravity sensing in positively and negatively gravitropic organs of the same plant.

The aim of the research was to study the dependence between the location and movement of amyloplasts (statoliths) in gravisensing cells (statocytes) of axial organs of

garden cress seedlings and the alterations of gravitational force, to compare the peculiarities (features) of gravity sensing in positively and negatively gravitropic organ of the same seedling.

Main tasks:

- to analyse and compare the formation of gravisensing cells in roots and hypocotyls of seedlings under real and simulated microgravity conditions;
- to evaluate the dependence of amyloplast location along statocytes of roots and hypocotyls on the magnitude of permanently acting gravitational force;
- to ascertain the dependence of amyloplast movement in statocytes of roots and hypocotyls on the short-term alterations of gravitational force acting the seedlings in longitudinal direction;
- to determine the peculiarities of amyloplast sedimentation in root and hypocotyl statocytes during the stimulation of seedlings by the natural gravitational force in transverse direction.

Scientific novelty of the research:

The induction of amyloplast movement by varying the magnitude of gravitational signals modelled by an original device – a centrifuge-clinostat 'Neris-7' with special equipment for the fixation of the seedlings under the same gravitational conditions was applied for the first time in research of plant gravity sensing processes on the Earth.

The effects of permanent and short-term gravitropic stimulation on the location and movements of amyloplasts were compared for the first time in statocytes of hypocotyls and roots of the same seedling.

The original method for the formation of gravitropic signals (through changes in the magnitude and action direction of gravitational force) to provoke the movements of amyloplasts, allowed us to evaluate the significance of the other intracellular structures for the location and mobility of plastids in the cells of positively and negatively gravitropic organs.

The analysis of seedlings, grown in space centrifuge 'Neris-5' during the flight of unmanned biosatellite 'Bion-10', allowed to evaluate for the first time the significance of gravity on the structure and formation of gravisensing tissue in the axial organs of the same seedling under real microgravity conditions.

In this study, the dependence of amyloplast location on the magnitude of gravitational force was determined for the first time in endodermal cells of positively gravitropic organs – hypocotyls.

The new data on the gravity-sensing mechanisms in the oppositively gravitropic axial organs of plants were obtained.

The statements for defence:

- Real and simulated microgravity affects the growth and structure of gravisensing cells in roots and hypocotyls differently.

- Location of statoliths in statocytes of roots and hypocotyls is not random and depends on the magnitude of gravitational force during permanent gravitropic stimulation in root tip direction.

- Simulated microgravity and 180° inversion provoke immediate lifting of statoliths in gravisensing cells of roots and hypocotyls of 1-*g* seedlings. The 1-*g* gravity acting longitudinaly the seedlings after growth in simulated microgravity provokes the immediate sedimentation of statoliths from their initial location towards its action direction; however, the sedimentation proceeds more actively in hypocotyl than in root statocytes.

- Gravitropic stimulation of 1-*g* seedlings in transverse direction causes the sedimentation and simultaneous longwise sliding of amyloplasts in gravisensing cells of both organs. The combined trajectories and velocities of statolith movements in root and hypocotyl statocytes differ.

Approbation of the results. The main research data were presented in nine publications, of these, three – in the journals of ISI WOS database, three – in the journals included in the ISI database. The main results were presented and approved at seven international conferences organized by COSPAR/Committee on Space Research (2004); ESA/European Space Agency and ELGRA/European Low Gravity Research Association (2005, 2008, 2010, 2011) and at two international conferences in Lithuania.

Structure of the dissertation. The dissertation consists of the following chapters: Introduction, Literature review, Research object, materials and methods, Research results, Discussion, Conclusions, References (250 sources), List of publications. The dissertation is illustrated with 7 tables, 51 figures. Volume of the dissertation is 146 pages. The text of dissertation is written in Lithuanian with the abstract in English.

RESEARCH OBJECT, MATERIALS AND METHODS

Object of research. Ivestigations were carried out with seedlings of garden cress (*Lepidium sativum* L.).

Equipment for producing of altered gravitational conditions

Experiments with garden cress seedlings were carried out in unmanned space biosatellite 'Bion-10' and on the ground using original devices for modelling of altered gravity and gravitropic stimulation conditions.

Space centrifuge 'Neris 5' – an automatically operating device was used for the study of the effects of real microgravity on the germination and growth of seedlings during the space flight and ground control conditions. It consists of two parts: the stationary part for the growth in real weightlessness (microgravity) and rotating one (centrifuge) – for generating the centripetal force of 1 *g*, which acts permanently. Sixteen cylindrical containers, which consist of three hermetic volumes (water reservoir – 0.3 ml, seed

germination chamber – 4.5 ml and reservoir for the fixation of solution – 2.2 ml), were used for the growth and fixation of seedlings. All experimental procedures were performed accordingly to in advance programmed scheme.
Centrifuge-clinostat 'Neris-7' was used for producing of altered gravity (from simulated microgravity to 1 *g*) on the ground and the fixation of the seedlings during the growth under the same gravitational conditions. It is designed as a device with two orthogonal axes allowing independent or simultaneous rotation around four horizontal (clinostat) and one vertical (centrifuge) axes. The rotation rate of the clinostat and the speed of centrifugation can be adjusted and controlled by software commands. The rotation stability of the low-vibration electric drives was no less than 2 %. To sustain an appropriate orientation of growing seedlings during experimental manipulations, four closed biocontainers with a special inside equipment (glass funnels for seed planting; fluoroplastic holders for glass funnels; cylindrical holders for centring of foregoing equipment) were used.

Protocols of experiments and gravitropic stimulation of seedlings
Space experiment and ground control ones were performed using space centrifuge 'Neris 5' for the study of real microgravity effects on the formation of gravisensing tissues in axial organs of seedlings. Both experiments were carried out in the darkness at 22 ± 1 °C. Before the start of experiments, 16 cultivation containers were prepared through filling the reservoirs with water and fixative (4 % (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Three seeds were mounted in appropriate orientation on circle-shaped filter paper for each container, where growing seedlings could orient their axial organs freely. The experiments started automatically by water spraying into germination chambers both in space and on the ground. After 28 h of growth, the fixative was spread out on the seedlings what allowed to keep them until the launch of the biosatellite and delivering in to the laboratory.
Experiments on the centrifuge-clinostat were performed for the study of gravisensing system functioning in axial organs of seedlings under altered gravity conditions on the ground. For the elimination of unidirectional action of natural gravitational force, horizontal axes of centrifuge-clinostat rotated at 50 rpm (simulated microgravity conditions or clinorotation). Two sets of experiments were performed. The first set was devoted to reveal the effects of permanent stimulation by differently reduced gravitational force, the other – to study the kinetics of amyloplasts in gravisensing cells of seedlings during different short-term gravitropic stimulation procedures. Both experiment sets were carried out in the darkness, at 23 ± 1 °C
Permanent stimulation by reduced gravity from 1 g up to simulated microgravity. The seeds germinated and grew on the centrifuge-clinostat working in clinorotation and simultaneous centrifugation regime. Centrifugation by the speed of 0, 3.63, 5.31, 7.72, 17.61, 23.72 or 47.85 rpm provided the simulation of gravitational environment of simulated microgravity, 0.004, 0.008, 0.02, 0.1, 0.5 or 1 *g*, respectively. After 32 h of growth under appropriate conditions, the seedlings were chemically fixed without spinning of the centrifuge-clinostat.

Short-term gravitropic stimulation was applied to the seedlings, which grew 32 h vertically under the action of natural gravity or in simulated microgravity on the centrifuge-clinostat. *Stimulation along the longitudinal axis of seedlings.* For the

evaluation of amyloplast movement properties along statocytes, 1-*g* seedlings were exposed to simulated microgravity or after a 180° inversion for 1, 2, 4, 6, 12 and 24 min. In order to compare the features of statolith motion in the different regions of root and hypocotyl statocytes, the seedlings grown in simulated microgravity conditions were stimulated by the gravitational force in root-tip direction or *vice versa* for analogous periods.

Stimulation across the longitudinal axis of seedlings. After 30 h of vertical growth under natural 1-*g* gravity, the seedlings were reoriented right-wards at 90° for 1, 2, 4 and 6 min. The seeds were germinated in glass funnels to retain and mark their respective orientation during subsequent manipulations of stimulation, fixation and cutting of samples.

Preparation of histological sections of roots and hypocotyls

After stimulation procedures, the seedlings were fixed for 45 min in 4 % (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), preserving the same gravitational conditions as in the period of stimulation. Thereafter they were transferred into a fresh portion of glutaraldehyde solution for 12 h at 4 °C. Cut root apices and two hypocotyls pieces after wash procedures in sodium phosphate buffer were transferred into a fresh portion of glutaraldehyde solution for 12 h at 4 °C and post-fixed in 1 % (w/v) OsO_4. After dehydration, the samples were embedded in Epon by standard procedures (Luft, 1961). The semi-thin (1 µm) longitudinal sections of root apices and hypocotyls were prepared using III8800LKB (Sweden) ultramicrotome and stained with Toluidine-blue [1 % (w/v) in 0.1 % (w/v) $Na_2B_4O_7$].

Analysis of gravisensing tissues and amyloplast location

Morphometrical analysis of gravisensing cells was performed employing light microscopy on median longitudinal sections of hypocotyls and root apices. The sections were photographed with a PENTAX*ist D and MOTIC (Japan) digital cameras attached to an SMP 03 MICROSCOPE PHOTOMETER (Opton, Germany). The images were analysed using the SigmaScanPro 5 (Jandel Scientific Software) and MOTIC programmes. As a rule, 2–3 sections were analysed for 3–4 roots and hypocotyls of each test variant.

For the study of gravisensing tissues formation, dimensions of cells of the 3rd to 5th columella storeys in root caps, and dimensions of endodermal cells along their profiles in hypocotyls were determined.

Evaluation of statolith location during longwise gravitropic stimulation. The longwise location of amyloplasts was evaluated by measuring the distance of each plastid centre from the morphological cell bottom (distal statocyte wall in root or basal wall of endodermal cell in hypocotyl) and expressed in percent of the length of tested statocyte.

Evaluation of statolith location during transverse gravitropic stimulation. The location of each amyloplast was characterized by the horizontal (*x-position*) and vertical (*y-position*) coordinates in the statocyte as two-coordinate system. The *x-position* represents the distance of the plastid centre from the morphological cell bottom, which is expressed in percent of the tested cell length. The *y-position* represents the distance of the plastid centre from the right/lower longitudinal statocyte wall in organs of horizontally reoriented seedlings in percentof the tested cell width.

Statistical analysis. Data were analysed using the MS EXCEL 7 standart package (Microsoft Corporation). The values are represented as a mean ± standard error (SE). Statistical significance was determined using Student's test.

RESEARCH RESULTS AND DISCUSSION

Impact of different gravitational loads on the formation of gravisensing tissues in garden cress seedlings and intracellular location of amyloplasts

Effects of real microgravity were evaluated by comparing the cytomorphological indices of gravisensing tissues in hypocotyls and roots of seedlings grown during spaceflight in microgravity and under the action of simulated 1 *g* (space centrifuge) and/or natural 1 *g* gravity (ground control). It was determined that the length of statocytes in different cell layers of columella did not depend significantly on the action of gravitational force (Fig. 1). The effect of real microgravity conditions on the radial growth of root statocytes was negligible, too. The mean width of functioning statocytes of six columella storeys was similar under real microgravity and natural gravity conditions (15.2 ± 0.3 μm and 14.8 ± 0.2 μm, respectively), but smaller than in space centrifuge (16.1 ± 0.2 μm).

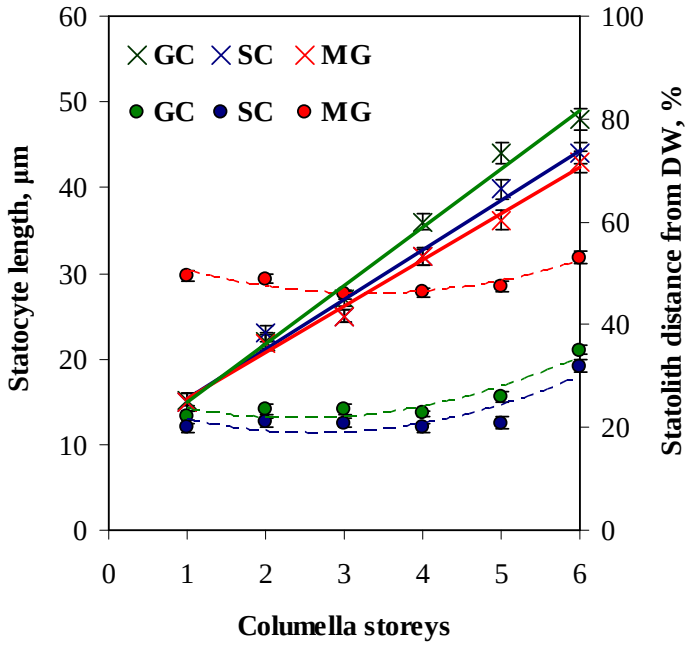

Fig. 1.The length (**×**) of statocytes and the distance (**o**) of statoliths from the distal statocyte wall (DW) in roots of garden cress seedlings**.** GC – ground control, SC – space centrifuge and MG – real microgravity

For the analysis of gravisensing tissue in hypocotyls, they were divided into apical and basal sections. Statistically significant dependence of endodermal cell size on the action of gravity as well as on the cell presence in the apical or the basal section of hypocotyls was determined (Table 1). Comparison of both space and ground control variants revealed the essential difference between the properties of endodermal cells growth. Under the action of the Earth's gravity, they were more than 3.5-fold longer and 2-fold wider in basal than in apical section of hypocotyls. The similar change in endodermal cell measurements along hypocotyls was determined under 1 *g* in space centrifuge, though the cells in the basal sections were smaller. Essential alterations were revealed in the linear growth of endodermal cells in real microgravity. As compared with the measurements of space centrifuge and ground control cells, they were about 1.5-fold longer in the apical sections and 2-fold shorter in basal sections of hypocotyls (Table 1).

Table 1. Effect of real microgravity on the size of endodermal cells in hypocotyls of garden cress seedlings

Growth conditions	Hypocotyl section					
	apical			basal		
	Cell			Cell		
	N	length, μm	width, μm	N	length, μm	width, μm
Ground control	110	24.3 ± 0.6	16.8 ± 0.4	47	86.4 ± 7.0*	34.3 ± 1.9*
Space centrifuge	80	26.1 ± 0.8^	19.4 ± 0.5^	100	79.9 ± 1.7*^	28.3 ± 1.5*
Real microgravity	53	40.1 ± 3.3	22.7 ± 1.2	79	46.6 ± 3.4	27.9 ± 1.9

* – difference between apical and basal hypocotyl sections statistically significant at $p \leq 0.01$; ^ – difference between space centrifuge and real microgravity variants is significant at $p \leq 0.01$; N – number of tested cells

Microgravity affected considerably the location of statoliths along statocytes of both axial organs. In 1-*g* seedlings of space and ground experiments, amyloplasts concentrated near the morphological cell bottom, whereas in microgravity grown seedlings they grouped in the central part of root and hypocotyl statocytes (Fig. 1 and 2).

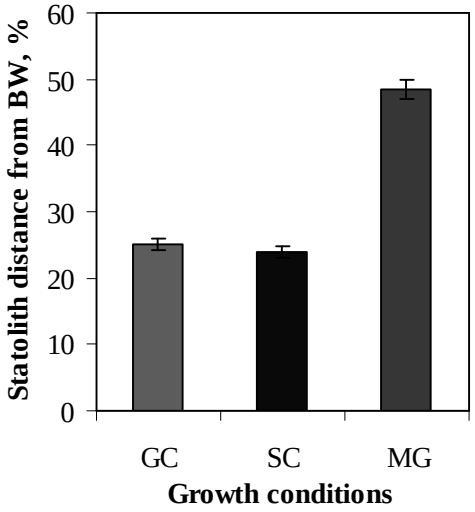

Fig. 2. The distance of statoliths from the basal cell wall (BW) in statocytes of hypocotyls of garden cress seedlings. GC – ground control, SC – space centrifuge and MG – microgravity

Effects of simulated microgravity were evaluated by comparing the cytomorphological indices of gravisensing tissues in hypocotyls and roots of seedlings after growth on the centrifuge-clinostat working in the regime of clinorotation (HC) and in vertical orientation under the action of natural 1 *g* gravity. It was found that the linear growth of root gravisensing cells did not depend significantly on the gravitational force. Under 1 *g* and microgravity conditions, the linear relationship between the mean length of cells and their position in statenchima was determined (Fig. 3).

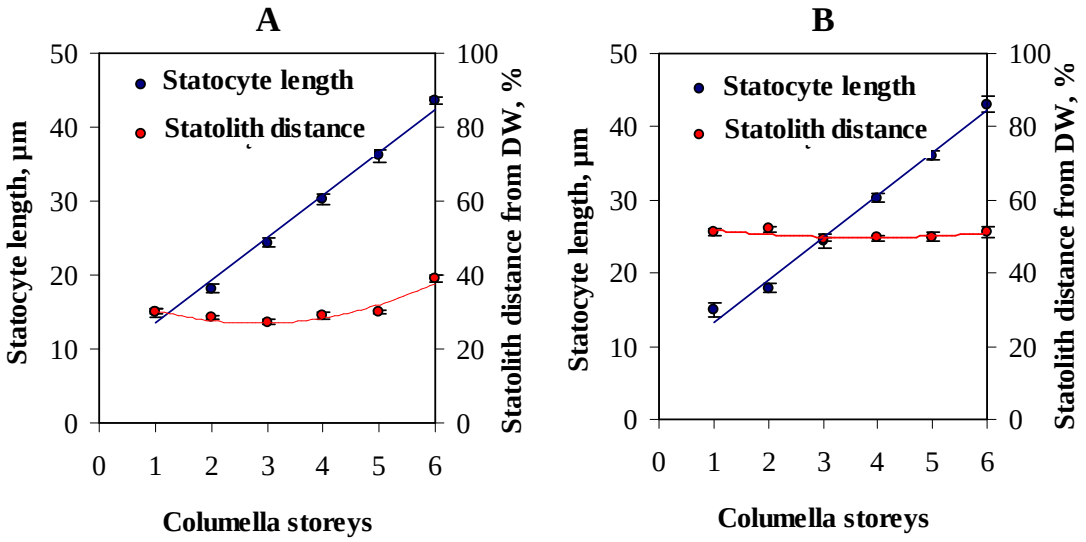

Fig. 3. The length • of statocytes and distance • of statoliths from the distal cell wall (DW) in roots of garden cress seedlings grown under 1 *g* (A) and simulated microgravity (B) conditions

The effect of simulated microgravity on the radial growth of gravisensing cells in roots was not significant, too.

Endodermal cells in hypocotyls of clinorotated seedlings grew more slowly, because they survived for a longer period of time in meristem zone, whereas under 1-*g* conditions they shifted faster to the elongation and differentiation phases (Fig. 4). As in the case of longitudinal growth, similar changes in radial growth were identified. The endodermal cells enlarged rapidly under 1 *g* conditions, but their growth in width was slower in simulated microgravity.

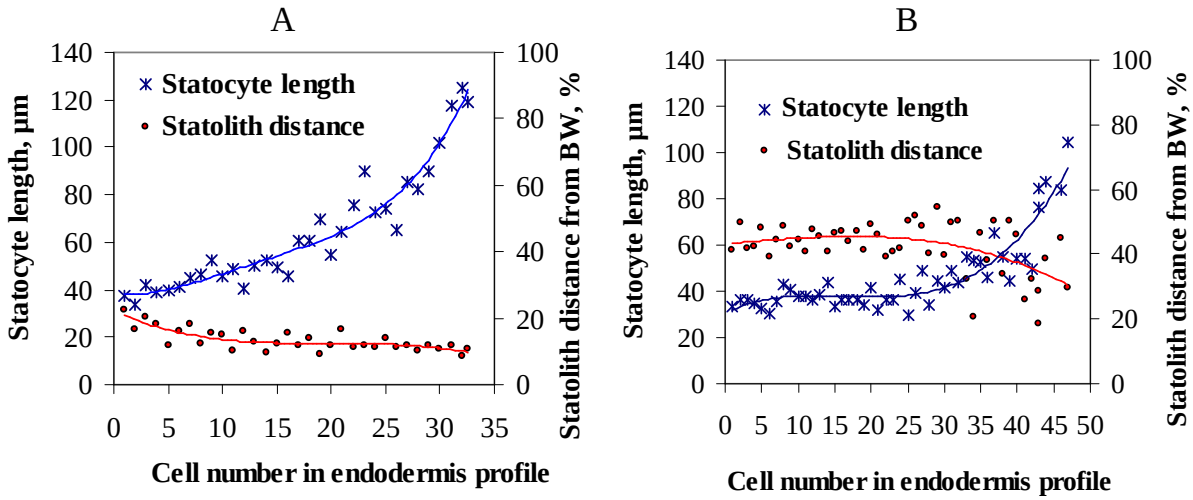

Fig. 4. The length * of statocytes and the distance • of statoliths from the basal cell wall (BW) in hypocotyls of garden cress seedlings under 1 *g* (A) and simulated microgravity (B) conditions

Simulated microgravity effected the positioning of amyloplasts in gravisensing cells of both axial organs. In 1-*g* root and hypocotyl statocytes, the statoliths were

concentrated near the morphological cell bottom (Fig. 3A, 4A). In the horizontal clinostat, the amyloplasts in root statocytes were concentrated around the cell centre (Fig. 3B), whereas in hypocotyl statocytes the statoliths were grouped in the central part of the cell or scattered throughout the cell periphery (Fig. 4B).

Gravitational loads had no effect on statolith quantity in root and hypocotyl statocytes (1 *g* roots – 4.7 ± 0.6, HC – 4.6 ± 0.9; 1 *g* hypocotyls – 5.9 ± 0.4, HC – 5.4 ± 0.2 per statocyte).

Positioning of amyloplasts in gravisensing cells of garden cress seedlings under differently reduced gravity

Under permanent gravitropic stimulation, the seedlings grew in simulated microgravity (horizontal clinostat) or were simultaneously exposed to 0.004, 0.008, 0.02, 0.1, 0.5 and 1 *g* mass acceleration in the root-tip direction. Cytomorphological analysis of gravisensing cells revealed the dependence of amyloplast intracellular location on the magnitude of mass acceleration (Fig. 5, 6).

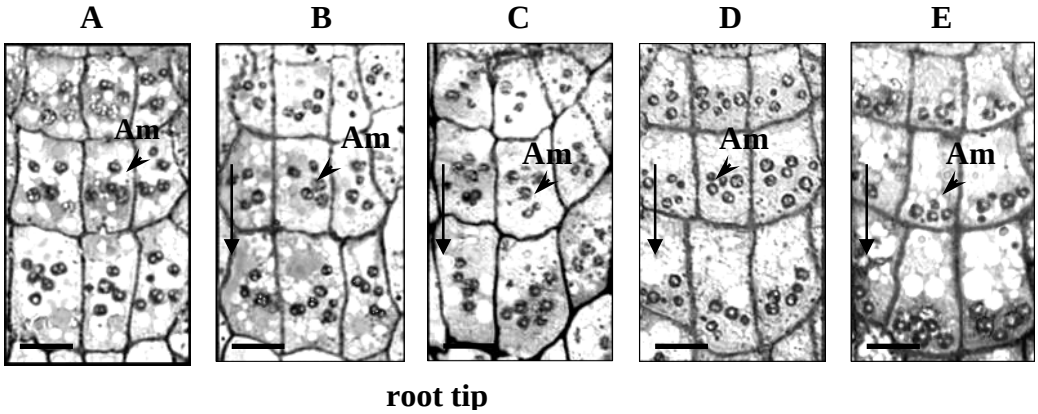

Fig. 5. Light micrographs of root statocytes after growth in simulated microgravity (A) and under the action of 0.004 *g* (B), 0.02 *g* (C), 0.1 *g* (D) and 1 *g* (E) mass acceleration. Am – amyloplasts. Arrowheads indicate the direction of gravity. Bars – 10µm

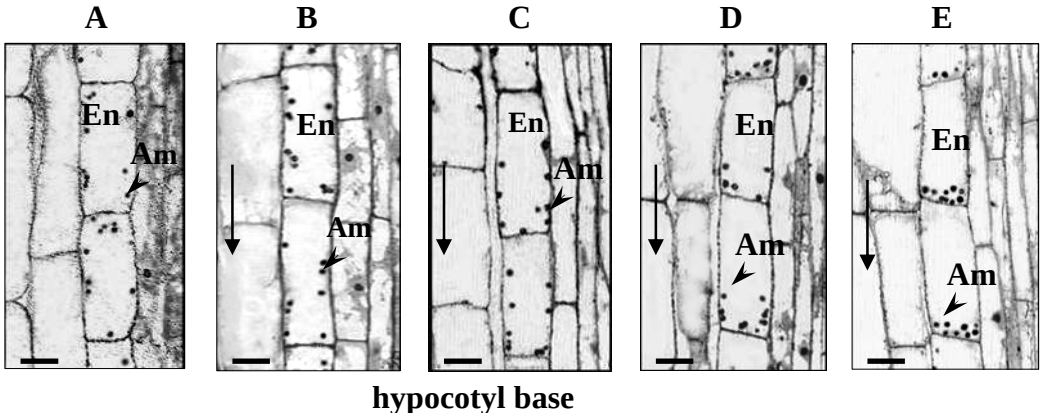

Fig. 6. Light micrographs of hypocotyl endodermal cells after growth in simulated microgravity (HC, A) and under the action of 0.008 *g* (B), 0.02 *g* (C), 0.1 *g* (D) and 1 *g* mass acceleration (E). Am – amyloplasts, En – endodermis. Arrowheads indicate the direction of gravity. Bars –10µm

It was determined that the mean location of amyloplasts in root statocytes under 0.004 *g* and in endodermal cells of hypocotyls under 0.008 *g* gravitational loads was comparable to that which was under simulated microgravity (Fig. 7). Under the permanent action of 0.02 *g* mass acceleration, most statoliths shifted significantly downwards from the initial position in the statocytes of both organs.

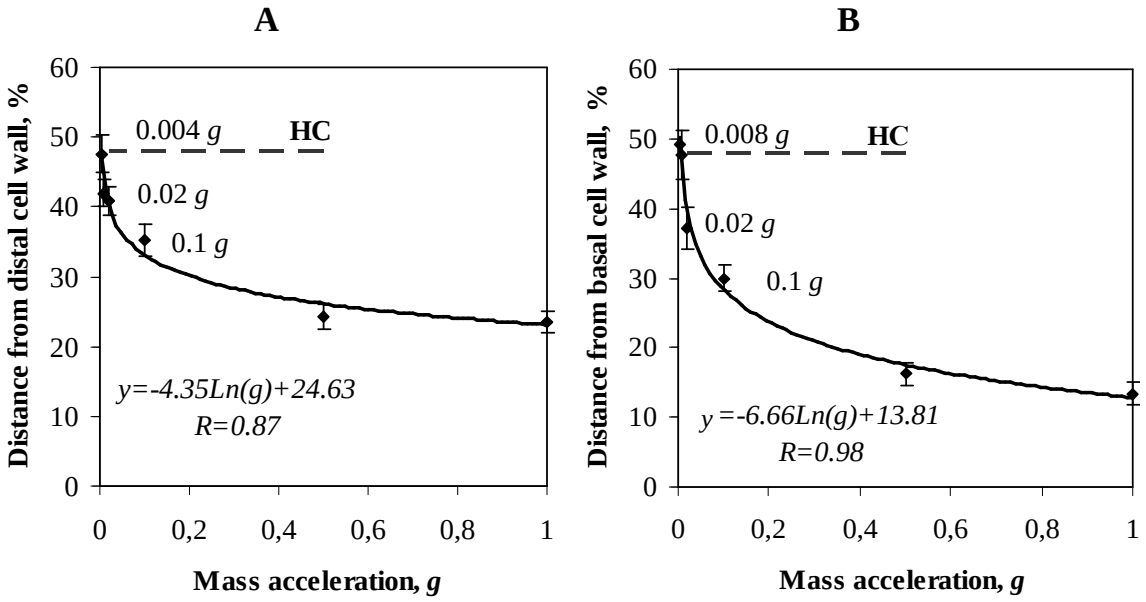

Fig. 7. Dependence of statolith location in gravisensing cells of roots (A) and hypocotyls (B) on the magnitude of mass acceleration. DW – distal cell wall, BW – basal cell wall, HC – simulated microgravity

A statistically significant lifting of amyloplasts from the morphological cell bottom was determined after the decrease of mass acceleration from 1 *g* to 0.1 *g* in root statocytes and after the decrease to 0.5 *g* – in hypocotyl endodermal cells

Movement of amyloplasts in gravisensing cells during short-term gravitropic stimulation along longitudinal axis of garden cress seedlings

Kinetics of amyloplasts in statocytes of 1-*g* seedlings during the exposition to simulated microgravity and after reorientation 180° (inversion). The statolith location was tested after 1, 2, 4, 6, 12 and 24 min. Before gravistimulation, the amyloplasts grouped in the distal part of statocytes of the third to fifth columella storeys (Fig. 8A) and in the basal part of endodermal cells of hypocotyls (Fig. 9A). Thus, the statoliths located near the morphological bottom of both types of gravisensing cells. Following elimination of the unidirectional gravity action or the inversion, the amyloplasts moved towards the cell centre in the statocytes of both organs (Fig. 8, 9).

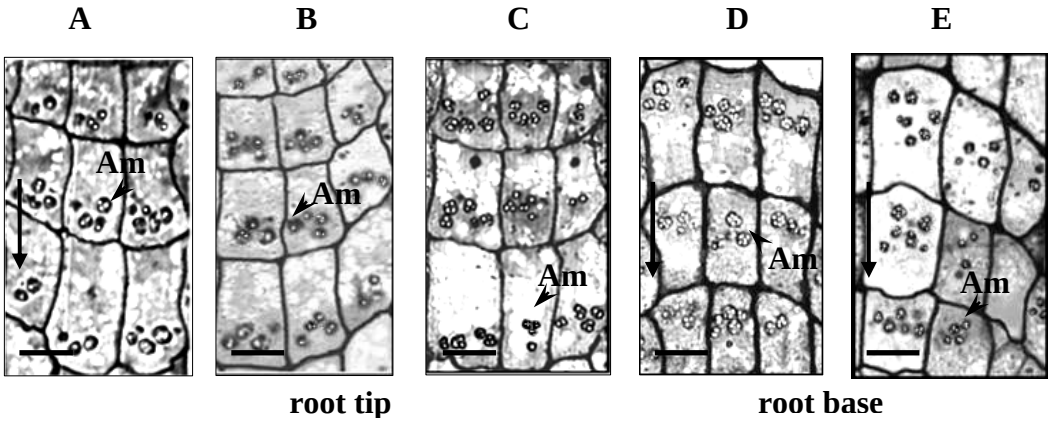

Fig. 8. Light micrographs of root statocytes of 1-*g* seedlings (A) exposed to simulated microgravity for 2 min (B) and 6 min (C) or reoriented 180º for 2 min (D) and 6 min (E). Am – amyloplasts. Arrowheads indicate the direction of gravity. Bars – 10µm

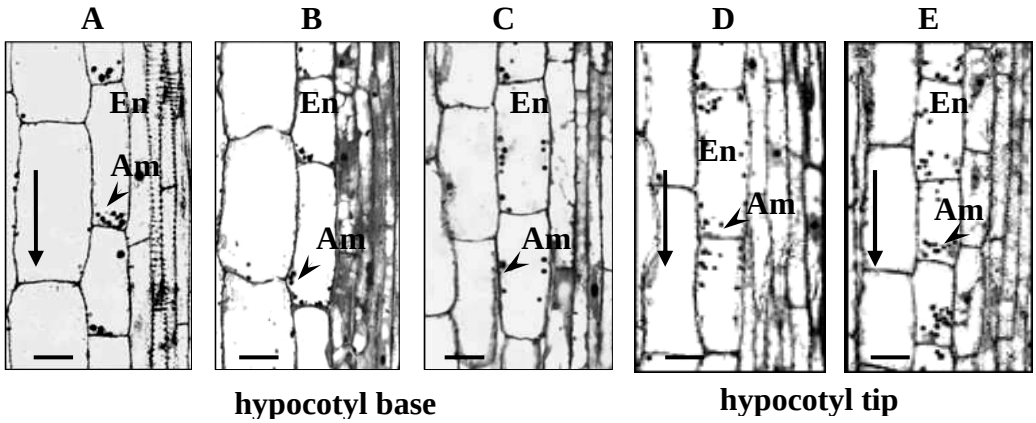

Fig. 9. Light micrographs of hypocotyl endodermal cells of seedlings grown at 1 *g* (A) and then exposed to simulated microgravity for 6 min (B) and 24 min (C) or reoriented 180° for 6 min (D) and 24 min (E). En – endodermis, Am – amyloplasts. Arrowheads indicate the direction of gravity. Bars – 12 µm

However, the displacement and the velocities of plastid motion differed significantly. In root statocytes, a statistically confirmed longwise displacement of amyloplasts was determined as soon as after the first and second minutes of clinorotation and inversion (Fig. 10A).

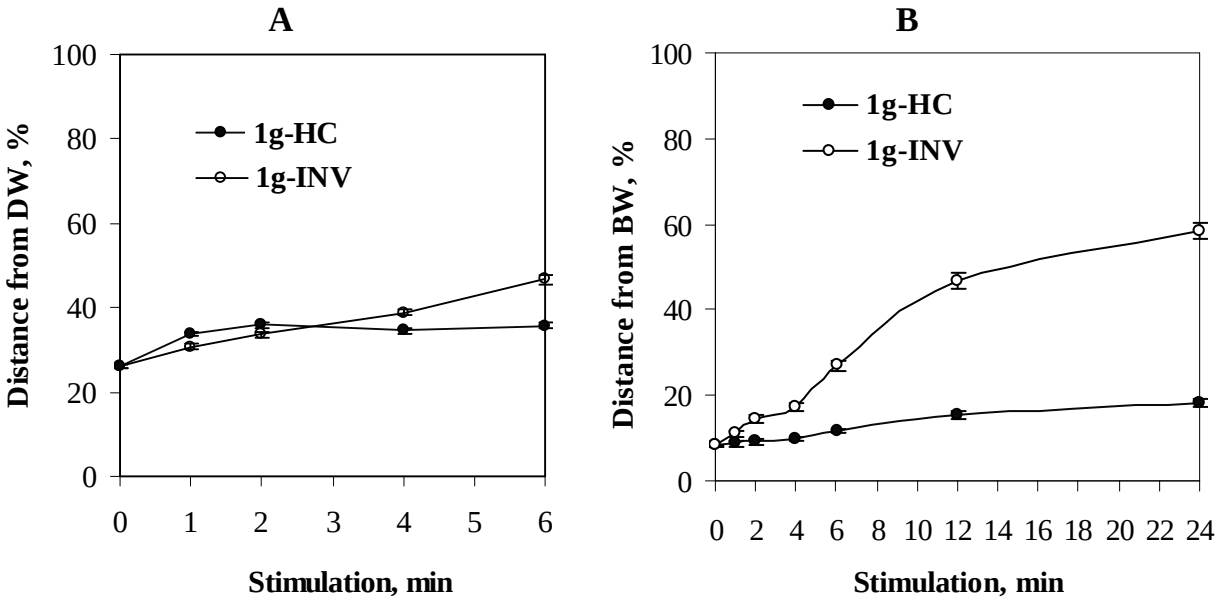

Fig. 10. Movements of amyloplasts along statocytes in roots (A) and hypocotyls (B) of 1-*g* seedlings exposed to simulated microgravity (1 *g*-HC) or inverted (1 *g*-INV). DW – distal cell wall, BW – basal cell wall

Later on, this statolith movement became significantly slower under clinorotation; however, it proceeded at a constant velocity during inversion. In hypocotyl statocytes, a significant shift of amyloplasts in longitudinal direction was found only after the 6-min period of clinorotation and as soon as after the first minute of inversion (Fig. 10B). A comparatively slow sliding of plastids continued until the 24th minute of clinorotation, and the mean distance of plastids from the bottom of hypocotyl statocytes increased up to 18.0 ± 0.9 % of the whole cell length. Under inversion, plastid sedimentation increased even more – 58.6 ± 1.7 % of the total length of endodermal cells.

Gravity-induced statolith sedimentation along statocytes of clinorotated seedlings. In order to compare the features of statolith displacement along the different regions of root and hypocotyl statocytes, seedlings grown in microgravity simulated by clinorotation were stimulated by the gravitational force in root-tip or root-base directions and fixed after 1, 2, 4, 6, 12 and 24 min. Before the stimulation, amyloplasts grouped in the central region of root statocytes (Fig. 11A), but dispersed mostly throughout the entire periphery of endodermal cells (Fig. 12A). The direction of amyloplasts movement along the statocytes of roots and hypocotyls of garden cress seedlings after growth on a fast clinostat depended on the direction of stimulating gravitational force (Fig. 11, 12).

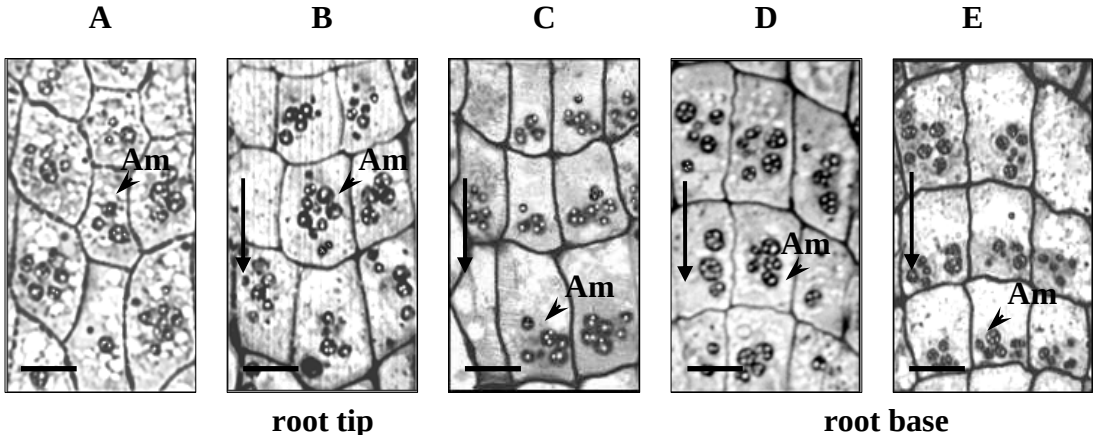

Fig. 11. Light micrographs of root statocytes of garden cress seedlings grown under simulated microgravity (A) and stimulated by gravity in root-tip direction for 1min (B), 6 min (C) or in root-base direction for 1 min (D) and 6 min (E). Am – amyloplasts. Arrowheads indicate the action direction of gravity. Bars – 10 µm

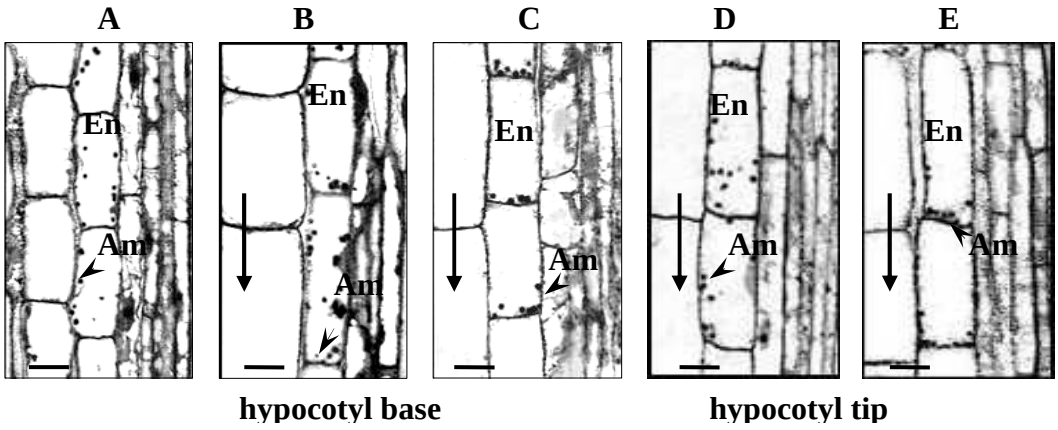

Fig. 12. Light micrographs of hypocotyl statocytes of garden cress seedlings grown under simulated microgravity (A) and stimulated by gravity in root-tip direction for 1min (B) and 24 min (C) or in root-base direction for 1 min (D) and 24 min (E). En – endodermis, Am – amyloplasts. Arrowheads indicate the action direction of gravity. Bars – 12 µm

The maximal longitudinal shift of amyloplasts from their initial location in root and hypocotyl statocytes was determined within the first minute of stimulation both in root-tip and root-base directions. (Fig. 13A, B), but the average velocity of statolith movement was different.

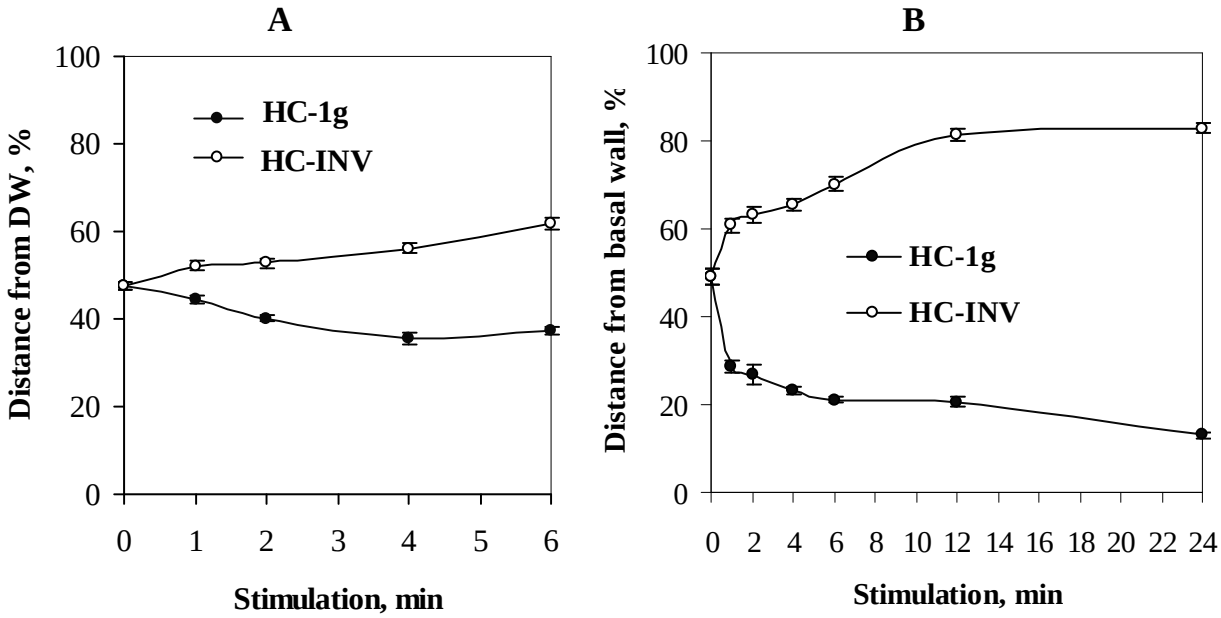

Fig. 13. Displacement of amyloplasts along statocytes in roots (A) and hypocotyls (B) during 1 *g* stimulation in root-tip (HC-1 *g*) and root-base (HC-INV) directions. DW – distal cell wall, BW– basal cell wall

In root statocytes, amyloplasts sedimented intensively in the direction of gravity to a comparable extent even during the first minutes of treatments. However, after the fourth minute, plastid displacement became more active towards the cell top as compared with that towards the cell bottom. In hypocotyl statocytes, during the first minute of stimulation, the longitudinal sliding of amyloplasts was more pronounced downwards under the action of gravity in root-tip direction. Later on, the plastid displacement slowed down in both directions. Finaly, after the 24-min period, the mean distance of amyloplasts from the bottom of hypocotyl statocytes reduced to 13.0 ± 0.6 % of the total cell length under the action of gravity in root-tip direction and increased up to 82.8 ± 1.2 % under its action in root-base direction.

Movement of amyloplasts in gravisensing cells during short-term transverse gravitropic stimulation of garden cress seedlings

Amyloplast motion was studied during a subsequent 6-min period of gravitropic stimulation at 90º by the analysis of plastid positioning in root and hypocotyl statocytes of garden cress seedlings grown vertically at 1 *g*. Before stimulation, the amyloplasts grouped near the morphological bottom of both cell types. The reorientation of seedlings had a considerable effect on the location of amyloplasts in transverse as well as longitudinal direction in statocytes of both organs (Fig. 14B, C and 15B, C).

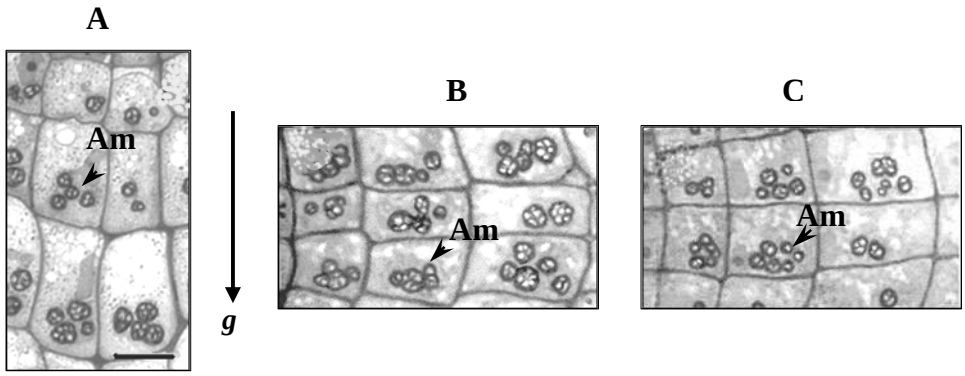

Fig. 14. Micrographs of root statocytes in seedlings grown vertically (A), after 90° reorientation for 2 min (B), and 6 min (C). Am – amyloplasts. Arrowhead indicates the direction of gravity. Bar – 10 µm

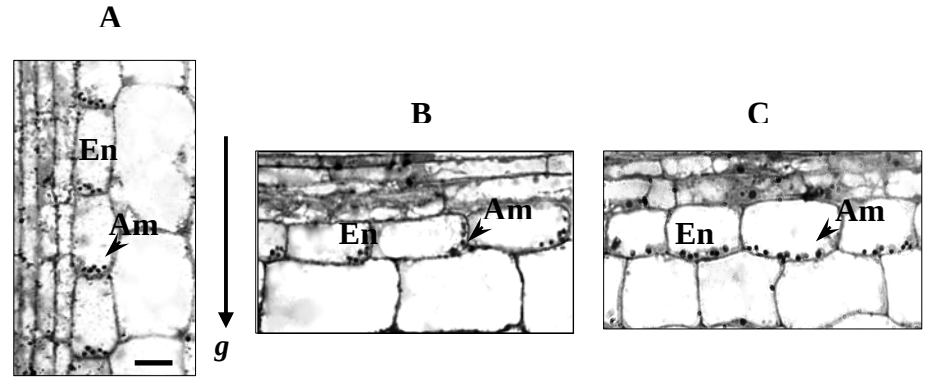

Fig. 15. Micrographs of endodermal cells in hypocotyls of seedlings grown vertically (A) and after 90° reorientation for 1 min (B), 6 min (C). En – endodermis, Am – amyloplasts. Arrowhead indicates the direction of gravity. Bar – 20 µm

The statistical analysis of this motion allowed characterizing the statolith sedimentation kinetics (Fig. 16A, B). A more rapid amyloplasts displacement towards the gravity from the initial *y-position* of 48.5 % to 37.2 % ($p \leq 0.01$) of the total cell width and simultaneous sliding along the cells from initial *x-position* of 15.2 % to 20.4 % ($p \leq 0{,}05$) were determined within the first minute of gravitropic stimulation in hypocotyl endodermal cells (Fig. 16B) as compared with root statocytes, where the *y-position* decreased from 49.7 % to 43.1 % ($p \leq 0.01$), the *x-position* from 27.7 % to 32.3 % (Fig.16A). During the second minute, the amyloplasts location remained almost unchanged in endodermal cells, while they continued to slide intensively along columella cells slightly downwards.

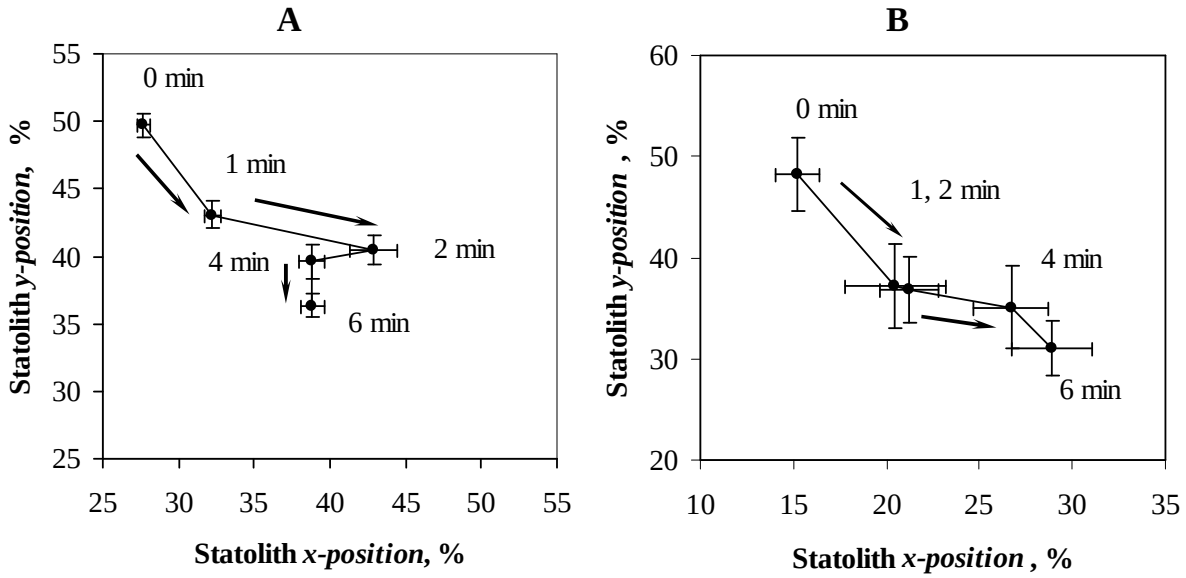

Fig. 16. Movement of amyloplasts in root (A) and hypocotyl (B) statocytes during the 6-min period of gravitropic stimulation at 90º of garden cress seedlings after growth under natural gravity conditions

Later, the movement of statolith changed considerably in the statocytes of both axial organs. In root statocytes, a significant shift of statoliths was observed in the direction of distal cell wall and simultaneously downwards sedimentation within a 2–6 min interval of gravitropic stimulation. In hypocotyls, only after 4 min a marked displacement of amyloplasts was visible in the longitudinal direction of the cell.

The aim of the study was to compare the peculiarities of root and hypocotyl gravisensing of the same seedling under different gravistimulation conditions. Indirect experimental method, i.e. quantitative analysis of the dependence of amyloplast statics and kinetics on the direction and magnitude of gravitational force, was applied to assess the cytoskeleton role in the maintenance of polarized root and hypocotyl statocyte structure and amyloplast movements during seedling gravistimulation. The experiments were performed with garden cress (*Lepidium sativum* L.) seedlings in space biosatellite 'Bion-10' (in-flight centrifuge 'Neris-5') and under laboratory conditions in the dark by changing the magnitude of the gravitational force from microgravity (real in space or simulated by horizontal clinostat) to 1 *g* (simulated by centrifugation in space or natural Earth's gravity) and its action direction at 90° or 180° inversion with respect to the longitudinal axis of the seedlings.

Growth and development of gravisensing tissues ensure the ability of plants to sense and accept gravitational signals. Root cap statenchima and hypocotyl endodermis

are compact tissues, but their sources, growth direction and structural organization of cells differ substantially. Therefore, it was possible to expect different growth reactions of these tissues to alterations of gravitational force from 1 *g* to weightlessness. The 28-hour-old seedlings of garden cress were used in space and 32-hour-old in ground experiments. The effect of real (Fig.1) and simulated (Fig. 3) microgravity on the linear growth of root gravisensing cells was minimal. Thus, it can be stated that the gravitational 1-*g* force is not necessary for the formation of root gravisensing tissues. The obtained data are in compliance with the notion that the gravity is not essential for growth and development of root statenchima (Merkys et al., 1981; Perbal, Driss-Ecole, 1989; Laurinavičius et al., 1996; Sievers, 2000; Kiss, 2000; Laurinavičius, Švegždienė, 2000; Driss-Ecole, 2000; Driss-Ecole et al., 2003).

In hypocotyls, gravisensing-endodermal tissue, surrounding the stele', formed and differentiated into functioning statocytes under both microgravity and 1-*g* conditions, but in different ways. The presence of significantly smaller endodermal cells in apical and larger ones – in basal hypocotyl sections suggest that under simulated or natural 1-*g* gravity, a certain number of these cells were in the phases of division, elongation and differentiation (Table 1). In real microgravity, the growth of hypocotyl endodermal cells was significantly slower than under 1 *g* conditions because of the inhibition of cell division process. A similar dependence of endodermal cell growth on the gravity was observed in 32-hour-old seedlings (Fig. 4). Under natural gravity as compared with the simulated microgravity, the endodermal cells transfer from the division phase to that of elongation significantly faster and much more intensively. Similar positive impact of natural gravity was obtained for radial growth of endodermal cells, too.

The obtained data on the formation of gravisensing tissues imply that altered gravity may affect the growth and functioning of plants at different structural levels. The gravisensing is not an exception, either. The roots of lentil and garden cress seedlings after growth in microgravity are known to be significantly more sensitive and react faster than the roots of seedlings which grow under simulated and natural gravity of 1 *g* (Driss-Ecole et al., 2000, 2008; Laurinavičius et al., 2001). Laurinavičius and co-authors assumed that intracellular distribution of amyloplasts could be the reason for the change in the sensitivity of roots to gravitropic stimulation.

Under the action of simulated or natural gravity, gravisensors – amyloplasts in root and hypocotyl gravisensing cells of cress seedlings were grouped close to the morphological cell "bottom" (Fig. 1, 2, 3A, 4A). Therefore, the longitudinal polarization of cell structure is obvious. Thus, the location of amyloplasts in both negatively and positively gravitropic organs is largely impacted by 1-*g*-magnitude gravitational force, which balance the intracellular forces (buoyant, drag, the elastic forces generated by cytoskeleton elements, etc.) as a whole (Björkman, 1988; Todd, 1994; Yoder et al., 2001). Under real or simulated microgravity, amyloplasts in root statocytes were grouped in the central part of the cells, whereas in hypocotyl endodermal cells they were scattered throughout the cytoplasm. However, their mean distance from the morphological cell bottom in relative units was about 48 % of the cell length (Fig. 1, 2, 3B and 4B) both in root and hypocotyl statocytes. Thus, it can be assumed that when cress seedlings grow without gravity, amyloplasts location, although different, depends upon the activity of the above-mentioned intracellular force.

In recent years, extensive analyses have been carried out on actin filaments and motor protein – myosin structural state, their possible contacts with amyloplasts (Palmieri et al., 2007; Kiss, 2009; Morita, 2010). However, it is not known whether the significance of these cytoskeleton elements on the amyloplast location in root and hypocotyl statocytes is the same under reduced gravity conditions. Our data demonstrated, although indirectly, the effect of cytoskeleton network on the longitudinal distribution of statoliths in root statocytes and hypocotyl endodermal cells.

The studies on relationship between intracellular distribution of statoliths upon gravitational force of 0.004, 0.008, 0.02, 0.01, 0.5 or 1 *g*, continuously acting the seedlings in the normal direction, revealed that this dependence is satisfactorily described by the logarithmic function both in the root and hypocotyl statocytes (Fig. 7). As mentioned above, 1-*g* gravitational force keeps the statoliths at the morphological statocyte bottom in both axial organs of cress seedlings (Fig. 5E and 6E). What magnitude of gravitational force will keep positioning of amyloplasts, i.e. will not compensate the opposite-direction intracellular forces? According to our data, statistically significant lifting of plastids from the morphological bottom of cells in root statocytes proceeded under the action of ten times less than 1 *g* force (Fig. 5D, 7A). A similar shift of amyloplasts in hypocotyl endodermal cells proceeded under the action of

half less than 1-*g* force, i.e. 0.5 *g* (Fig. 7B). These facts suggest that in the statocytes of oppositively gravitropic organs the magnitude of intracellular forces, affecting the location of amyloplasts, is different. In root statocytes, the statoliths are transported up by the force, the magnitude of which is reduced from 1 *g* to 0.1 *g*, in hypocotyl endodermal cells it is up to five times stronger, i.e. reduced from 1 *g* to 0.5 *g*.

There is no doubt that discussed-above location of statoliths depends not only on the gravitational loads, but also on the structural organization of gravisensing cells. Most part of endodermal cells in hypocotyls or footstalks is ocupped by central vacuoles (Volkmann et al., 1993; Kiss, 2000; Kato et al., 2002; Morita, Tasaka, 2004), where active cytoplasmic streaming associated with the activity of actin-myosin system proceeds (Groling, Pierson, 2000). If in the functioning root statocytes many small vacuoles are distributed throughout the cytoplasm (Fig. 5), most of the hypocotyl endodermal cells are occupied by central vacuoles, which push the cytoplasm and the organelles, including the amyloplasts as well as the elements of cytoskeleton, to the cell periphery or to transvacuolar strands penetrating vacuoles (Fig. 6). For this reason, the interaction of statoliths and cytoskeleton elements in statocytes of roots and aboveground organs can also differ under directional changes of gravity.

The above-discussed data on the dependence of amyloplast statics upon the magnitude of gravitational force confirm the involvement of intracellular forces, particularly elastic forces generated by cytoskeleton, in the maintenance of amyloplast location along the statocytes in both positively and negatively gravitropic organs of cress seedlings. Do these forces effect amyloplast movement in the statocytes of both organs during early phases of gravity sensing and, if they do, how? Are the conditions for statolith movements in different areas of statocytes of both organs similar? Responses to these questions were sought by comparing the kinetics of statolith in statocytes of seedlings, which grew naturally under gravity or without it, after change of gravitational force direction at 180° or 90° inversion, and its magnitude – from simulated microgravity to 1 *g* and vice versa. The first technique of gravity stimulation made it possible to assess the peculiarities of longitudinal movements, the second – transverse movements of amyloplast in the statocytes.

When 1-*g* seedlings were transferred in simulated microgravity or 180° inverted, the statoliths lifted up from their initial location both in hypocotyls and in roots (Fig. 8

and 9). It is shown that in root statocytes this lifting of statoliths is provoked by the action of intracellular, cytoskeleton generated forces if reducing the magnitude of gravity from 1 *g* to microgravity in the space or on the Earth (Volkmann et al., 1991; Laurinavičius et al., 1997, 2001; Driss-Ecole et al., 2000; Gaina et al., 2003; Perbal et al., 2004). Our data allow applying the above-mentioned hypothesis to endodermal cells of 1-*g* hypocotyls as well, because the direction and speed of statolith movement, like in root statocytes, depended on the changes in gravitational force (Fig. 9 and 8, respectively).

On the other hand, a few key differences between statoliths movements along root and hypocotyl statocytes are also observed. In roots, after above-mentioned stimulations, the amyloplasts moved away from the distal cell region to the comparable location within 2 min period (Fig. 10A). It can be expected that the displacement of amyloplasts would be more intense at 180° inversion than clinorotation, because in the former case to the proximally directed intracellular forces the gravity force is added. On the other hand, the velocity of statolith movements along statocytes depends not only on the magnitude of acting force, but also on the rheological properties of the cytoplasm, which can change near the moving statolith within a short period of time (Björkman, 1988; Leitz et al., 2008). Therefore, it can be assumed that the increase of total intracellular forces acting proximally had a limited impact on the statolith movement within 1–2 min of inversion.

In endodermal cells of 1-*g* seedling hypocotyls within the first two minutes after inversion the amyloplasts lifted up from cell bottom significantly faster than after the transfer into simulated microgravity (Fig.10B). Obviously, in the statocytes of hypocotyls, gravitational force affected the movement of statoliths much stronger than intracellular forces and contrary to the root statocytes, enhanced their impact. It may be supposed that the rheological properties of cytoplasm in morphologically "lower" regions of root and hypocotyl statocytes vary. Thus, the effect of gravitational force on amyloplast movement is not similar. On the other hand, differences in the structural organization of these cells are obvious. In root statocytes, small vacuoles are distributed throughout the whole volume of cells (Fig. 8) and the statoliths can move along the cells evenly. In hypocotyl statocytes, the amyloplasts, regardless of the gravity situation, were distributed and moved mostly through the periphery and trasvacuolar strands of cells

(Fig. 9). It is, therefore, likely that the central vacuoles dislocate amyloplast movement in hypocotyl statocytes driven by intracellular forces.

According to the data obtained by the other authors (Merkys et al., 1981; Laurinavičius et al., 1996, 2001; Driss-Ecole et al., 2000, 2003) and our results (Fig. 11A), the amyloplasts in statocytes of cress roots grown both in real or simulated microgravity are grouped in the central cell region. In hypocotyl statocytes under these conditions, the amyloplasts, differently from root statocytes, are scattered throughout the cell and most of them, supposedly due to pressure of the central vacuole, are concentrated on the periphery of the cell (Fig. 12A). Under longitudinal stimulation of such seedlings by the 1 *g* gravity, the statoliths showed remarkable shifts in its action direction within the first minute in the statocytes of both organs (Fig.11B, D, 12B, D, 13A, B). Thus, plastids can move easily from their initial location towards any of the cell poles and, possibly, of actin filaments. This finding suggests the cytoplasm of the central statocyte parts in both organs to be almost homogenous. Later on, in hypocotyl endodermis, the sedimentation of statoliths slowed down towards the cell bottom, but proceeded at the comparable velocity until the 12^{th} minute towards the cell top. In root statocytes, similar change in plastid sedimentation proceeded later, i. e. from the 4^{th} minute. Consequently, the conditions for the movement of statoliths along statocytes are different closer to the opposite cell poles in roots and hypocotyls. In our opinion, the statolith sedimentation slowed down in both organs of gravisensing cells under the action of gravitational force in root-tip direction due to the opposite-directed intracellular forces, i.e. elastic forces generated by the cytoskeleton, which transport plastids towards the cell centre, and the drag effect provoked by the interaction of moving amyloplasts with the surrounding cytoplasm.

Considering that the length of statocytes in roots was approximately fivefold smaller than in hypocotyls, we suppose that the elastic forces of the cytoskeleton affect the amyloplast motion induced by gravity in the statocytes of hypocotyls in a similar manner.

In order to detail the above-discussed properties of gravity sensing, the motion of statoliths in root and hypocotyl statocytes during transverse gravistimulation of seedlings was studied. There is no doubt that gravity is responsible for amyloplast sedimentation. However, numerous studies on root gravisensing (Iversen, Larsen, 1971; Volkmann et

al., 1991; Laurinavičius et al., 2001; Driss-Ecole et al., 2003; Gaina, 2003; Perbal, 2004; Švegždienė et al., 2005; Leitz et al., 2009) show that statoliths move not directly towards gravity. As discussed previously, it is supposed that the gravity-dependent transport of amyloplasts along root statocytes of garden cress seedlings is modulated by elastic forces of the cytoskeleton. The obtained data on kinetics of amyloplast sedimentation during transverse gravistimulation of 1-*g* seedlings also suggest that the gravitational force acts together with cytoskeleton-dependent elastic forces, which actively transport the plastids in longitudinal direction in statocytes of both axial organs (Fig. 14, 15). On the other hand, the 6-min period of transverse stimulation revealed the differences of statolith movement trajectory and velocity in hypocotyl and root statocytes (Fig. 16A, B). Within the first minute, the amyloplast movement in gravity vector direction and at the same time sliding towards the cell centre was significantly faster in hypocotyl endodermal cells (Fig. 16B). However, over the next minute, the positioning of amyloplasts almost did not change in endodermal cells, while they continued to slide intensively along the root statocytes (Fig. 16A). These and the above-described data confirm our hypothesis that structure, rheological properties of the cytoplasm near the morphological bottom of the endodermal cell are different than in root statocytes and the action of intracellular forces in these cells may be modulated by the central vacuole.

In summary, the obtained results confirm the hypothesis that gravity-dependent positioning and movement of amyloplasts are modulated by the cytoskeleton both in hypocotyl and root gravisensing cells of cress seedlings despite the differences in the structure of statocytes and organization of gravisensing tissues. The data of research provide a basis for further investigations on gravisensing processes in oppositively gravitropic organs of plants.

CONCLUSIONS

1. The action of gravitational force is not an essential factor for the formation of gravisensing tissues in hypocotyls and roots of garden cress seedlings; however, under real and simulated microgravity, the growth of endodermal cells in hypocotyls is slower, the location of amyloplasts changes significantly with respect to the morphological bottom of statocytes in roots and hypocotyls.

2. The logarithmic dependence between the positioning of amyloplasts along statocytes of roots and hypocotyls and the magnitude of gravitational force (from 0,004 *g* to 1 *g*), which acted permanently on seedlings in root-tip direction, was determined. Unimodal regularity is characteristic of amyloplast distribution in root statocytes under tested gravitational loads. In hypocotyl statocytes, unimodal regularity of amyloplast distribution changes in-to bimodal if the gravitational force is reduced to 0.02 *g*.

3. The magnitude of intracellular forces, which lift up the amyloplasts from the morphological cell bottom is in the range from 0.1 *g* to 1 *g* in root statocytes. In hypocotyl endodermal cells, it is in the range from 0.5 *g* to 1 *g*. The magnitude of intracellular forces maintaining the positioning of amyloplasts under microgravity conditions is in the range from 0.004 *g* to 0.02 *g* in roots and in the range from 0.008 *g* to 0.02 *g* in hypocotyls.

4. Short-term exposition to simulated microgravity or 180° inversion of seedlings grown vertically under natural gravitational force provoke the lifting of amyloplasts from the morphological bottom of gravisensing cells. In root statocytes, within the first two minutes the plastids move at comparable rate towards the cell centre, but later on their motion slows down under microgravity and proceeds under inversion. In hypocotyl statocytes, the amyloplasts lift up slowly during the entire period of exposition to microgravity, but much more rapidly during the first two minutes of inversion.

5. Short-term longwise stimulation of seedlings grown in simulated microgravity by the gravitational force in root-tip or opposite direction provokes the movement of

amyloplasts in gravisensing cells parallel to its action. In root statocytes, the amyloplasts sediment intensively by a comparable extent during the first four minutes of both stimulations. In hypocotyl statocytes, the sedimentation of amyloplasts proceeds about two times faster towards the morphological cell bottom than in opposite direction already during the first minute.

6. Short-term transverse stimulation of seedlings grown vertically under natural gravitational conditions by the gravity of 1 *g* induces the sedimentation of amyloplasts with simultaneous longwise sliding towards the centre of gravisensing cells. In root statocytes, during the first minute, the amyloplasts move by the comparable rate in both directions, during the second– faster longwise than downwards. In hypocotyl statocytes, within the first minute of stimulation the movement of statoliths occurrs more quickly downwards than longwise, and it proceeds by the comparable rate in both directions during the second minute.

7. The impact of interaction between the gravitational force and intracellular forces on the kinetics of amyloplasts depends on the structural peculiarities and real size of statocytes in roots and hypocotyls of a seedling.

8. The obtained data on the statics and kinetics of amyloplasts confirm that gravity-dependent positioning and movement of amyloplasts, both in hypocotyl endodermal cells and root statocytes of garden cress seedlings, are modulated by intracellular forces, firstly generated by the cytoskeleton.

LIST OF PUBLICATIONS

ARTICLES

in the journals of ISI WOS database

1. Švegždienė D., **Koryznienė D**., Raklevičienė D. Comparison study on gravity-dependent longwise positioning of amyloplasts in statocytes of cress roots and hypocotyls. – Microgravity Sci. Technology 2011; 23: 235–241.
2. Švegždienė D., **Koryznienė D**., Raklevičienė D. Structural polarity of gravisensing cells in garden cress seedlings under varying gravity conditions. – Acta Physiologiae Plantarum 2009; 3(Supl.1*): 112–113.
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Sėjamosios pipirnės gravitacijos jutimo tyrimas

Santrauka

Pirmą kartą buvo palyginti to paties daigo šaknies ir hipokotilio gravitacijos jutimo savitumai skirtingomis gravitacinio dirginimo sąlygomis. Netiesioginiu eksperimentiniu metodu, t.y. kiekybiškai analizuojant amiloplastų statikos ir kinetikos priklausomybę nuo gravitacinės jėgos veikimo krypties ir dydžio, buvo įvertinta citoskeleto reikšmė poliarizuotos šaknų ir hipokotilių statocitų struktūros palaikymui ir viduląsteliniams amiloplastų judesiams daigų gravitacinio dirginimo metu. Eksperimentai atlikti su sėjamosios pipirnės (*Lepidium sativum* L.) daigais tamsoje, gravitacijos pokyčių generavimui naudojant originalius prietaisus – borto centrifugą „Neris-5“ kosminio skrydžio sąlygomis (biopalydovas „Bion-10“) ir dviejų ortogonalių ašių centrifugą-klinostatą. Gravitacinės jėgos dydis buvo keičiamas nuo mikrogravitacijos lygmens (reali kosmose arba imituota horizontaliu klinostatavimu) iki 1 *g* (imituota centrifugavimu arba natūrali Žemės gravitacija), o jos veikimo kryptis buvo keičiama 90° arba 180° kampu daigo išilginės ašies atžvilgiu.

Nustatyta, kad gravitacinės jėgos buvimas nėra būtina sąlyga sėjamosios pipirnės daigų šaknų ir hipokotilių gravisensorinio audinio formavimuisi, bet jai neveikiant (reali ir imituota mikrogravitacija) sulėtėja hipokotilių endodermio ląstelių augimas, pakinta gravisensorinių ląstelių poliškumas dėl esminio amiloplastų pakilimo ląstelių centro kryptimi.

Nuolatinio gravitropinio dirginimo metu, kuomet sėjamosios pipirnės daigus šaknies viršūnės kryptimi veikė skirtingo dydžio gravitacinė jėga, nustatyta logaritminė priklausomybė tarp veikiančios jėgos dydžio ir amiloplastų išsidėstymo išilgai šaknų ir hipokotilių statocitų. Nustatyta, kad abiejų ašinių organų gravisensorinėse ląstelėse amiloplastai juda ne pasklidai, o kaip judrus kompleksas, kuriam šaknų statocituose nepriklausomai nuo gravitacijos dydžio būdingas unimodinis pasiskirstymas, o hipokotilių statocituose unimodinis amiloplastų pasiskirstymas pakinta į bimodinį, kuomet gravitacinė jėga sumažėja nuo 1 *g* iki 0,02 *g*. Šaknų statocituose viduląstelinių jėgų, kurios tempia amiloplastus priešinga gravitacijos vektoriui kryptimi iš apatinės ląstelių srities, dydis yra nuo 0,1 *g* iki 1 *g*, hipokotilių endodermio ląstelėse šių jėgų dydis penkis kartus didesnis, t.y. nuo 0,5 *g* iki 1 *g*. Viduląstelinių jėgų, palaikančių mikrogravitacijos sąlygoms būdingą amiloplastų išsidėstymą šaknų statocituose, dydis yra nuo 0,004 *g* iki 0,02 *g*, o hipokotilių – nuo 0,008 *g* iki 0,02 *g*.

Pakeitus gravitacinės jėgos dydį nuo 1 *g* į imituotą mikrogravitaciją arba pakeitus įprastinę veikimo kryptį 180° (inversija) nustatyta, kad amiloplastai kyla nuo morfologinio ląstelių dugno abiejų ašinių organų statocituose. Šaknų gravisensorinėse ląstelėse per pirmąsias dvi minutes amiloplastai juda panašiu greičiu abiem dirginimo atvejais. Hipokotiliuose pirmosiomis dirginimo minutėmis inversijos metu nustatytas žymiai spartesnis amiloplastų kilimas nuo morfologinio ląstelių dugno nei mikrogravitacijos atveju.

Imituotos mikrogravitacijos sąlygomis augusius sėjamosios pipirnės daigus veikiant 1 *g* jėga šaknies apekso arba priešinga kryptimi (inversija), amiloplastai abiejų ašinių organų gravisensorinėse ląstelėse judėjo iš mikrogravitacijai būdingos padėties gravitacijos veikimo kryptimi, tačiau skirtingu greičiu. Jau pirmąją minutę abiejų organų

gravisensorinėse ląstelėse stebėtas akivaizdus statolitų slinkimas veikiančios jėgos kryptimi, kuris šaknų statocituose panašiu intensyvumu abiem kryptimis tęsėsi iki 4 minutės. Nustatyta, kad hipokotilių statocituose amiloplastai pirmąją minutę morfologinio ląstelių dugno link judėjo apie du kartus greičiau nei priešinga kryptimi.

Nustatyta, kad 1-g daigus dirginant skersine kryptim natūralia (1 g) gravitacine jėga, amiloplastai juda jos veikimo kryptimi, tačiau judėjimo trajektorija ir greitis hipokotilių ir šaknų gravisensorinėse ląstelėse skiriasi. Šaknų statocituose pirmąją minutę vyko panašus amiloplastų judėjimas gravitacijos veikimo, ir ląstelės centro kryptimi, o antrąją minutę - intensyvesnis ląstelės centro kryptimi. Hipokotilių statocituose amiloplastai pirmąją minutę žymiai greičiau judėjo gravitacijos veikimo kryptimi nei ląstelės centro link, o antrąją minutę – panašiu greičiu abiem kryptimis.

Gauti rezultatai patvirtina prielaidą, kad, nepriklausomai nuo gravisensorinio audinio organizacijos ir statocitų struktūros, viduląstelinės jėgos, visų pirma generuojamos citoskeleto moduliuoja nuo gravitacinės jėgos priklausomą amiloplastų išsidėstymą ir judėjimą ir šaknų, ir hipokotilių gravisensorinėse ląstelėse.

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