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MAGNETOTROPISM OF ROOTS AND STRUCTURE OF THEIR STATOCYTES EXPOSED TO HIGH GRADIENT MAGNETIC FIELD

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In most living organisms, gravity perception is based on the response of the gravisensing system to displacement of a specific mass induced by a gravitational force. The amyloplasts in higher plants are known to play the important role in gravisensing cells, statocytes. Effects of the HGMF on roots result in their curvatures similar to those produced by gravity. It was suggested that the HGMF could allow to imitate the effects of gravity in microgravity and/or to change them in laboratory conditions correspondingly. The parameters of kinetics of *Pisum sativum* L. root curvatures under the HGMF action were recorded by video system and measured by means of image analysis software. It was shown that application of the HGMF could deflect roots. At cellular level, we observed significant changes in the position of the individual cellular components in statocytes, while the locations of statoliths in columella cells were similar to those in horizontally-oriented roots up to 1 h stimulation. Thus, the root curvature in the HGMF is the plant response to displacement of amyloplasts by ponderomotive force. The data indicate similarity of the effects of magneto- and gravistimulation at the organ and cellular level.

Key words: *Pisum sativum L., high-gradient magnetic field, plant gravisensing, statocyte, ultrastructure, morphometry*

According to relevant statements, the processes of plant orientation under the Earth's gravitational field are controlled by the relative position of statoliths in the gravisensitive cells. In roots of higher plants, the amyloplasts (starch-containing plastids, whose densities are higher than that of the cytoplasm) play a role of statoliths within the columella cells of the root cap. A widely known starch-statolith hypothesis suggests that the sedimentation of the amyloplasts is one of the initial acts of gravity sensing (Salisbury, 1993; Sack, 1997; Kiss, 2000). However, the mechanisms by which plants transform the physical signal resulting from the amyloplast sedimentation into a biochemical signal to initiate the bending response have been elusive and remain an area of intense research (Blancaflor et al., 1998; Boonsirichai et al., 2002; Chen et al., 2002; Hou et al., 2003).

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In order to study experimentally the starch-statolith hypothesis, the plant position relative to gravity vector may be changed and then compared with changes in the intracellular location of statoliths. However, the plant response to the changes in the position in gravitational field can not be limited only by the statolith displacements but a complex response of the whole plant organism could not be excluded. Therefore, noninvasive methods that allow to control the statolith locations and analyze their interactions with other cellular components open new possibilities for an examination of the starch-statolith hypothesis and studying the mechanisms of the plant graviperception. In addition, such methods can play a significant role in studying the mechanisms related to the alteration in plant graviperception during orbital space flight and can be used to simulate the microgravity effects on plants in the experiments on the Earth.

One of the most perspective noninvasive methods affecting the relative position of the amyloplasts in graviperceptive cells of plants is using a High Gradient Magnetic Field (HGMF). The amy-

loplast magnetic susceptibility differs from a magnetic susceptibility of the cytoplasm due to presence of starch in the plastids. Therefore, a ponderomotive force induced by the HGMF can affects the amyloplasts. The amount of the force is proportional to the difference between their magnetic susceptibilities, the magnetizing magnetic field and the field gradient. Since the difference between the magnetic susceptibilities of the amyloplasts and cytoplasm is negative, the ponderomotive forces will push the amyloplasts out of maximum of the HGMF. The effects of ponderomotive forces and a gravitational force on the statoliths in plant cells are similar. It was shown in experiments of Kuznetsov and Hasenstein (1996) that the root response (curvature) to the HGMF had correlated with the transient change in the gravitropic curvature. A light-microscopic observation on root sections of plants exposed to the HGMF demonstrated that the responses of the plant roots were correlated with displacements of statoliths inside their graviperceptive cells (Kuznetsov, Hasenstein, 1996). Further, these authors have demonstrated that the HGMF can mimic the gravitropic response of different plant organs (Kuznetsov, Hasenstein, 1997, 2001; Weise et al., 2000). Recently, new insights in the field of magnetotropic effects on plant systems have been successfully provided (Kondrachuk, Belyavskaya, 2001a, b; Kondrachuk, Hasenstein, 2001; Білявська, Даневич, 2005).

However, the following questions still remain without reply: what changes at the cellular level occur in columella cells of plant roots exposed to the ponderomotive force and what cellular components of statocytes besides the amyloplasts have the modifications in their topography? Therefore, the objective of this study was to determine the HGMF effects on magnetotropism, structure and topography of cellular components in root statocytes of pea seedlings.

METHODS

Pea (*Pisum sativum* L.) cv. Damir-2 was used in this work. Seeds of pea were imbibed for 2 h in running water. After imbibitions the seeds were germinated and grown vertically at room temperature in special chamber lined with filter paper moistened with distilled water and wrapped round by a black paper. Control seedlings were kept vertically while other seedlings were reoriented at the angle of 45º from the gravity vector for 5, 30 or 60 min.

In the HGMF experiments, a pair of permanent magnets (NdFeB, $10 \times 15 \times 60$ mm^3 each) produced by the ROMSAT firm was used. A ponderomotive force generated by the HGMF at the edge of the gap between two magnets acted away from the depth of the gap. The system consisted of two magnets mounted at an angle of 44º from the gravity vector that reduced the force required to induce root curvature. The gap width of 1.5 mm was used in the experiments where magnetic induction in working zone was 0.7 T. At the start of each experiment, the root tip was positioned 2 mm above the center of the gap between two magnets and fixed vertically on a plastic mat, which was covered with a wet filter paper. In the control experiments, two aluminium plates of similar size were used instead of the magnets.

Roots of 4-day pea seedlings were prepared for electron-microscopic investigations according the following procedure. The samples were picked out from experimental sets for vertical control, gravistimulation (for 5, 30 or 60 min) and magnetostimulation in the same time intervals. Root tips were excised in a manner (at angle to the root axis) so that their original orientation with respect to gravity could be determined in all subsequent manipulations. Root apices were fixed with mix of 2.5% (w/v) glutaraldehyde and 2% (w/v) paraformaldehyde on 0.1 M sodium cacodylate buffer, pH 7.8 (for 3 hours at room temperature). After three washes of 10 min each, the root tips were postfixed in 1% (w/v) OsO_4 on the same buffer for 2-3 h at 4º C. Tissues were then dehydrated in a graded series of acetone solutions and soaked in mixes of acetone and epone-araldite resin and in the pure resin. The root apices were put in molds and filled with fresh-prepared resin and polymerized for 48 h at 60º C in thermostat. Sections were stained with uranyl acetate and lead citrate and examined in transmission electron microscope JEM 1200 EX at 80 kV.

For morphometric analyses, the columella cells on random sections of root caps were photographed and scanned on an Epson Perfection 3200 Photo scanner. Photos were prepared using Adobe Photoshop 7.0 and Corel Photo-Paint 11 computer programs. Measurements of organelles' areas for determining relative volumes of cellular components (i.e., the ratio of areas of all organelles of the certain type to the area of whole cell) were performed on scanned micrographs using an UTHSCSA ImageTool (the University of Texas Health Science Center, San Antonio, Texas, USA) software program. For each experimental set and vertical control, 6-8 median longitudinal root cap sections, in which 4-6 columella cells were randomly chosen, were analyzed from micrographs with a total magnification of 2000×. Calculations

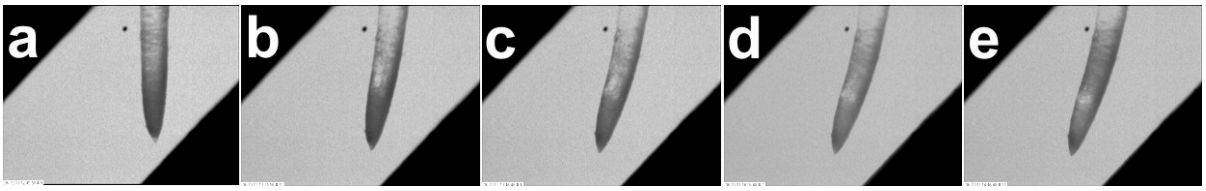

Fig. 1. Induction of curvature by the HGMF in root of *Pisum sativum*.
The root was placed over a gap between two square magnets so that their upper edges were at a 44º angle to a gravity vector (magnet is seen as a black triangle in a right lower corner). Successive stages of the curvature are shown for 0 min (a), 30 min (b), 60 min (c), 90 min (d), and 120 min (e).

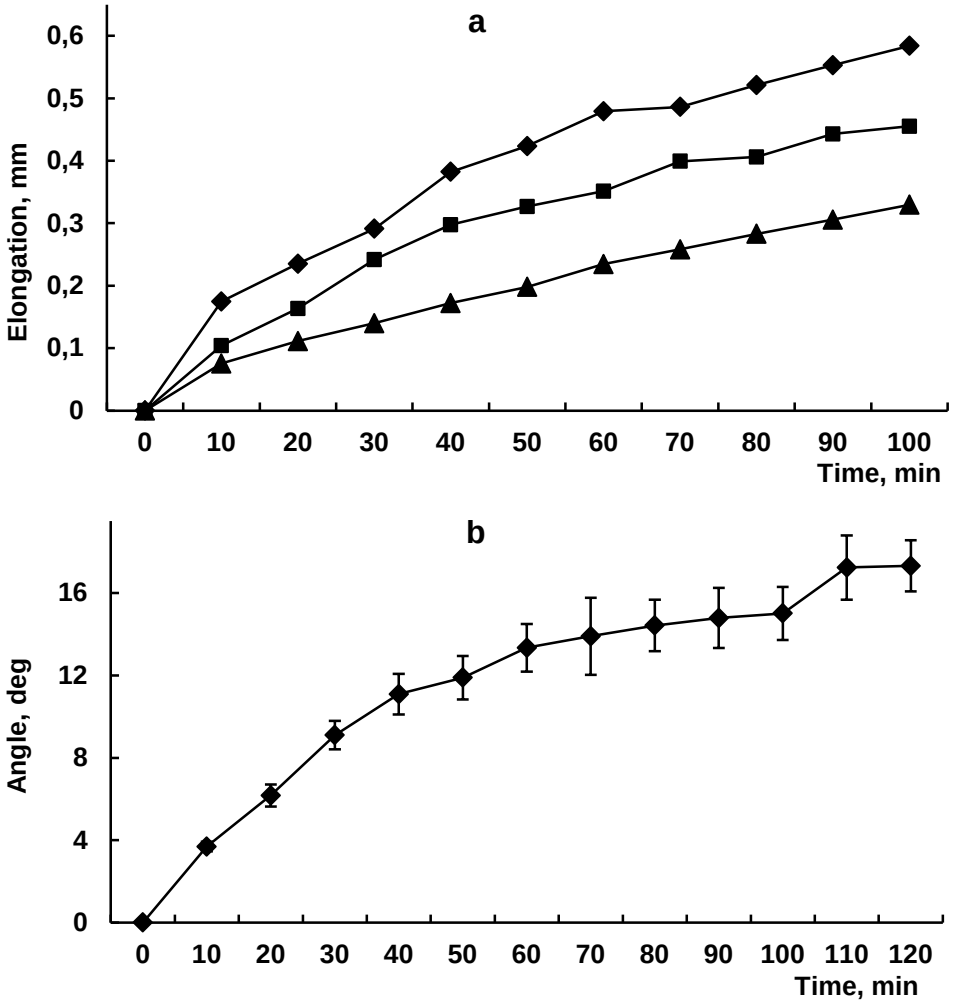

Fig. 2. Effects of magnetostimulation on the growth and curvature of pea roots. a – elongation of upper (▲), median (■), and lower (◆) parts of pea roots that were placed in the HGMF. **b** – time course of curvature development in the HGMF. Data are means for 15 roots; the bar represents the average standard error.

were made on a whole cell basis as well for the upper, middle, and lower thirds of the cells, and proximal and distal halves of the same cells.

RESULTS AND DISCUSSION

The pea root curvature recorded at 0.5-h intervals for 2 h in the HGMF is presented in Figure 1a-e. Deviations of pea roots elongating away from magnetic fields were found. The roots subjected to the exposition in the HGMF showed a significant decrease in elongation growth. Control roots (n = 12) were elongated at the rate of 1.25 ± 0.11 mm h^{-1}. In contrast, the average growth rates of gravistimulated (n = 15) and especially HGMF-treated roots (n = 16) were reduced to 1.20 ± 0.09 and 0.35 ± 0.01 mm h^{-1}, respectively. The time course of growth along concave (upper), median and convex (lower) side of the roots in the HGMF is shown in Fig. 2a. Despite a strong growth inhibitory effect of a ponderomotive force, root curvature in the HGMF was promoted during the first hour (14.2 ±

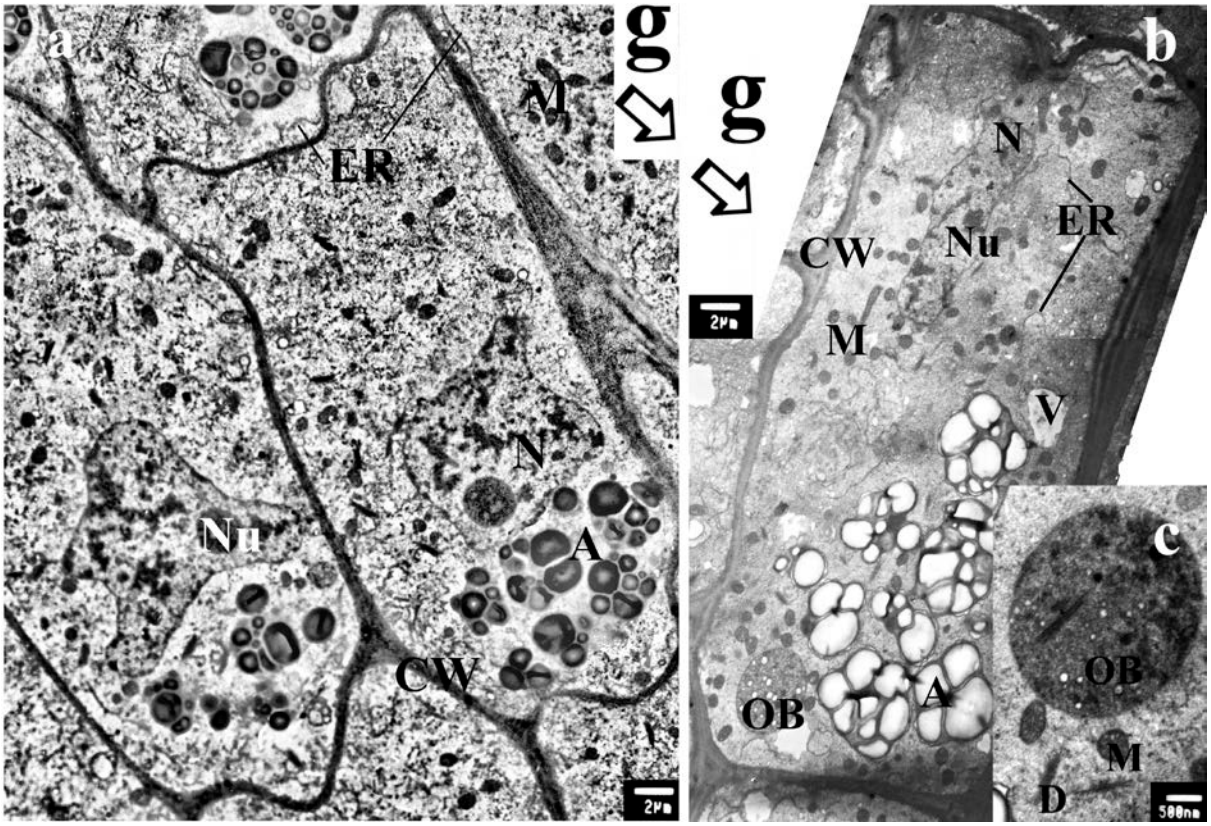

Fig. 3. Columella cells in roots of pea seedlings. (a), Vertical control; (b-c), Gravistimulation for 30 min. (a), The amyloplast sedimentation is seen. (b), The amyloplasts are shifted to physically lower cell wall. There is the oval body (OB) in the cytoplasm. (c), The oval body at a higher magnification. Abbreviations: A – amyloplast, CW – cell wall, D – dictyosome, ER – endoplasmic reticulum, M – mitochondria, N – nucleus, Nu – nucleolus, V – vacuole.

1.16 deg h^{-1}) and retarded to 4.01 ± 1.02 deg h^{-1} during the second hour (Fig. 2b). The reorientation of pea seedlings at the angle of 45º resulted in root curvature of 14.1 ± 1.3 and 26.4 ± 2.4 degrees after 1 and 2 h. These experimental data were similar to previously reported results which showed that flax roots responded by curving in the HGMF and curvature rate reduced in time (Kuznetsov, Hasenstein, 1996). On the other hand, our experiments were carried out without using the clinorotation as opposed to experiments of Kuznetsov and Hasenstein (1996) with roots of *Linum usitatissimum* rotated on clinostats. In contrast to the asymmetric growth observed in graviresponding roots, magnetotropic curvature in pea roots showed time-dependent reduction of curvature rate. It seems to be due to the cessation of stimulation when root tips get out a zone of the HGMF action. The reduction in growth rate and curvature of roots exposed to the HGMF suggested that these roots exhibited a reduced response as compared to gravistimulated roots. We cannot exclude a possibility of the HGMF effects on the auxin transport and/or other processes related to the bending development. Thus, our data reflected the fact that in the HGMF, variable resultants of a superposition of two forces (gravitational and ponderomotive) can act on root caps in each point (where columella cells locate) and induce tropic responses.

Columella cells of vertically-oriented pea roots had a typical ultrastructure for the statocytes (Fig. 3a). These cells were characterized by the presence of sedimented amyloplasts on distal pole. Extensive descriptions of these cells are provided elsewhere (for review, see Sack, 1991).

Figure 3b presents the columella cell of the pea seedling root that has been gravistimulated for 30 min. In this case, the structure of individual cell components has not been affected. The appearance of the oval bodies in the cytoplasm was a significant phenomenon. Their inner contents had an electron density greater than that of surrounding cytoplasm. These bodies could be observed in 11 % of the columella cells (one per cell). The localization of the bodies within the cell was random. The oval bodies were separated from the cytoplasm by a monolayer membrane. The dictyosome-like structures, multiple vesicles with electron-translucent content and other electron-dense structures of various shape and size could be observed in these bodies (Fig. 3c). They resembled

Table 1. Effects of gravistimulatiom and magnetostimulation on relative volumes of cellular components in columella cells of pea roots

Cellular components	Control	Gravistimulation			Magnetostimulation		
	0 min	5 min	30 min	60 min	5 min	30 min	60 min
Amyloplast	9.23±1.35 a	9.47±1.44 a	10.95±1.01 a	10.65±1.1 a	9.29±1.08 a	9.86±1.19 a	9.92±1.06 a
Vacuole	3.24±0.31 a	3.87±0.44 a	7.37±0.59 b	9.02±0.63 c	7.77±0.61 b	7.62±0.59 b	8.23±0.71 bc
Nucleus	8.86±1.05 a	10.35±1.08 a	10.89±1.21 a	10.55±1.26 a	10.87±1.18 a	10.51±1.3 a	10.42±1.12 a
Cell wall	7.38±0.82 a	7.84±0.98 a	7.89±1.05 a	7.66±1.24 a	7.92±1.16 a	7.30±1.28 a	7.51±1.31 a
Mitochondria	3.18±0.29 a	3.01±0.21 a	3.84±0.33 b	3.04±0.28 a	3.12±0.23 a	3.15±0.30 a	3.36±0.30 ab
Endoplasmic reticulum	2.94±0.13 ab	2.90±0.15 a	2.89±0.13 a	2.86±0.18 a	2.93±0.17 ab	3.31±0.22 b	3.21±0.24 ab
Dictyosome	0.51±0.06 ab	0.44±0.04 ab	0.57±0.05 b	0.49±0.04 ab	0.50±0.04 ab	0.43±0.04 a	0.53±0.05 b
Lipid globule	0.41±0.04 bc	0.33±0.03 a	0.36±0.03 ab	0.36±0.04 ab	0.41±0.04 bc	0.43±0.05 bc	0.44±0.04 c
Cytoplasm	64.25±5.59 a	61.79±5.05 a	55.24±4.08 a	55.28±4.24 a	57.19±5.17 a	57.39±5.29 a	56.38±5.08 a

N o t e . All volumes are expressed as a percentage of the cellular volume ± SE, n = 20 cells for each treatment. Means with different letters are significantly different ($P < 0.05$, Student's test).

cytosegresomes. However, we have never observed lysis of the oval body contents that is a main characteristic of cytosegresomes. It should be mentioned that such structures occurred firstly in pea root statocytes after 30- and 60-min gravistimulation. They were not observed in pea columella cells gravistimulated for 5 min. Thus, these structures seem to be a characteristic of pea root statocytes at later stages of gravistimulation. It should be noticed that the appearance of the oval bodies was correlated with the decrease in relative volume of dictyosomes at the later stages of gravistimulation (Table 1). If the appearance of these structures is actually associated with sequestration of the dictyosomes it can suppose that the phenomenon indicates the tendency of cell wall polysaccharides supplied by the Golgi vesicles to decrease.

The vacuolar compartment is affected by the gravistimulation among the cell structures of pea root statocytes (Table 1). Its relative volume is gradually increased from 3.87 (by 5 min) up to 9.02 % (by 60 min) during gravistimulation. It may be suggested that the alterations in osmotic pressure are possibly associated with the modifications in ion balance and/or in the intracellular pH in response to gravistimulation (Fasano et al., 2001, 2002; Plieth, Trewavas, 2002; Scott, Allen, 1999). It appears to correlate with the data concerning participation of the vacuoles in stem gravitropism (Kato et al., 2002). This allows us to suggest that the vacuome can also participate in gravity-induced responses of statocytes in roots of higher plants. Insignificant increase in relative volumes of nuclei in pea root statocytes was registrated at all stages of gravitropism in comparison with vertical control. The increase in the relative volume of the mitochondria was demonstrated by 30 min of gravistimulation. The relative volumes of other cell components (amyloplasts, nuclei, cell wall, ER, cytoplasm, and lipid globules) vary insignificantly during gravistimulation (Table 1).

Probabilities that the cellular components were located in one of the thirds in pea root columella cells during gravistimulation showed substantial changes in distribution of these components in response to gravistimulation (Table 2). The amyloplasts moved from the lower part to the middle third while some part of them even reached the upper one after 5 min of gravistimulation. At later terms (30 and 60 min), the most portion of amyloplasts sedimented upon physically lower part of the statocytes after root reorientation. The same pattern in redistribution of the plastids was revealed between the proximal and distal half of the columella cell. The vacuoles, which were located in most cases in the upper and middle parts of the statocytes of roots grown vertically, could be found in all three parts with almost equal probabilities after 5 and 30 min reorientation and mostly in the middle one after 60 min of gravistimulation. In direction parallel to the root axis, the vacuoles remained practically in the proximal part of the cell as in control. The nuclei changed their position partially, shifting firstly (5 min of gravistimulation) toward the middle and then (30 and 60 min) toward the upper part of the statocyte. The same pattern of their relocations could be observed in the proximal-distal direction (Table 3). After 5 min of the root reorientation, most of the mitochondria could be seen in the upper part of the statocyte.

Table 2. Probability that a cellular component is located in the upper (U), middle (M), or lower (L) portion of columella cells of pea roots oriented vertically (control) and at the angle of 45º (gravistimulation)

Cellular components	Control			Gravistimulation								
	0 min			5 min			30 min			60 min		
	U	M	L	U	M	L	U	M	L	U	M	L
Nucleus	40.0± 4.5 ef	41.2± 4.0 ef	18.8± 1.7 d	35.3± 2.1 e	55.8± 1.9 h	8.9± 0.8 c	44.9± 3.5 fg	49.2± 3.1 g	5.9± 0.4 b	52.9± 4.8 gh	47.1± 3.9 f	0.0± 0.0 a
Vacuole	37.5± 3.6 cd	42.4± 4.1 d	20.0± 1.9 a	40.1± 4.0 cd	34.5± 3.1 c	25.4± 2.2 b	44.0± 4.7 d	32.4± 3.1 c	23.6± 1.3 b	21.0± 1.9 ab	57.7± 4.8 e	21.3± 2.0 ab
Mitochondria	26.8 1.3 ab	36.0± 2.4 de	37.2± 2.9 e	40.4± 3.8 e	26.3± 2.1 ab	33.3± 1.5 cd	34.1± 2.9 cde	30.1± 2.5 bc	35.8± 2.9 cde	30.3± 2.3 bc	22.9± 2.1 a	46.9± 1.2 f
Amyloplast	0.0± 0.0 a	7.3± 0.6 b	92.7± 5.8 f	6.3± 1.1 b	48.8± 2.4 e	45.0± 4.5 e	6.2± 0.9 b	45.1± 4.9 e	48.7± 4.4 e	13.3± 1.6 c	31.3± 2.1 d	55.4± 4.9 e
Dictyosome	0.0± 0.0 a	100.0± 0.0 f	0.0± 0.0 a	100.0± 0.0 f	0.0± 0.0 a	0.0± 0.0 a	61.6± 3.1 e	20.1± 1.3 b	18.3± 1.6 b	30.0± 2.6 c	26.8± 1.4 c	43.2± 3.8 d
Cytoplasm	34.9± 3.9 bc	39.5± 4.0 c	25.7± 2.3 a	34.7± 3.1bc	33.5± 2.7 bc	31.8± 2.7 b	33.2± 1.7 bc	33.1± 1.9 bc	33.8± 1.5 bc	31.8± 2.3 bc	32.4± 1.5 b	35.8± 1.7 b

Table 3. Probability that a cellular component is located in the proximal (P) or distal (D) halves of columella cells of pea roots oriented vertically (control) and at the angle of 45º (gravistimulation)

Cellular components	Control		Gravistimulation					
	0 min		5 min		30 min		60 min	
	P	D	P	D	P	D	P	D
Nucleus	50.0±4.8 c	50.0±4.8 c	70.1±5.7 d	29.9±2.7 c	77.3±7.6 d	22.7±2.3 b	100.0±0.0 e	0.0±0.0 a
Vacuole	69.0±6.1 c	31.0±3.4 b	76.8±6.8 c	23.2±2.1 a	63.5±6.0 c	36.5±3.9 b	66.9±5.7 c	33.1±3.5 b
Mitochondria	41.8±4.1 ab	58.2±4.3 d	40.0±4.1 a	60.0±5.5 d	52.4±4.0 cd	47.6±3.7 abc	51.6±5.4 bcd	48.4±4.2 abc
Amyloplast	0.0±0.0 a	100.0±0.0 d	12.6±1.8 b	87.4±6.6 c	10.5±1.0 b	89.5±6.3 c	9.4±1.4 b	90.6±7.1 cd
Dictyosome	64.1±4.2 e	35.9±2.6 d	100.0± 0.0 g	0.0±0.0 a	82.7±5.1 f	17.3±2.8 b	72.2±4.3 e	27.8±2.2 c
Cytoplasm	57.2±3.7 d	42.8±2.8 a	52.5±4.0 b	47.5±2.1 abc	53.3±3.2 cd	46.7±1.5 ab	53.0±4.1 bcd	47.0±3.2 abc

The mitochondria were evenly distributed within the cell at 30-min root reorientation, while their amount became the highest in the bottom part after 60-min gravistimulation. They shifted somewhat along the root axis toward the proximal part for 30 and 60 min of gravistimulation. The dictyosomes went up abruptly toward the upper part of the cell. They sedimented gradually with time from their position mostly in central part of the statocyte as in vertical control. Most of them was located in the bottom part at 60 min of gravistimulation. The same pattern of displacement for the dictyosomes was observed along the root axis, but their localization was like to the vertical control at the 60 min of gravistimulation. The common tendency seems to be even distribution of the cytoplasm in all three parts. The difference in the cytoplasm distribution was not demonstrated along direction of the root axis during the gravistimulation of pea seedlings.

In general, the exposition of pea seedlings to the HGMF had resulted in no changes in ultrastructure of individual cellular components in their root columella cells (Fig. 4). However, the modifications in the quantitative indices of the relative volumes of some organelles occurred (Table 1). These values for the vacuolar compartment increased significantly from 5 min in the HGMF to 60 min as compared to 2.24 % in vertical control.

Figure 4a shows the columella cell in root of pea seedling after 5-min exposition in the HGMF. Its peculiar feature is a displacement of the amylo-

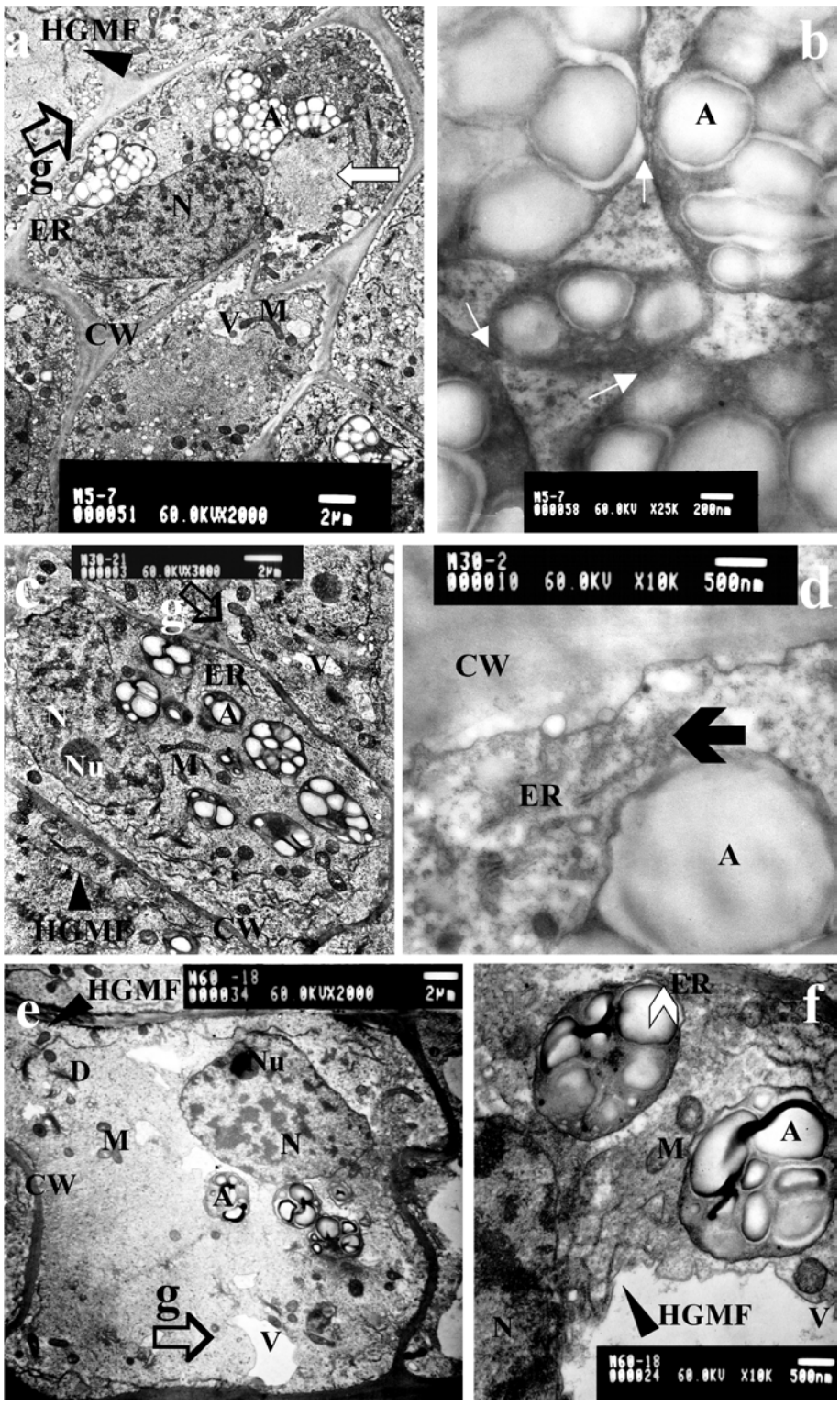

Fig. 4. (a-f), Columella cells in pea seedlings exposed to the HGMF (black arrow heads). Time of magnetostimulation is following: 5 min (a, b), 30 min (c, d) and 60 min (e, f). (a), White arrow shows the separate zone of the cytoplasm. (b), White arrows show contacts between the amyloplasts. (d, f), The amyloplasts frequently contact with the endoplasmic reticulum nearly the cell wall (white arrow heads). Transparent arrow shows a gravity vector (g).

plast population along a gradient of the nonhomogenous magnetic field, i.e., to the most distant from the magnets and to the middle third of the cell; most amyloplasts have located in proximal halves of the statocytes (Tables 4, 5). In addition, it should be worthy of note that the separate zones of the cytoplasm are formed within the cells and have no limiting membranes and cellular organelles,

excluding ribosomes and polysomes (Fig. 4a, white arrow). The fact can be explained by 1) the release of the zones of the cytoplasm from the amyloplasts that have been pushed out by the ponderomotive force from their places to the periphery of the statocyte, and 2) short term of magnetostimulation (5 min), by which the amyloplasts cannot move into these zones. It is possible that mechanical disruptions of the cytoskeleton components in the moment of pushing out the amyloplasts by this force occur because of the plastid redistribution delay. It should be noticed that in the HGMF, the amyloplasts combined into groups where all plastids clustered tightly each to other to form the contacts between them (Fig. 4b, white arrows). Such contacts appear to have exclusively mechanical nature; however, they can have both physical consequences to increase a total mass of a body with diamagnetic characteristics and physiological ones to promote the transport of some substances inside such "megaamyloplasts". After 30 min of magnetostimulation, the amyloplasts continued to locate nearly cell wall distally from magnets (Fig. 4c). Enhancement in the vacuolization of the statocytes occurred during this period of magnetostimulation. The peripheral endoplasmic reticulum, which had

Table 4. Probability that a cellular component will be located in the proximal (P) to the HGMF or distal (D) halves of columella cells in comparison to those in pea roots oriented vertically (control)

Cellular components	Control		Magnetostimulation					
	0 min		5 min		0 min		5 min	
	P	D	P	D	P	D	P	D
Nucleus	50.0±4.8 c	50.0±4.8 c	86.9±7.6 d	13.1±1.8 b	97.1±9.1 d	2.9±0.6 a	48.6±4.6 c	51.4±4.6 c
Vacuole	69.0±6.1 e	31.0±3.4 a	60.9±6.3 de	39.1±3.8 b	48.6±4.7 c	51.4±5.4 d	44.0±4.3 bc	56.0±5.2 cd
Mitochondria	41.8±4.1 ab	58.2±4.3 c	65.3±4.8 d	34.7±3.6 a	55.8±5.2 c	44.2±4.8 b	42.3±3.3 b	57.7±4.9 c
Amyloplast	0.0±0.0 a	100.0±0.0 f	10.4±1.2 d	89.6±5.7 e	6.4±0.8 c	93.6±6.3 ef	2.2±0.4 d	97.8±5.2 ef
Dictyosome	64.1±4.2 c	35.9±2.6 b	100.0±0.0 d	0.0±0.0 a	100.0±0.0 d	0.0±0.0 a	100.0±0.0 d	0.0±0.0 a
Cytoplasm	57.2±3.7 c	42.8±2.8 a	51.3±5.3 bc	48.7±4.2 ab	49.2±2.7 b	50.8±3.1 bc	57.4±3.7 c	42.6±3.5 ab

Table 5. Probability that a cellular component will be located in the upper (U), middle (M), or lower (L) portion of columella cells of pea roots oriented vertically (control) and magnetostimulated by the HGMF

Cellular components	Control			Magnetostimulation								
	0 min			5 min			30 min			60 min		
	U	M	L	U	M	L	U	M	L	U	M	L
Nucleus	40.0± 4.5 e	41.2± 4.0 e	18.8± 1.7 d	51.7± 2.4 f	48.3± 2.4 f	0.0± 0.0 a	58.2± 5.9 g	35.9± 3.6 e	5.9± 0.5 b	45.4± 3.9 f	43.9± 4.8 ef	10.6± 1.9 c
Vacuole	37.5± 3.9 e	42.4± 4.1 e	20.0± 1.9 c	7.9± 1.1 a	61.6± 5.9 h	30.6± 2.0 d	38.8± 3.1 ef	48.5± 3.9 g	12.7± 1.3 b	27.8± 2.6 d	26.9± 2.7 d	45.3± 3.4 fg
Mitochondria	26.8± 1.3 bc	36.0± 2.4 df	37.2± 2.9 df	33.1± 4.6 ce	23.4± 2.9 ab	43.5± 4.7 fg	34.8± 3.9 de	26.1± 2.5 bc	39.1± 3.5 e	32.1± 3.2 de	19.6± 1.6 a	48.3± 4.0 g
Amyloplast	0.0± 0.0 a	7.3± 0.6 bc	92.7± 5.8 h	10.3± 2.3 c	18.5± 1.8 d	71.2± 6.1 f	22.7± 1.9 e	24.8± 3.0 e	52.5± 3.7 g	6.0± 0.8 b	47.8± 4.4 g	46.2± 4.9 g
Dyctiosome	0.0± 0.0 a	100.0± 0.0 d	0.0± 0.0 a	0.0± 0.0 a	22.6± 2.0 b	77.4± 6.6 c	100.0± 0.0 d	0.0± 0.0 a	0.0± 0.0 a	100.0± 0.0 d	0.0± 0.0 a	0.0± 0.0 a
Cytoplasm	34.9± 3.9 b	39.5± 4.0 c	25.7± 2.3 a	25.4± 1.9 a	33.8± 4.4 b	40.8± 3.9c	29.0± 2.0 a	25.7± 2.9 a	45.4± 3.7 c	30.9± 2.0 b	30.6± 3.7 b	38.5± 3.0 c

almost uninterruptedly lined interior surface of the statocytes, served as a mount for the amyloplasts that were pressed to it by a ponderomotive force (Fig. 4d, white arrow head). After 60-min exposition to the HGMF, ultrastructure of individual cellular components of the statocytes in pea roots was virtually unchanged compared to those from vertically oriented seedlings (Fig. 4e). At a 30- and 60-min exposition, the contacts between outward membranes of the amyloplasts and adjacent membranes of the endoplasmic reticulum had observed too (Fig. 4f, white arrow head).

Among the cellular components, magnetostimulation had changed their localization in pea root statocytes along the root axis for the nuclei and the mitochondria (by 5 and 30 min), the vacuoles (at all sampling times), the dictyosomes (at all terms of observation), and the cytoplasm (by 30 min) (Table 4). It was noted that besides the dictyosomes located exclusively in proximal half and the vacuoles, whose localization probability was higher in distal half of the statocytes, all other organelles almost stabilized their distribution by 60 min, turning back to the bottom of the cell as in the statocytes of vertical control (Table 4). Such a pattern of redistribution of the cellular components indicated that after 60-min exposition to the HGMF, the root tips that went on to grow could leave the zone where the ponderomotive force operated and therefore the organelles, first of all the amyloplasts, came back in lower (distal) part of the statocytes under the influence of gravity.

As regards topography of the cellular components along the gradient of the ponderomotive force, the differences in the localization of almost all components occurred in columella cells as compared to the vertical control (Table 5). In such a way, the amyloplasts that mainly move away from the magnets for first 30 min, as it had been shown in root caps of *Linum usitatissimum* at the light-optical level (Kuznetsov, Hasenstein, 1996), had nearly uniformly distributed in middle and remote from the magnets thirds of cell by 60 min. It may be considered as a further support of the above mentioned arguments concerning withdrawal of the columella of roots from the zone where the ponderomotive forces act in this time. The nuclei had mainly located in the third of the statocyte near magnets during all term of the exposition to the HGMF. Most vacuoles had been revealed in the middle third of the cell by 5 and 30 min of such an exposition while the tendency to their redistribution in the third part far from magnets might be registered by 60 min of magnetostimulation. The mitochondria had redistributed from the middle of the cell to the lateral thirds for 5 min in the HGMF. However, the most amounts of them had shifted in the third of the statocyte far from the magnets after 60 min of the exposition to the HGMF. The dictyosomes firstly (by 5 min) moved in third of the cell near the magnets whereas all organelles of the Golgi apparatus had been observed in far third of statocytes by 30 and 60 min of magnetostimulation. The cytoplasm had also redistributed in gradient of the ponderomotive force during all period of the observation, although its percentage had been the most in the remote third of the cell from the magnets. If we compared the cellular part remote from the magnets with the lower third of the gravistimulated statocyte the positive correlation of these data was demonstrated for the mitochondria only. All other cellular components showed the redistribution in statocytes as the individual responses to the exposition in the HGMF. It was evident that the impetuous movements of the amyloplasts in the moment of the HGMF application to pea root caused the redistribution not only of small dictyosomes but also the organelles of greater mass and volume (the nucleus and the vacuoles). Their complex kinetics of the movements could reflect the continuous changes in the direction and value of the resultant force (due to superposition of the ponderomotive and gravitational forces) in the course of root growth in the HGMF.

Comparative analysis of the experimental data of gravistimulation and HGMF effects on quantitative indices of the cellular components has shown that besides the amyloplasts, at least vacuolar compartment can respond to both factors in the same pattern to increase relative volume in the statocytes. It allows suggesting that the significant transformations can occur at the level of vacuome and it can be related to the changes in the localization of the amyloplasts. The suggestion is in agreement with assumptions of other authors (Kato et al., 2002; Morita et al., 2002). Their investigations showed that the structure of the vacuoles was altered in sgr2, sgr3, and zig/sgr4 mutants, suggesting a role for these proteins in vacuolar biogenesis and/or function. Furthermore, although amyloplasts were surrounded by flexible vacuolar membranes in wild-type statocytes, they were located in the cytoplasm and did not sediment in mutant statocytes (Kato et al., 2002). Therefore, it was concluded that the large vacuoles presented in shoot statocytes might be involved in gravity perception or signal transduction (Morita et al., 2002).

We have demonstrated here the contacts between the amyloplasts and membranes of the endoplasmic reticulum in the HGMF (Fig. 4d, f). The

similar ER-plastid associations were revealed in the statocytes of *Arabidopsis* (Sack, Kiss, 1989); such contacts have been hypothezised to trigger perception such as by a pressure-induced calcium release. The identity of the HGMF-induced signal is currently unknown but can include the transient cytoplasmic calcium and pH changes that occur immediately after magnetostimulation (Білявська, Даневич, 2005).

We have first demonstrated the HGMF effects on growth and curvature of seedling roots, structure and topography of cellular components of root statocytes in higher plants without using the clinostats. It was shown that the growth rate in the HGMF was less than in control experiments and further declined when the root tips came to the magnets. Therefore, a superposition of two forces, gravitational and ponderomotive, can induce tropic responses. No significant differences in ultrastructure of individual organelles between gravi- or magnetostimulated and control statocytes were detected. Morphometric analysis revealed that ponderomotive force induced by the HGMF resulted in the redistribution of all cellular components in statocytes. The correlation in the amyloplast distribution between gravistimulation and magnetostimulation in direction of both gravitational and ponderomotive force by 30 min as well as in proximal-distal direction by 60 min was established. The increase in related volumes of vacuoles after seedling reorientation or magnetostimulation can indicate that these organelles appear to take part in signaling events related to changes in localization of amyloplasts. Our results show that the HGMF *per se* is capable to displace the amyloplasts against a gravitational force and induce gravitropic-like curvature. It can be used as adequate non-invasive tool for studying the processes related to gravitropism at the cellular and subcellular level.

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МАГНИТОТРОПИЗМ КОРНЕЙ И СТРУКТУРА ИХ СТАТОЦИТОВ, ЭКСПОНИРОВАННЫХ В ВЫСОКОГРАДИЕНТНОМ МАГНИТНОМ ПОЛЕ

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Влияние высокоградиентного магнитного поля (ВГМП) на корни растений приводит к их изгибам, как и в условиях стимуляции гравитацией. Предполагалось, что ВГМП сможет имитировать влияние гравитации в космическом полете и/или изменять его в лабораторных условиях. Параметры кинетики роста и изгибов корней *Pisum sativum* L. под влиянием ВГМП регистрировались видеосистемой и измерялись с помощью программы анализа изображений. Было показано, что применение ВГМП может вызывать отклонения корней. На клеточном уровне мы наблюдали существенные изменения в размещении отдельных клеточных компонентов в статоцитах, локализация статолитов в клетках колумеллы была подобной таковой у горизонтально ориентированных корней. Таким образом, изгибы корней в ВГМП являются реакцией растений на смещение амилопластов пондеромоторной силой. Полученные данные указывают на сходство влияния магнито- и гравистимуляции как на органном, так и на клеточном уровне.

Ключевые слова: *Pisum sativum L., высокоградиентное магнитное поле, гравичувствительность растений, статоцит, ультраструктура, морфометрия*

МАГНІТОТРОПІЗМ КОРЕНІВ ТА СТРУКТУРА ЇХ СТАТОЦИТІВ, ЕКСПОНОВАНИХ У ВИСОКОГРАДІЄНТНОМУ МАГНІТНОМУ ПОЛІ

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Вплив високоградієнтного магнітного поля (ВГМП) на корені рослин призводить до їх вигинів, як і за умов стимуляції гравітацією. Очікувалося, що ВГМП зможе імітувати вплив гравітації у космічному польоті і/або змінювати його за лабораторних умов. Параметри кінетики росту та вигинів коренів *Pisum sativum* L. під впливом ВГМП реєструвалися відеосистемою та вимірювалися за допомогою програми аналізу зображень. Було показано, що застосування ВГМП може викликати відхилення коренів. На клітинному рівні спостерігалися істотні зміни у розміщенні окремих клітинних компонентів у статоцитах, локалізація статолітів у клітинах колумели була подібною до такої у горизонтально орієнтованих коренів. Таким чином, вигин кореня у ВГМП є реакцією рослини на зміщення амілопластів пондеромоторною силою. Отримані дані вказують на подібність впливів магніто- і гравістимуляції як на органному, так і на клітинному рівні.

Ключові слова: *Pisum sativum L., високоградієнтне магнітне поле, гравічутливість рослин, статоцит, ультраструктура, морфометрія*