ВІСНИК ХАРКІВСЬКОГО НАЦІОНАЛЬНОГО АГРАРНОГО УНІВЕРСИТЕТУ
СЕРІЯ БІОЛОГІЯ, 2009, вип. 2 (17), с. 47-55

УДК 581.144.2:581.17:582.739

PROPERTIES OF THE PLASMA MEMBRANE FROM ROOTS OF PEA SEEDLINGS GROWN UNDER ALTERED GRAVITY

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In this study the properties of pea (*Pisum sativum* L.) root plasma membrane (PM) were examined to determine how the membrane properties are maintained in response to clinorotation (chronic disorientation of biological object in relation to the gravity vector) conditions. The membrane preparations enriched by PM vesicles were obtained from 6-day seedlings using aqueous two-phase partitioning. The characteristics of PM ATP-hydrolysis activity, lipid and fatty acid composition and the seedlings growth indices were analyzed. Compared with controls, clinorotated seedlings have shown the change in an orientation of the primary roots and an increased growth of lateral roots. Additionally, the intensity of lateral roots development varied in depending on the orientation of the primary roots. ATP-hydrolysis activity was inhibited by vanadate and activated by potassium and lysophosphatidilcholyne in preparations from both clinorotated and the control seedlings. The phospholipids and neutral lipids were the two major components as well as sitosterol and stigmasterol were predominant free sterol species in PM of both treatments. At the same time, PM preparations from clinorotated seedlings contained a higher level of unsaturated fatty acids and were characterized by a higher double bound index as compared with control ones.

Key words: *Pisum sativum L., clinorotation, plasma membrane, ATP-hydrolysis activity, lipid and fatty-acids composition*

Although the plant cell metabolism is affected by microgravity (Brown et al., 1995; Glaassen, Spooner, 1994; Klymchuk et al., 1999; Korduym, 1997), but it's not clear yet whether the PM is directly targeted to microgravity. At the same time, it's well known that plasma membrane (PM) is involved in many important functions in plant adaptation to environmental stimuli and developmental signals (Navari-Izzo et al., 1993; Palladina, Pedchenko, 1997; Serano, 1989). It suggests that microgravity also can affect at the PM level (Cogoli, 1990; Korduym, 1997; Schatz et al., 1994). PM can be involved in the gravity perception process and differential cell growth of upper and lower tissues in responding region of plant roots. In cooperation with cytoskeleton, it can be also involved in the gravity sensitivity of unspecialized to gravity cells of intact plants or plant cells *in vitro*, whose metabolism has been shown to be also sensitive to gravity environment (Korduym, 1997; Rasmussen et al., 1994).

Unfortunately, little is known about the effects of altered gravity on membrane properties. It might be caused by the difficulties to adapt the experimental techniques to the requirements of spaceflight experiments. Nevertheless, it has been reported that the PM from roots of clinorotated pea seedlings has showed changes in fatty acid composition (Polulyakh, 1988; Polulyakh et al., 1989)

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and lipid peroxidation intensity (Zhadko et al., 1992) as compared with stationary controls. In the experiment with artificial planar lipid bilayers, the activity of alamethicin pores in microgravity was lower than 10% of their ground control activity (Hanke, 1996). Ground-based studies have revealed that PM lipid composition influences the activity of protein kinase C that alters the functions of membrane-associated molecules such as receptors, enzymes, integrin molecules, etc (Nishizuka, 1992).

The plant plasma membrane H^+-ATPase is the primary transporter that pumps protons outside the cell generating the H^+ and electrical potential gradients, which in turn activate the uptake and efflux of ions and metabolites across the membrane (Tanner, Caspari, 1996). The activity of the PM H^+-ATPase is modulated by many factors such as plant hormones, stress conditions (Navari-Izzo et al., 1993; Palladina, Pedchenko, 1997; Serano, 1989). Although the mechanisms by which ATPase activity is regulated are still uncertain, PM phospholipids and sterols have been reported to define the fluidity and permeability of lipid bilayer (Cooke et al., 1993; Pedchenko et al., 1990; Palmgren et al., 1988) and thus the activity of membrane-bound enzymes (Cooke et al., 1993).

This research aims to analyze the physical-chemical properties of the PM to elucidate a possible its role in the cell rearrangements occurring under altered gravity conditions. The specific properties and characteristics of the H^+-ATPase, lipid and fatty acid content and composition in PM-enriched vesicles isolated by aqueous two-phase partitioning from roots of pea seedlings grown under clinorotation (2 rev/min) and stationary conditions were examined.

MATERIALS AND METHODS

Plant material. Pea (*Pisum sativum* L., cv "Intensivniy") seeds were surface-sterilized in 0.01% (v/v) potassium permanganate solute for 20 min and 5% sodium hypochlorite for 10 min, rinsed in distilled water and imbibed for 36 h with running tap water. Then the seeds were rolled in filter paper and placed into clinostat (2 rev/min). Seedlings were daily supplied with distillate water and grown in darkness at 23±1°C. 6-day-old seedlings were harvested for determination of the main root length and root fresh and dry weight. 45 g of roots harvested from approximately 350 seedlings were used for membrane preparations.

PM isolation and assessment of membrane purity and sidedness. PM preparations were isolated using a two-phase aqueous polymer partition system as described by Kjellbom and Larsson (Kjellbom, Larsson, 1984) with some modifications. Roots were homogenized by a blender Multiquick/Minipimer MR 404 (Type 4185) for 90 sec. The homogenate was filtered through nylon cloth with pore size 150 and 100 nm. The filtrate was centrifuged at 10 000 g for 20 min to yield a crude pellet. The supernatant was further centrifuged at 105 000 g for 60 min to yield a microsomal pellet.

For the phase partitioning, 6 ml of the microsomal fraction was applied to 18 g of phase mixture to give a phase system with further purification using a tree-step batch procedure (Kjellbom, Larsson, 1984). PM vesicles from U_3 and U'_3 fractions were combined and pelleted at 105 000 g for 60 min. The pellets were resuspended in 5 mM Mes-Tris buffer, pH 6,5 containing 0,33 M sucrose, 1 mM DTT and used in assay of enzyme activity or stored at -80°C until used. All steps of the isolation procedure were carried out at 4°C.

For plasma membrane purity assessment, the activity of the vanadate-sensitive ATPase as well as dependence of ATP-hydrolysis activity on pH (Briskin et al., 1987) was determined. Cytochrome-c oxidase, NADPH-cytochrome-c reductase, latent IDPase and NO_3^--sensitive ATPase activities were used as markers of mitochondria, endoplasmic reticulum, Golgi membranes and tonoplast (Briskin et al., 1987). The membrane vesicles orientation was determined by assaying ATP-hydrolysis activity in absence and presence of Triton X-100 detergent (Larsson et al., 1994).

H^+-ATPase activities were measured as indicated by Pedchenko et al. (1990). The content of protein was performed according to Bradford (1976) with bovineserum albumin as a standard.

Electron microscopy assays. Isolated membrane pellets were mixed with 1.6 % melted agar (pH 7.2) (1:1, v/v) at 40ºC. The agar drop was cooled and cut with a stale razor. Agar pieces with membrane vesicles and root apices were fixed in a solution of 2,5 % (v/v) glutaraldehyde in 0,05 M sodium cacodylate buffer, pH 7,2, for 1,5 hour, followed by 1 hour incubation in 1 % (v/v) osmium tetroxide in the same buffer. The specimens were dehydrated in a graded ethanol series and embedded in Epon 812-Araldite resins. Silver-gold sections (60±10 nm) were obtained with LKB 8800 ultramicrotome, collected on formvar-coated copper and gold grids, stained with uranyl-lead citrate (Reynolds, 1963) and silicotungstic acid (Roland, 1978), respectively. Specimens were then examined in a JEM-I2OO EX transmission electron mi-

croscope at 60 kV (JEOL, Japan). Morphometric analysis was performed by the method of Wiebel et al. (1966).

Lipid extraction and separation. Lipids were extracted from the PM vesicles in the chloroform-methanol solvent system according to Bligh and Dyer (1959). The extracted lipids were separated into neutral lipids, phospholipids and glycolipids by thin-layer chromatography (TLC). Samples were spotted on silica gel G plates (Silica Gel 60, 0,25 mm thickness; Merck, Germany). The chromatoplates were developed with the solution mixture of 10% methanol in acetone. Lipids were identified by running the commercial standards simultaneously with samples.

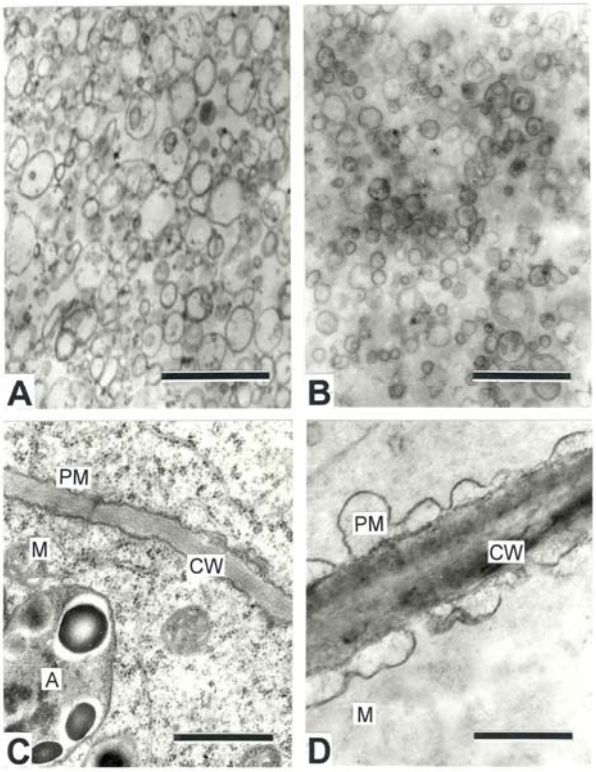
Quantitative analysis of lipids separated by TLC was performed as described by Novitskaya and Ruzkaya (1976). All procedures were performed in the presence of silica gel from TLC plates. Phospholipids were determined by P_i analysis using KH_2PO_4 as standard. Glycolipids were quantified by measuring sugar content using glucose and galactose as standards.

Sterol lipids were fractionated with solvent system benzol:ethanol (90:10). Chromatographic zones of sterols were detected by compare with standard compounds (cholesterol, β-sitosterol, campesterol and stigmasterol) according to their eluation speeds on chromatograms. Sterols were extracted from silica gel, dissolved in chloroform, steamed, weighed, dissolved in hexane and assayed with gas-liquid chromatography technique (Berch, Storrs, 1964) with capillary column.

Fatty acid analysis. For analysis of fatty acid composition, the total lipids were extracted as described above and methylated with 5% H_2SO_4 in methanol. Methyl esters were analysed (Berch, Storrs, 1964) with a gas chromatograph Tsvet-100. The fatty acids were identified by comparing R_ts with those of methyl esters standards. The content of each fatty acid was calculated from the peak area and expressed as a percentage of the total fatty acids. Double bond index (DBI) was calculated according to Lyons et al. (1964).

RESULTS

ATP-hydrolysis activity was inhibited by orthovanadate and was insensitive to nitrate in PM preparations from clinorotated and the control seedlings. The optimum of the ATP-hydrolysis activity was at pH of 6,5 in the both clinorotated and control specimens. Activities of specific markers for tonoplast, mitochondria, endoplasmic reticulum and Golgi apparatus membranes were low or not detectable. The purity of PM preparations composed not less than 90 and 92 % from clinorotated and control seedlings respectively. Using Triton X-100 detergent as a measure of structurally linked latency, 97 and 93 % of the PM vesicles in preparations from clinorotated and the control seedlings respectively were inside out orientation.



Electron micrographs of plasma membrane vesicles (A, B) and fragments of root apex cell (C, D) from 6-day pea seedlings. Sections were stained with lead citrate (A, C) or STA (B, D) specific for plasma membrane. PM – plasma membrane, CW – cell wall, A – amyloplast, M – mitochondrion. Bars equal 1 μm.

Results of electron microscope assays showed that vesicles of microsomal and PM fractions had a rounded form and were soldered. Figure presents the electron micrographs of the PM vesicles (A, B) and fragment of root apex cell (C, D) from 6-day control seedlings. Quantitative morphometric analysis has shown that membrane vesicles stained by silicotungstic acid composed more than 95 % silicotungstic acid staining vesicles (Figure, B). Silicotungstic acid staining of cells from root apices stained only the PM and any other cellular membranes were not seen (Figure, D). These data support the results of biochemical studies that U_3 and U'_3 membrane fractions are composed of PM-derived vesicles.

Table 1

The effects of 6-day clinorotation on length of main roots as well as root fresh and dry weight of pea seedlings

Growth parameters	Control	Clinorotation	Clinorotation / Control
The length of main root, cm	19,1 ± 0,2	16,0 ± 0,3*	83,9 %
Fresh weight of one root, mg	287 ± 12	254 ± 5*	88,3 %
Dry weight of one roots, mg	18,0 ± 0,6	17,0 ± 0,7	96,8 %
Fresh / dry weight	16,1	14,7	91,3 %

Data are means ± SE of twelve independent experiments.
* – significant difference from the control at $P < 0,05$.

Table 2

ATP hydrolysis activity and protein content in PM vesicles isolated from roots of pea seedlings grown 6 days under clinorotation

Indices	Control	Clinorotation
Protein content, μg ml^{-1}	1189 ±11	999 ± 36*
ATP hydrolysis activity, μmol P_i mg^{-1} protein h^{-1}	21 ± 2	25 ± 3

Data are means ± SE of five independent experiments.
* – significant difference from the control at $P < 0,05$.

The analysis of pea seedlings growth showed that approximately a half of clinorotated seedlings had well developed primary roots with usual orientation and developed lateral roots. The primary roots of the other half clinorotated seedlings were oriented in direction of stem growth and with noticeably developed lateral roots of first order. The control seedlings were characterized by well developed primary and faintly lateral roots. The average length of main roots in clinorotated seedlings was 83,9% of the control (Tab. 1). The total fresh and dry weight of roots from clinorotated seedlings consisted 88,3 and 96,8% of the control correspondingly. Root fresh/dry ratios in the clinorotated and control seedlings were 14,7 and 16,1 respectively.

The contents of protein in vesicles of PM preparations from clinorotated seedlings were lower then in the control ones (Tab. 2). By comparison, ATP-hydrolysis activity in the PM vesicles from clinorotated seedlings showed a tendency to higher activity than in the control ones. ATP-hydrolysis activity in vesicles of microsomal membranes from clinorotated seedlings was approximately 2,5 times lower than in the PM vesicles.

The analysis of lipid composition showed that the phospholipids (PL) and the neutral lipids (NL) (primarily the free sterols) were two major components in PM preparations (Tab. 3). The glycolipids such as mono- and digalactosyldiacylglycerols were also founded in PM preparations in very low quantities (3,2-3,3%). PM preparations from clinorotated and control seedlings contained the similar quantities of the phospholipids and glycolipids. The major PM phospholipids were phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with lesser amounts of phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol (PS) and phosphatidyc acid (PA). PC and PE contained more than 80% of the total phospholipids. The PA and PS were detected in low quantities (~1-2 %).

Free sterols composed 0,187±0,016 and 0,204±0,018 mg per mg PM protein in clinorotated and control seedlings respectively (Tab. 3). In sterol fractions, β-sitosterol was the key sterol and composed 55,1 %, stigmasterol and campesterol occupied intermediate place (26,0 and 16,3 % accordingly), amount of cholesterol was 2,6 %.

Fatty acid analysis of total lipids showed that the linoleic and palmitic acid were the predominant fatty acids in PM vesicles from clinorotated and control seedlings (Tab. 4). Stearic, oleic and linolenic linolenic fatty acids were detected in the lesser amounts. Lauric and myristic fatty acids were detected in trace amounts. The amount of unsaturated fatty acids in PM of clinorotated seedlings was increased in comparison with controls. Therefore, the double bound index was higher in

Table 3

Lipid content and composition in PM vesicles isolated from roots of pea seedlings grown 6 days under clinorotation. Lipid content is expressed in mg lipid molecules per mg PM protein

Lipids content	Control	Clinorotation
Total phospholipids	0,614 ± 0,031	0,600 ± 0,029
Total galactolipids	0,033 ± 0,004	0,032 ± 0,004
Total free sterols	0,204 ± 0,018	0,187 ± 0,016
Total neutral lipids	0,374 ± 0,028	0,346 ± 0,024
Total lipids	1,021 ±0,006	0,978 ± 0,005

Data are means ± SE of four independent experiments.

Table 4

Fatty acid composition of the total lipids in PM isolated from roots of 6-day pea seedlings grown under clinorotation. The content of each fatty acid was calculated from the peak area and expressed as a percentage of the total fatty acids

Fatty acids	Total content, %	
	Control	Clinorotation
Lauric (C12:0)	3,3 ± 0,2	trace**
Myristic (C14:0)	trace**	trace**
Myristoleic (C14:1)	4,4 ± 0,2	3,6 ± 0,2*
Palmitic (C16:0)	23,3 ± 1,4	19,6 ± 1,4
Palmitoleic (C16:1)	2,4 ± 0,1	3,0 ± 0,1*
Stearic (C18:0)	4,8 ± 0,2	3,5 ± 0,3*
Oleic (C18:1)	10,9 ± 0,9	7,6 ± 0,5*
Linoleic (C18:2)	25,5 ± 1,8	32,9 ± 2,1*
Linolenic (C18:3)	8,7 ± 0,8	17,4 ± 1,0*
Arachidic (C20:0)	8,2 ± 0,7	7,4 ± 0,8
Eicosenoic (C20:1)	6,6 ± 0,5	4,6 ± 0,2*
Saturated fatty acids	39,6 ± 2,2	30,5 ± 2,5*
Unsaturated fatty acids	58,4 ± 2,2	69,0 ± 1,9*
Saturated fatty acids / unsaturated fatty acids	0,45	0,65
Double bound index	1,18	1,49

Data are means ± SE of three independent experiments.
* – significant difference from the control at $P < 0,05$.
** – total content less than 1 %.

the membrane preparations from clinorotated seedlings in comparison with controls (Tab. 4).

DISCUSSION

The present study shows that an effect of clinorotation on pea seedling growth was associated with an inhibition of primary root growth and the intensification of lateral roots growth. These changes accompanied by reduction in length of main roots and the reduction in root fresh and dry weight as compared with stationary control. It is suggested that exposure of pea seedlings to clinorotation results in their disorientation with respect to gravity vector and the primary roots of the half seedlings grew in the direction of stem growth. When the root tips run up to the edge of filter paper tubes, their growth stopped and forming of lateral roots was intensified. Nevertheless, an intensification of lateral roots growth occurred also in the clinorotated seedlings with usual orientation of primary roots. Therefore, it is suggested that exposure of pea seedlings to clinorotation prevents the

gravistimulation of primary root that stimulates the development of lateral roots.

The changes in growth of roots were revealed under different unfavourable conditions such as altered gravity conditions (Klymchuk et al., 1999), flood (Niki, Gladish, 2001) and hypoxia (Drew et al., 1994). Acclimation of plants to flooding was immediate with forming of vascular cavities in vascular cylinder (Niki, Gladish, 2001) in the elongation region of primary roots. It has been established that development of aerenchyma in flooded tissues is associated with increased activity of cellulases, which are mediated by ethylene (He et al., 1994). It is interesting to note that decreased root growth of soybean seedlings exposed during 6 days in spaceflight conditions was accompanied by increased production of ethylene and characterized by significant reduction in growth of lateral roots in comparison with the controls (Klymchuk et al., 1999).

The reduction in root growth under clinorotation was accompanied by a decrease in the PM total protein amount. At the same time, it is revealed that the ATP-hydrolysis activity in PM preparations from clinorotated seedlings has tendency to an increase in comparison with controls. These data suggest that the differences in the PM ATP-hydrolysis activities are mediated by changes in an activity of H^+-ATPase rather than by changes in the amount of their enzyme molecules. A lower total protein content and higher ATP-hydrolysis activity has been found in membranes of microsomal fractions isolated from roots of corn seedlings under clinorotation and hypergravity (2 g) conditions (Tairbekov et al., 1980). At the same time, hypergravity effects were more essential than clinorotation (Tairbekov et al., 1980). Several studies have been also reported an enhanced plasma membrane ATP-hydrolysis activity in response of plants to environmental and developmental stimuli including fusicoccin (Olivari et al., 2000), salinity and phytohormones (Kabuzenko, 1997), high temperature and prooxidant paraqut (Veselov et al., 2002). Whereas, reduction in the ATP-hydrolysis activity was noted in PM preparations isolated from maize roots affected by growth regulators of N-oxide pyridine family (Belyaeva et al., 1996). Kerkeb at al. (2001) reported that tolerance of tomato calli to 50 mM NaCl resulted in the activation of H^+-pumping without changes in ATP-hydrolysis activity. The involvement of the PM H^+-ATPase activity in plant response to environmental and developmental stimuli including clinorotation may be associated with an intensification of transport processes under unfavourable conditions.

Clinorotation tended to the changes in the sterol content, but there were no noticeable changes in the phospholipid and glycolipid contents. It is known, that structural-functional organization of plant membranes are greatly influenced by its lipid composition, in particular, by sterols that stabilize membranes interacting with phospholipids (Schroeder, 1984). However, high sterol amounts in the lipid bilayer reduce membrane permeability and fluidity (Schroeder, 1984). The tendency to decrease in free sterols content in PM preparations in our studies may be one of the adaptive mechanisms of pea seedlings to clinorotation conditions.

The PM preparations obtained from roots of clinorotated seedlings are characterized by the higher level of unsaturated fatty acids as compared with control. The plant cells membrane functions under different unfavourable conditions correlate with maintenance of an increased levels of unsaturated fatty acids (Szalai et al., 2001). Therefore, noted enhancement of unsaturated fatty acid content may be also the adaptive mechanism of pea seedlings to clinorotation. It has been reported that phospholipids in PM from leaves of wheat seedlings grown in spaceflight were characterized by the higher level of unsaturated fatty acids as compared with the ground controls (Rumyantceva et al., 1990). The lesser level of PM unsaturated fatty acids in roots of clinorotated seedlings relatively to controls reported by (Polulyakh, 1988; Polulyakh et al., 1989) may be explained by the short experiments duration for 24 and 48 hours.

The increase in unsaturated fatty acid content in pea seedling in our study may be attributed to membrane permeability and fluidity, activation of lipid peroxidation (Zhadko et al., 1992). Activated oxygen species in elevated amounts can cause an oxidation of membrane lipid unsaturated fatty acids and consequently membrane injury, protein degradation, enzyme inactivity (Merzlyak, 1989). Therefore, it would be interesting to appreciate whether the alteration in fatty acids content under clinorotation could interfere with membrane permeability and fluidity.

Thus, the data presented here suggest that clinorotation inhibits the growth primary roots and stimulates the growth of lateral roots of pea seedlings. In addition, evaluation of the effects of clinorotation on the physical-chemical properties of the pea root PM suggests that H^+-ATPase activity and fatty acids content may be an important part of the

membrane rearrangements in an adaptation of pea seedlings to altered gravity conditions.

These studies were financially supported by the Science and Technology Center in Ukraine, (Project NN 03).

REFERENCES

Belyaeva N.V., Nasyrova G.F., Simchuk E.E., Ponomarenko S.P. Effect of pirydyn N-oxides and its metylated derivatives on active ion transport in corn seedling roots // Physiologiya Rasteniy. – 1996. – V.43, № 1. – P. 94-100. (In Russian).

Berch G., Storrs E. Gazovaia cromatographiia v biohimii. – Moscow: Mir, 1964. – 618 p. (In Russian).

Bligh E.L., Dyer W.J. A rapid method of total lipid extraction and purification // Canad. J. Biochem. and Physiol. – 1959. – V. 37, № 4. – P. 911.

Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // Anal. Biochem. – 1976. – V. 72. – P. 248-254.

Briskin D.P., Leonard R.T., Hodges T.K. Isolation of the plasma membrane: membrane markers and general principles // Methods Enzymol. – 1987. – V. 148. – P. 542-558.

Brown C.S., Hilaire E.M., Guikema J.A. et al. Metabolism, ultrastructure and growth of soybean seedlings in microgravity: result from the BRIC-01 and BRIC-03 experiments // ASGSB Bull. – 1995. – V. 61, № 9. – P. 93.

Cogoli M. The fast rotating clinostat: A history of its use in gravitational biology and a comparison of ground-based and flight experiment results // ASGSB Bull. – 1990. – V. 56, № 2. – P. 59-68.

Cooke D., Ros R., Burden R.S., James C.S. A comparison of the influence of sterols on the specific activity of the H^+-ATPases in isolated plasma membrane vesicles from oat, rye and rice shoots // Physiol. Plant. – 1993. – V. 88. - P. 397-402.

Drew M.C., Cobb B.G., Johnson J.R. et al. Metabolic acclimation of root tips to oxygen deficiency // Ann. Bot. – 1994. – V. 74. – P. 281-286.

Glaassen D.E., Spooner B.S. Impact of altered gravity on aspects of cell biology // Int. Rev. Cytol. – 1994. – V. 156. – P. 301-373.

Hanke W. Planar lipid bilayer as model system to study the interaction of gravity with biological membranes // 30th Plenary Meeting COSPAR. – 1996. – P. 283.

He C.J., Drew M.C., Morgan P.W. Induction of enzymes associated with lysigenous aerenchyma formation in roots of *Zea mays* during hypoxia or nitrogen starvation // Plant Physiol. – 1994. – V. 105. – P. 861-865.

Kabuzenko S.N. Effect of phytohormones on the resistance of cultivated plants // Physiologiya i Biohimiya Kul'turnih Rasteniy. – 1997. – V. 29, № 3. – P. 220-225. (In Russian).

Kerkeb L., Donaire J.P., Rodriguez-Rosales M.P. Plasma membrane H^+-ATPase activity is involved in adaptation of tomato call to NaCl // Physiol. Plantarum. – 2001. – V. 111. – P. 483-490.

Kjellbom P., Larsson C. Preparation and polypeptide composition of chlorophyll-free plasma membranes from leaves of light-grown spinach and barley // Physiol. Plant. – 1984. – V. 62, № 4. – P. 501.

Klymchuk D.O., Brown C.S., Piastuch W.C., Kordyum E.L.. Alteration in the root tip cells of soybean seedlings grown under microgravity // J. Gravit. Physiol. – 1999. – V. 12, № 1. – P. 97-98.

Korduym E. L. Biology of plant cells in microgravity and under clinostating // Int. Rev. Cytol. – 1997. – V. 171. – P. 1-78.

Larsson C., Sommarin M., Widell S. Isolation of highly purified plant plasma membranes and separation of inside-out and right-side-out vesicles // Methods Enzymol. – 1994. – V. 228. – P. 451-469.

Lyons J.M., Wheaton T.A., Pratt H.K. Relationship between the chilling sensitivity in plants // Plant Physiol. – 1964. – V. 39, № 2. – P. 262-268.

Merzlyak M.N. Activated oxygen and oxidative processes in plant cell membranes // Itogy Nauki i Techniki. VINITI. Seriya Physiologiya Rasteniy. – 1989. – V. 6. – P. 168. (In Russian).

Navari-Izzo F., Quartacci M.F., Melfi D., Izzo R. Lipid composition of plasma membranes isolated from sunflower seedlings grown under water-stress // Physiol. Plantarum. – 1993. – V. 87. – P. 508-514.

Niki T., Gladish D.K. Changes in growth and structure of pea primary roots (*Pisum sativum* L.cv.Alaska) as a result of sudden flooding // Plant Cell Physiol. – 2001. – V. 42, № 7. – P. 694-702.

Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C // Science. – 1992. – V. 258. – P. 607-613.

Novizkaya G.V., Ruzkaya L.A. Quantitaitive determination of chloroplast membrane lipids // Physiologiya Rasteniy. – 1976. – V. 23, № 5. – P. 899. (In Russian).

Olivari C., Albuni C., Pugliarello M.C., De Michelis M.I. Phenylarsine oxide inhibits the fusicoccin-induced activation of plasma membrane H^+-ATPase // Plant Physiol. – 2000. – V. 122. – P. 463-470.

Palladina T.A., Pedchenko V.K. Reconstruction of solubilase plasma membrane H^+-ATPase of corn root cells // Dopovidi Natsional'noyi Academiyi Nauk Ukrayiny. – 1997. – V. 7. – P. 166-170. (In Russian).

Pedchenko V.K., Nasyrova G.F., Palladina T.A. Lysophosphatidylcholine specifically stimulates plasma membrane H^+-ATPase from corn roots // FEBS. – 1990. – V. 275, № 1-2. – P. 205-208.

Palmgren M.G., Sommarin M., Ulvskov P., Jorgensen P.L. Modulation of plasma membrane H^+-ATPase from oat roots by lysophosphatidylcholine free fatty acids and phospholipase A_2 // Physiol. Plant. – 1988. – V. 74. – P. 11-19.

Polulyakh Yu.A. Phospholipid and fatty acid composition of the pea root cell membrane under clinostating // Doklady Academii Nauk Ukrayinskoy SSR. Seriya Biologiya. – 1988. – V. 10. – P. 67-69. (In Russian).

Polulyakh Yu.A., Zhadko S.I., Klimchuk D.A. et al. Plant cell plasma membrane structure and properties under clinostatting // Adv. Space Res. – 1989. – V. 9, № 11. – P. 71-74.

Reynolds S. The use of lead citrate at high pH as an electron opaque stain in electron microscopy // J. Cell Biol. – 1963. – V. 17. – P. 208-212.

Roland J.C. General preparation and staining of thin sections // Electron microscopy and cytochemistry of plant cells / Ed. Hall J.L. – Amsterdam, Oxford, New York: Elsevier, 1978. – P. 1-62.

Rasmussen O., Baggerud C.A., Larssen H.C. et al. The effect of 8 days of microgravity on regeneration of intact plants from protoplasts // Physiol. Plant. – 1994. – V. 92. – P. 404-411.

Rumyantceva V.B., Merzlyak N.N., Mashynsky A.L., Nechitaylo G.S. Influence of space flight on pigment and lipid content of wheat plants // Kosmicheskaya Biologiya i Aviakosmicheskaya Meditsina. – 1990. – V. 2. – P. 53-55. (In Russian).

Schatz A., Reitstetter R., Linke-Hommes A. et al. Gravity effects on membrane processes // Adv. Space Res. – 1994. – V. 14, № 8. – P. 835-843.

Schroeder F. Fluorescent sterols probe molecules of membrane structure and function // Prog. Lipid Res. – 1984. – V. 23. – P. 97-113.

Serano R. Structure and function of plasma membrane ATPase // Annu. Rev. Plant Physiol. Plant Mol. Biol. – 1989. – V. 40. – P. 61-94.

Szalai G., Janda T., Paldi E., Dubacq J.P. Changes in the fatty acid unsaturation after hardening in wheat chromosome substitution lines with different cold tolerance // J. Plant Physiol. – 2001. – V. 158. – P. 663-666.

Tanner W., Caspari T. Membrane transport carriers // Annu. Rev. Plant Physiol. Plant Mol. Biol. – 1996**. –** V**.** 47. – P. 595-626.

Tairbekov M.G., Kabitski E.N., Mailyan E.S. ATPase activity in root cells of maize grown under altered gravitation // Physiologiya Rasteniy. – 1980. – V. 27, № 4. – P. 883-885. (In Russian).

Veselov A.P., Kurganova L.N., Lichacheva A.V., Suschkova U.A. Possible regulator effect of lipid peroxide oxidation on plasma membrane H^+-ATPase activity under the stress conditions // Physiologiya Rasteniy. – 2002. – V. 49, № 3. – P. 385-389. (In Russian).

Wiebel E.R., Kistler G.S., Scherle W.F. Practical stereological methods for morphometric cytology // J. Cell Biol. – 1966. – V. 30. – P. 23-38.

Zhadko S.I., Klimchuk D.A., Kordyum E.L. et al. Lipid peroxidation and the ultrastructural organization of Haplopappus tissue culture cells // Resultati Issledovaniy na Biosputnikach / Ed. Gasenko O. – Moscow: Nauka, 1992. – P. 321-323. (In Russian).

Received
05.05.2009

ВЛАСТИВОСТІ ЦИТОПЛАЗМАТИЧНОЇ МЕМБРАНИ КЛІТИН КОРЕНІВ ГОРОХУ ЗА УМОВ ЗМІНЕНОЇ СИЛИ ТЯЖІННЯ

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Досліджено вплив кліностатування (постійної дезорієнтації біологічного об'єкта відносно вектора сили тяжіння) на фізико-хімічні властивості цитоплазматичної мембрани (ЦМ), виділеної з коренів 6-добових проростків гороху (*Pisum sativum* L.) методом двофазової водно-полімерної системи. Проаналізовано ростові показники проростків, АТФ-гідролітичну активність та ліпідний склад препаратів ЦМ. АТФ-гідролітична активність пригнічувалася ортова-

надатом, підвищувалася в присутності калію, лізофосфатидилхоліну в ЦМ препаратах дослідних і контрольних варіантів. Фосфоліпіди і нейтральні ліпіди були основними ліпідними компонентами, як і ситостерин та стигмастерин – переважаючими вільними стеринами в ЦМ обох варіантів. Порівняно з контрольними, дослідні проростки містили більшу кількість ненасичених жирних кислот, характеризувалися вищим індексом подвійних зв'язків.

Ключові слова: *Pisum sativum L., кліностатування, цитоплазматична мембрана, АТФ-гідролітична активність, ліпідний та жирнокислотний склад*

СВОЙСТВА ЦИТОПЛАЗМАТИЧЕСКОЙ МЕМБРАНЫ КЛЕТОК КОРНЕЙ ГОРОХА В УСЛОВИЯХ ИЗМЕНЕННОЙ СИЛЫ ТЯЖЕСТИ

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Исследовано влияние клиностатирования (постоянной дезориентации биологического объекта по отношению к вектору силы тяжести) на физико-химические свойства цитоплазматической мембраны (ЦМ), выделенной из корней 6-суточных проростков гороха (*Pisum sativum* L.) методом двухфазной водно-полимерной системы. Проанализированы ростовые показатели проростков, АТФ-гидролитическая активность, липидный состав препаратов ЦМ. АТФ-гидролитическая активность ингибировалась ортованадатом, усиливалась в присутствии калия, лизофосфатидилхолина в препаратах опытных и контрольных вариантов. Фосфолипиды и нейтральные липиды были основными липидными компонентами, как и ситостерин и стигмастерин – преобладающими свободными стеринами в ЦМ обоих вариантов. Опытные проростки по сравнению с контрольными содержали большее количество ненасыщенных жирных кислот и характеризовались более высоким индексом двойных связей.

Ключевые слова: *Pisum sativum L. клиностатирование, цитоплазматическая мембрана, АТФ-гидролитическая активность, липидный и жирнокислотный состав*