ФІЗІОЛОГІЯ РОСЛИН

Expression of aquaporin *PIP2;1* as an indicator of *Zea mays* L. cultivar tolerance to reduced soil moisture

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Purpose. To investigate expression of aquaporin *PIP2;1* in maize cultivars 'Pereiaslavskyi' and 'Dostatok', (moderately drought-resistant) and 'Yachta' and 'Flahman' (drought-resistant), which grew for 10 days in low humidity substrate (30%). To evaluate possible influence of lipids and fatty acids on the functional activity of *PIP2;1* under above humidity conditions. **Methods.** Biochemical: study of lipids and fatty acids in cytoplasmic membrane fraction from the roots (liquid chromatography); molecular: detection of the relative expression of aquaporin *PIP2;1* in the roots (polymerase chain reaction, PCR); morphometric measurements and statistical methods for result processing. **Results.** Studies showed that in moderately drought-tolerant maize cultivars 'Pereiaslavskyi' and 'Yachta', *PIP2;1* expression decreased, while in drought-tolerant 'Dostatok' and 'Flahman', on the contrary, it increased. In 'Dostatok' and 'Flahman' smaller root water deficit compared with 'Pereiaslavskyi' and 'Yachta' in conditions of low humidity of the substrate was recorded. In addition, the quantity of sterols and phospholipids increased in the plasma membrane of all hybrids. **Conclusions.** Reduced expression of *PIP2;1* observed in 'Pereiaslavskyi' and 'Yachta', is a characteristic feature of not drought tolerant plants and indicates reaction to a decrease in substrate moisture and counteraction to dehydration, since a smaller amount of aquaporins ensures water retention in the cells. Contrary, at a substrate moisture content of 30%, *PIP2;1* expression in drought-resistant hybrids 'Dostatok' and 'Flahman' increased which was accompanied by lesser root water deficiency (comparing to that of 'Pereiaslavskyi' and 'Yachta'). It is quite probable that the enhanced expression of the *PIP2;1* isoform in cultivars 'Dostatok' and 'Flahman' is a specific indicator of hybrids drought resistance. The obtained data are important for improving the selection of drought resistant maize hybrids.

Keywords: corn; aquaporin PIP2;1, sterols; phospholipids; unsaturated fatty acids; roots; water deficiency; drought tolerance.

Introduction

Moisture deficit is one of the main abiotic stresses affecting a yield, therefore the study of mechanisms of adaptation possibilities in plants, including crops, becomes relevant. It is known that one of the primary targets of external stress is the cellular membrane [1], which reacts to the lack of moisture by biochemical alterations of the lipid bilayer and the change in its viscosity. The fluid state of the cytoplasmic membrane and the activity of aquaporines ensure the stable functioning of the membrane under conditions of lack of moisture. Small hydrophobic aquaphorin pro-

teins (27–30 kDa) in cell membranes are organized in the form of highly conserved tetrameric structures [2–4]. Aquaporins form transmembrane channels through which passive transport of water and dissolved substances occurs among cells, which is the main waterway under drought conditions [5]. 36 genes encoding aquaporines have been identified in the *Zea mays* L. genome [6]. The most numerous family of aquaporines in plants is plasma membrane intrinsic proteins (PIP) aquaporines, divided into two groups: PIP1 and PIP2. The aquaporines of the PIP2 group are expressed primarily in plant roots and characterized by a higher ability to pass water than PIP1-aquaporines [5, 7].

Since PIP2-aquaporines play an important role in the water balance of cells, the level of their genes expression may change during soil dehydration. It is known that moisture deficiency enhances the functioning of aquaporines and increases the number of transcripts

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[8]. The function of aquaporines depends on the stiffness of the lipid bilayer of the cytoplasmic membrane; therefore, in order to understand the nature of the drought resistance of maize hybrids, it is important to investigate the composition of the membrane lipids, determine the level of aquaporin function and reveal their role in the reaction of plants to reduce soil moisture.

The purpose of the research was to study the molecular signs of maize hybrids drought resistance, namely, the quantitative and qualitative composition of the lipids of the root cytoplasmic membrane and the level of expression of the aquaporin PIP2;1 isoform. In the experiment, drought-tolerant hybrids of corn ('Dostatok', 'Flahman') were compared with moderately drought-tolerant ('Pereiaslavskyi', 'Yachta') under conditions of substrate low humidity (30%). Along with this, the morphological parameters of the roots (length) were evaluated, the water deficit of the roots and leaves was determined, as well as the composition of the fatty acids of the cytoplasmic membrane.

Materials and methods

Conditions for plants growing. In vegetative experiments, four hybrids of *Z. mays* were grown: 'Dostatok' (droughtproof and ecologically plastic), 'Pereiaslavskyi' (moderately drought-resistant) – the originator of the Institute of Plant Physiology and Plant Genetics of the National Academy of Sciences of Ukraine (Kyiv), as well as 'Flahman' (high drought tolerance) and 'Yachta' (moderate drought tolerance) – the originator Plant Breeding and Genetics Institute – National Center of Seed and Cultivars Investigation (Odesa). Maize hybrids were harvested in 2014–2017, kept at a temperature of 12–14 °C in the darkness condition in paper envelopes with silicagel.

Corn seeds were presoaked with water on filter paper in the darkness condition for three days. Sand was chosen as a substrate for cultivation, its full moisture capacity was determined and the required humidity was calculated, which amounted to 15.9 g of water per 100 g of dry substrate (sand) 70% of the total capacity of moisture (control) and 6.8 g of water per 100 g of dry substrate 30% of full capacity (experiment) [9]. Three-day-old corn sprouts were planted in vegetation vessels (28 cm in diameter, 20 plants each, filled with a sandy substrate with a humidity of 70%. The plants grew under a transparent tent during the growing season (May-July); the moisture content of the substrate was monitored every other day. In the experiment, the humidity of the substrate was gradually brought to 30% of the total moisture capacity by stopping irrigation and plants were grown on this substrate for the next 10 days. For the experiments plants were used in the fifth leaf phase (according to F. M. Kuperman), 21–22 day age. Material for molecular biological reactions was taken at 10:00 on the day of plant RNA extraction from, the length of the main root was measured (n = 75 for each sample) and the water deficiency of leaves and roots was determined (n = 30 for each sample), which was calculated by the formula: water deficit = 100 × [(amount of water that saturates the organ) − (initial amount of water)] / amount of water that saturates the organ [9]. The experiments were performed in three biological replicates.

PIP2;1 gene expression study. The search for the *PIP2;1* aquaporin gene was performed using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in the international GenBank database, the BLAST online service (http://www.ncbi.nlm.nih.gov/) was used during the selection and testing of primers to the DNA site encoding the conserved part of the protein *PIP2;1*. Primers were synthesized by commercial company Metabion International AG (Germany) on request.

The expression of the aquaporin gene *PIP2;1* was evaluated by performing semi-quantitative reverse transcription polymerase chain reaction (PCR) based on the accumulation of PCR products (amplicon). To isolate the total RNA, 100 mg of the roots of the plants under study were taken, homogenized in a mortar on ice, and isolated by applying the innuPREP Plant RNA kit (AJ Innuscreen GmbH, Germany) according to the manufacturer's specification. Quantitative RNA analysis was carried out using the spectrophotometric method (260 and 280 nm), and the RNA integrity was checked through electrophoresis in a 1% agarose gel. RNA was stored at -70 °C for no longer than three days. Reverse transcription of RNA (1 µg) was performed on the Tercyk amplifier (DNA technology, Russia) using the Revert AidH Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) kit.

To determine the expression of the aquaporin gene *PIP2;1*, specific primers were used: 5'-GTT CCA GAG CGC CTA CTT C -3', 5'- GGG CTT GTC CTT GTT GTA GAT -3' (product length 297 pairs of nucleotide sequences). Density of the aquaporin amplicon *PIP2;1* was compared to that of the 18S rRNA gene

(internal control) with the primers: 5'- GCG AAA GCA TTT GCC AAG G-3', 5'-ATT CCT GGT CGG CAT CGT TTA-3' (product length – 104 pairs of nucleotide sequences). The amplification program included the following steps: initial denaturation – 95 °C, 3 minutes, 25 cycles: 95 °C, 30 seconds; association of primers 59 °C, 30 s; synthesis: 72 °C, 30 s; incubation 72 °C, 1 min. The reaction was stopped by cooling the samples to 4 °C. A solution for amplification without DNA encoding of the sample was taken as a negative control. The amplification product was separated in a 1.5% agarose gel with a TRIS-acetate buffer in the presence of ethidium bromide and visualized in UV light using the Bio-Vision (VILBER LOURMAT) system. DNA Marker – 1 Kb Plus DNA Ladder, Thermo Fisher Scientific. The density of the amplitudes was measured using the GelAnalyzer2010a program (www.GelAnalyzer.exe). The level of relative expression of aquaporin *PIP2;1* was calculated taking into account the density ratio in the gel of the sample products with the product density of the 18S rRNA gene in the samples taken per unit (Fig. 1).
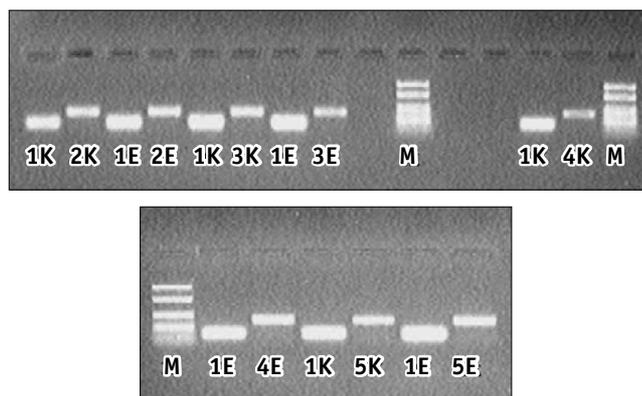

Fig. 1. Agarose gel electrophoresis of products of the reverse transcription reaction of aquaporin *PIP2;1*:
M – molecular marker; 1 – *18S rRNA* internal control for all hybrids; 2 – *PIP2;1* 'Pereyaslavskyi' hybrid; 3 – *PIP2;1* 'Dostatok' hybrid; 4 – *PIP2;1* 'Yachta' hybrid; 5 – *PIP2;1* 'Flahman' hybrid. K – control, substrate humidity of 70%, E – experiment, substrate moisture of 30%

Study of the cytoplasmic membrane fraction. The microsomal fraction enriched with plasmalemma fragments was obtained from the maize roots by partition in an aqueous polymer two-phase system [10] using Optima L-90K centrifuge (Beckman). The purity of the fraction was checked by electron microscopy after the specific coloration of the cytoplasmic membrane vesicles with phosphotungstic acid (PTA) [11]. For this, the plasmalemma fraction was fixed in glutaraldehyde and

processed according to a standard procedure for electron microscopy. On ultrathin sections, the ratio of stained PTA vesicles to unpainted (Fig. 2) was counted. The fraction purity was on average 52–55% (n = 5).
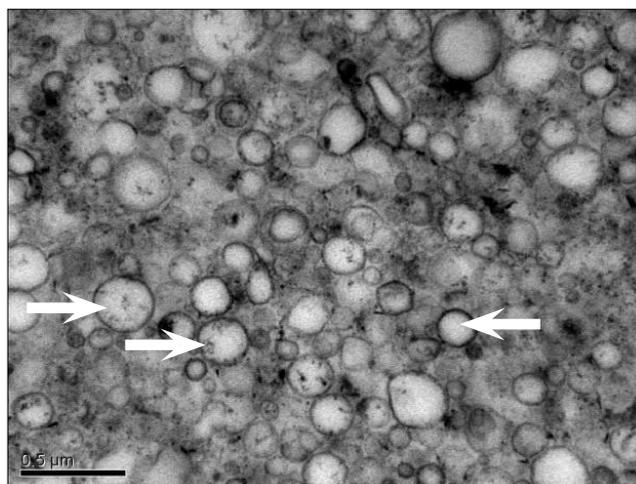

Fig. 2. Microsomal fraction of corn roots enriched with plasma membrane fragments (The arrow indicates plasma membrane vesicles. Scale: 0.5 μm)

The lipid composition of the cytoplasmic membrane fraction was analyzed by high performance phase liquid chromatography on the Agilent 1100 system in the center of HPLC, N. N. Hryshko National Botanical Garden National Academy of Sciences of Ukraine (Kyiv). To isolate lipid components, cytoplasmic membrane preparations separated from the buffer were extracted with isopropanol at a rate of 2 ml per 50 mg of the weight of the isolated membrane. Medium-, low- and non-polar metabolites, in particular sterols and phospholipids, were evaluated semiquantitatively (total pool, chromatographic profile).

To obtain lipid profiles, including sterols and their esters with FA and phospholipids, a tri-eluent scheme was used (eluent A = 0.01 M aqueous solution of orthophosphoric acid, B – was not used, C = acetonitrile, D = isopropanol) on the Thermo Scientific Hypersil™ BDS C18, 3μm, 2.1 × 100 mm. Text and graphic data processing was performed in MS Word 2010, MS Excel 2010 and Corel Draw X3. The position of phospholipids on the chromatogram was determined by the standard preparation of soybean phospholipids (Sigma) and sterols according to the stigmasterol standard (standard stigmasterol solution 10 μg/ml, in chloroform, Supelco) (Fig. 3).

The analysis of fatty acids (FA) was carried out after alkaline hydrolysis of membrane
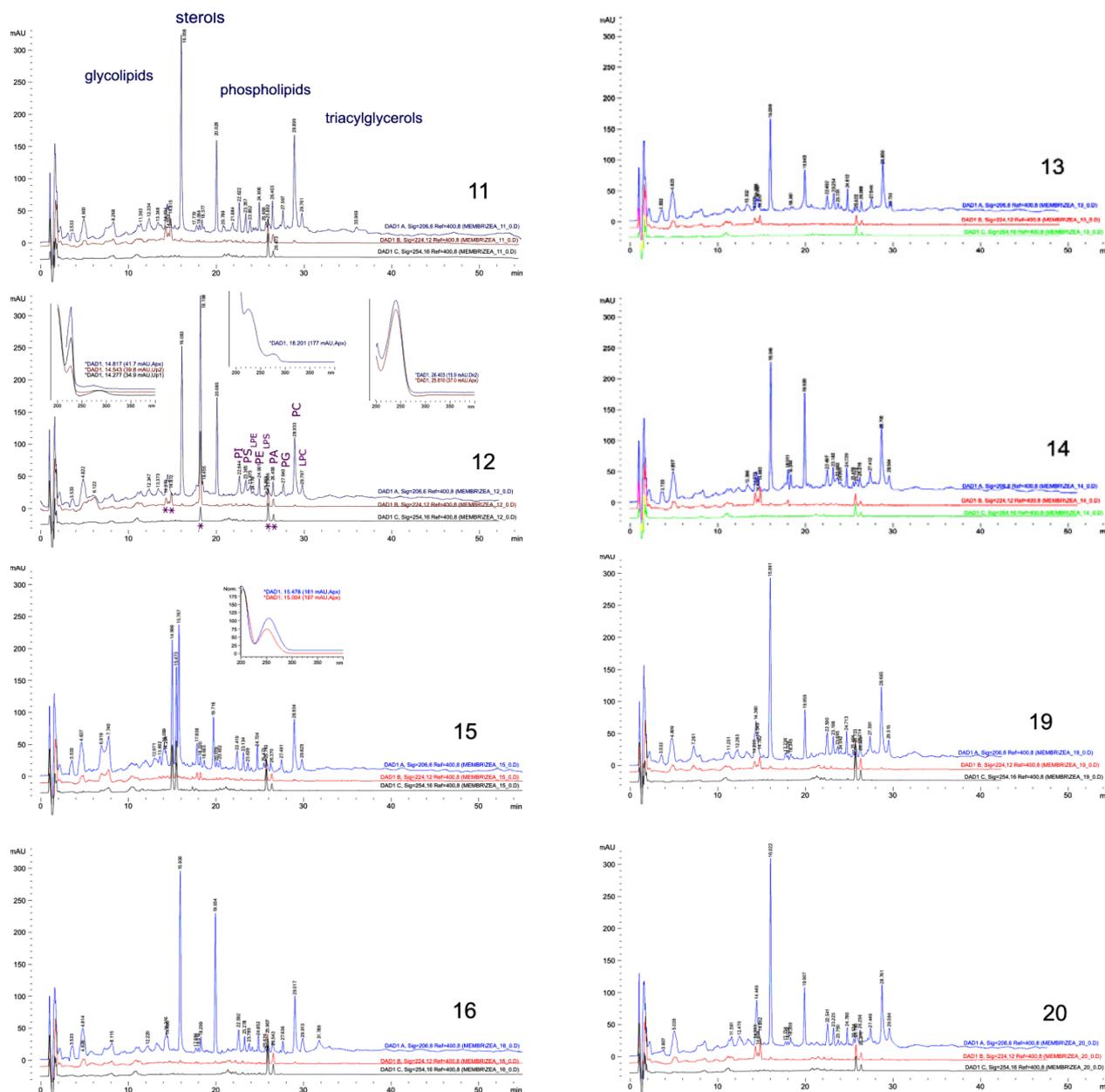
Fig. 3. Chromatographic profiles of plasmalemma lipids, including sterols and phospholipids of maize hybrids:
'Pereiaslavskyi' (11 – control: 70% of substrate moisture, 12 – experiment: 30% of substrate moisture content); 'Yachta' (13 – control, 14 – experiment); 'Dostatok' (15 – control, 16 – experiment); 'Flahman' (19 – control, 20 – experiment)

phospholipids in the form of n-phenacyl bromide derivatives using reverse-phase high performance liquid chromatography on the Angilent 1100 system. A di-eluent system (eluent A was 0.05 M aqueous solution of orthophosphoric acid, eluent B – methanol) on a column of Angilent ZORBAX Eslipse XDB-C18, 5 μm, 4.6 × 250 mm. The basic detection of FA was carried out at 258 nm, the detection threshold of FA > 0,02–0,03 mol%, the analytical error < 2%. Fatty acids were identified by comparing the relative peaking time to the standards [12]. The unsaturated coefficient (C) of the FA was determined as the ratio: Σ unsaturated FAs / Σ saturated FAs.

The experiments were performed in three biological repetitions. The statistically derived data was processed using the Microsoft Excel 2013 program. For each indicator, its mean value and the standard deviation from the mean value were determined.

Results

The study of growth parameters of corn hybrids roots showed a tendency to increase the total length of the main root both in drought-resistant hybrids – 'Dostatok' and 'Flahman', and a moderately drought-tolerant hybrid 'Yachta' under conditions of substrate humidity of 30% (Fig. 4).
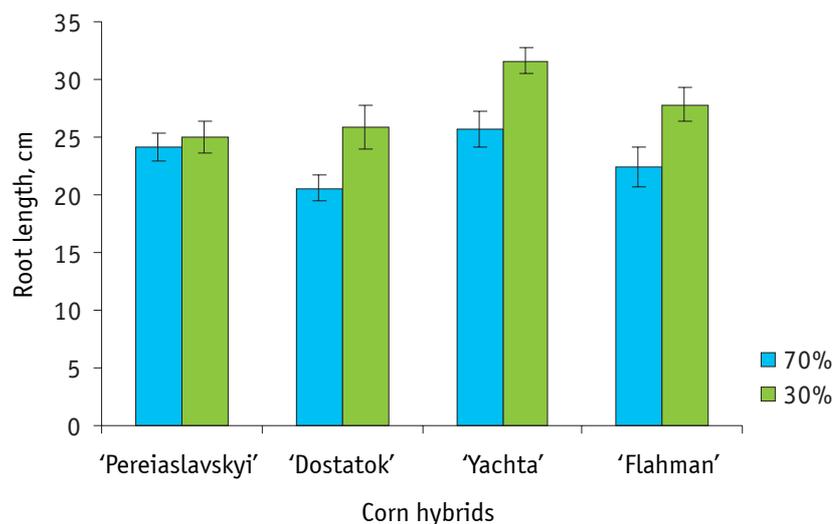
**Fig. 4. Average length of the roots of *Z. mays* L. hybrids
under conditions of substrate humidity of 70 and 30%**
(n = 75 for each specimen)

An increase in the length of the main root is a nonspecific reaction of plants to conditions of decreasing substrate moisture, because the longer and more spacious the root system ensures survival in conditions of water deficiency [13]. In this regard, the almost constant length of the main root of the hybrid 'Pereiaslavskyi' is interesting.

Research on water deficiency of the roots showed an increase in this indicator in moderately drought-resistant hybrids 'Pereiaslavskyi' and 'Yachta' (in 'Yachta' – significantly), which indicates the reaction of plants to stressful conditions (Fig. 5).

In drought-resistant hybrids 'Dostatok' and 'Flahman' root water deficiency with a substrate moisture of 30% was insignificant, which proves the resistance of plants to such conditions. Measurements of leaves water deficiency, on the contrary, did not reveal significant changes in all maize hybrids (not shown), which suggests that 30% moisture of the substrate for 10 days does not noticeably affect the state of the leaves of the studied hybrids.

The determination of the relative expression of aquaporin *PIP2;1* revealed its decrease in the moderately drought-resistant hybrids 'Pereiaslavskyi' and 'Yachta' (Fig. 6), what is the result of the plant resistance to the loss of water.

This is a non-specific feature of drought-tolerant plants, since similar observations were made for many crops [14]. According to [15], and in *Arabidopsis thaliana*, six PIP2 group genes that are expressed in roots, under
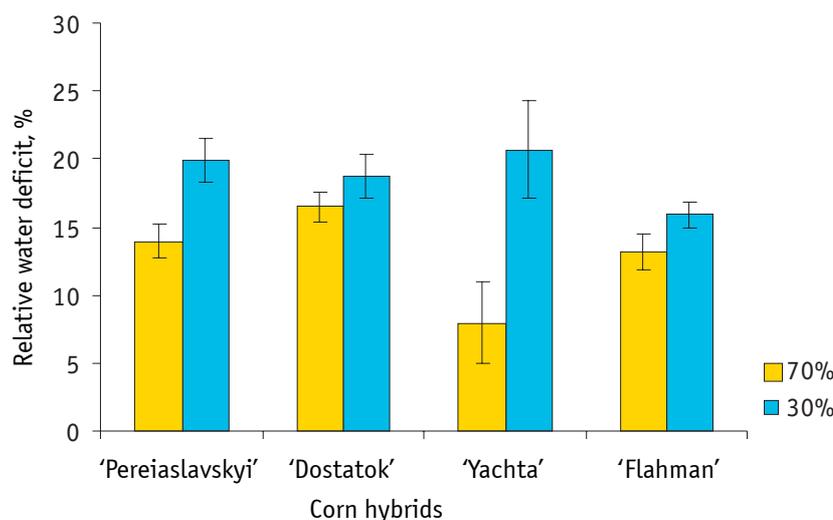

**Fig. 5. Water deficit in *Z. mays* L. hybrids roots under conditions
of substrate humidity of 30 and 70%**
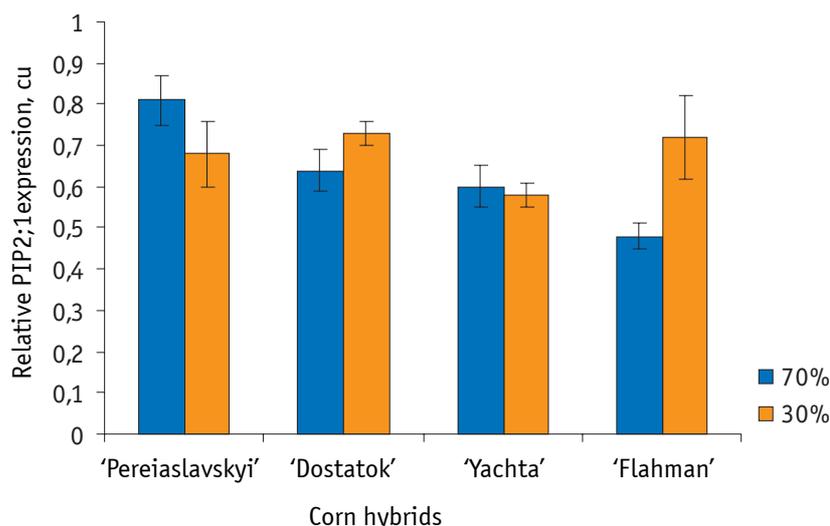(n = 30 for each sample)

Fig. 6. Relative expression level of *PIP2;1* in the roots of the *Z. mays* L. hybrids under conditions of substrate humidity of 30 and 70%

the influence of drought, showed a significant decrease in mRNA accumulation. Similarly, in *Nicotiana tabacum*, the expression of aquaporins of the PIP-family decreased according to the level of drought [8], which is also characteristic of species such as *Fragaria vesca* [16] and *Camellia sinensis* [17].

Artificial stress modeling with 250 mM mannitol solution also affected the rapid decline by almost ten times of *PIP2;2*, *PIP2;3*, and *PIP2;6* and prolonged decrease in *PIP2;7* and *PIP2;8* expression in *A. thaliana* L. [10]. In turn, the ectopic hyperexpression of *PIP2;5* in *N. tabacum* led to increased water loss during dehydration [19].

According to our research, in conditions of low humidity of the substrate (30%) expression of *PIP2;1* increased in drought-resistant hybrids 'Dostatok' and 'Flahman', in contrast to moderately drought-resistant hybrids ('Pereiaslavskyi' and 'Yachta'), (Fig. 6). It should be noted that the level of water loss did not increase in plants (Fig. 5), which proves their resistance to these conditions. The revealed increased expression of *PIP2;1* in drought-resistant maize hybrids somewhat contradicts the general trend of a decrease in the amount of aquaporin proteins in plants under dehydration and drought [8, 14–17]. Therefore, it is possible that increased expression of *PIP2;1* in conditions of reduced substrate moisture is a characteristic feature of drought-resistant hybrids 'Dostatok' and 'Flagman' and may be a specific sign of drought resistance in corn hybrids as a whole.

Increased expression of *PIP2;1* was also noted when wild plants responded to different soil moisture. In particular, a significant dif-ference in the expression of *PIP2;1* was found in the upland *Sium latifolium* L., in which *PIP2;1* expression remained elevated during ontogenesis compared with that in air-aquatic *Sium sizaroideum* L. [20]. An increase in the *PIP2;1* expression level in the upland *S. latifolium* L. may be a species sign of plant adaptation to the land. This is consistent with reports [21] that the expression of the *TaAQP7* aquaporin gene from *Triticum aestivum*, which also belongs to the PIP2 subgroup, determined resistance to the drought of transgenic *N. tabacum*, while the plants were characterized by a stable water status, a decrease in the accumulation of reactive oxygen species and the prevention of membrane destruction [21]. It should be noted that despite the rather long history of studies of aquaporins, many of drought resistance mechanisms with their participation still remain undisclosed. Different gene expression of aquaporins of the PIP2 subgroup in response to water stress suggests that different aquaporin isoforms play different roles in the regulation of water transport [22], which, in general, greatly expands the adaptive capacity of plants.

It is known [23, 24] that the transport of water through aquaporins is regulated by the physical properties of the cytoplasmic membrane, namely its rigidity / fluidity, which contributes to the functioning of proteins. The physical state of the membrane also affects the diffusion of water through the lipid bilayer, and diffusion, in turn, is determined by changes in the composition of lipids (the number of phospholipids, sterols and unsaturated fatty acids) and their packaging in the membrane [25]. Some authors [26] suggest

that changes in the composition of membrane lipids lead to changes in permeability through aquaporins.

Our studies revealed an elevated level of sterols in all hybrids under conditions of 30% moisture content of the substrate, indicating the response of the plasmalemma of the root cells to external stress, namely, the weakening of fluidity and some "thickening" of the membrane. It is known that the functioning of sterols is directly related to their ability to influence the structure of the membrane and its permeability. By interacting with the side chains of fatty acids of phospholipids and the integral proteins of the steroid membrane, they affect the packaging of the membrane bilayer, thereby altering membrane fluidity [27]. It is revealed that the increase in membrane stiffness due to the raise in the number of sterols contributes to the increase of water permeability and gas exchange precisely because of aquaporins proteins.

In particular, it is believed that the efficacy of the aquaporins is closely related to their interaction with the domains of the membrane enriched with sterols [28]. This assertion is based on the fact that the high local concentration of aquaporins in the membrane is observed in domains enriched with sterols with a characteristic high permeability [29]. In addition, it was reported that an increase in the proportion of aquaporins in a detergent-resistant membrane fraction (DRM) enriched in sterols and substantially different from the general fraction of the plasma membrane increases the osmotic penetration of water in the cell membrane at low temperatures and freezes, thereby increasing the survival of the organism [30].

Investigations on the regulation of intracellular transport of aquaporins and their subcellular localization in response to external stresses, such as dehydration and salt stress, revealed that domains enriched with sterols are key in the dynamics of cell surface and endocytosis of aquaporins of the plasma membrane [28, 31]. In particular, the content of sterols is associated with increased or weakened resistance to salt stress, to which aquaporins are also involved [26, 32]. It has been shown that increased salt content generally results in an increase in the total amount of sterols [26, 33].

Based on a decrease in the membrane fluidity with increasing functioning of aquaporins, Frick et al. [24] suggested that membrane rigidity may affect the conformational state of *PIP2;1* shifting the equilibrium toward its open conformation, which facilitates the passage of water. It was also found that the hydration of the membrane increases the space between the acyl chains of fatty acids [34]. Thus, with an increased number of water molecules at the boundary of the lipid / protein compound, due to the increased unsaturation of fatty acids, an increase in permeability through aquaporins was observed [35]. In our studies, it was found that the substrate moisture content of 30%, which increases the expression of *PIP2;1* in general, does not affect water loss in the roots of drought-tolerant hybrids 'Dostatok' and 'Flahman'. It is possible that in these hybrids, the isoform *PIP2;1* participates in the regulation of a different function associated with the transport of soluble substances through the membrane during dehydration. Therefore, it is possible that the increased expression of *PIP2;1* is a specific sign of drought resistance of the hybrids 'Dostatok' and 'Flahman' and differs from that in moderately drought-tolerant 'Pereiaslavskyi' and 'Yachta'.

In all studied hybrids, the number of phospholipids structurally and functionally involved in the plant response to external stress increased. It is known that the composition of phospholipids, their major groups and acyl chains affects the physical properties of plasma membraine, which is essential for the functioning of proteins. The ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) is indicative. It is believed that an increase in the ratio of PC / PE is a sign of maintaining the integrity of the membrane and normalizing of proteins functioning [36].

In experiments, PC / PE ratio significantly increased in moderately drought-resistant hybrids 'Pereiaslavskyi' and 'Yachta', while drought-resistant vice versa: did not undergo changes in the 'Dostatok' hybrid or even decreased in the 'Flahman' hybrid (Table 1).

An increase in the ratio of PC / PE is a non-specific response and indicates the response of plants to the reduced soil moisture. In particular, studies of wheat plasmalemma under drought conditions revealed an increase in the ratio of PC / PE and the level of unsaturation of phospholipid fatty acyl chain. It is believed that this provides a more fluid matrix, preserves the physiological functions of the lipid bilayer [36]. An increase in the PC / PE index was also observed in the sunflower cytoplasmic membrane under conditions of water stress [37]. In our case, an increase in the ratio of PC / PE to moderately drought-tole-

**The content of phosphotidylcholine and phosphotidylethanolamine
in the plasma membrane fraction of corn hybrids roots under conditions
of low moisture content of the substrate**

Corn hybrid	Substrate moisture, (%)	The content of phospholipids, µg/ml		Correlation PC / PE
		Phosphatidylcholine (PC)	Phosphatidylethanolamine (PE)	
'Pereiaslavskyi'	70	26.10	14.20	1.80
	30	26.40	6.90	3.80
'Dostatok'	70	34.00	4.70	7.20
	30	16.40	5.20	3.20
'Yachta'	70	0.64	3.32	0.19
	30	0.45	0.51	0.88
'Flahman'	70	0.77	0.13	5.90
	30	0.57	0.77	0.74

rant hybrids 'Pereiaslavskyi' and 'Yachta' may indicate a counteraction to dehydration in unadapted to drought plants, which is aimed at improving the fluidity of the membrane maintaining its functionality. However, regardless of the fact that the composition of phospholipids can modulate the functioning of aquaporins due to the effect on the plasma membrane flow, in general it is believed that the adaptation of plants to stress depends to a greater extent on aquaporins than on lipid composition [23].

The composition of fatty acids, especially unsaturated ones, also ensures the maintenance of the functional state of the plasmalemma under conditions of dehydration (Table 2).

**Qualitative and quantitative composition of fatty acids of the roots plasma membrane fraction
of corn hybrids under conditions of low substrate moisture content**

The content of phospholipids, µg/ml	Corn hybrids							
	'Pereiaslavskyi'		'Dostatok'		'Yachta'		'Flahman'	
	Substrate *moisture*, %							
	70	30	70	30	70	30	70	30
Saturated fatty acids								
Palmitic acid 16:0	34.1	34.6	36.9	36.4	25.5	25.3	30.6	29.7
Stearic acid 18:0	10.4	8.9	8.1	9.3	4.4	2.9	4.2	6.1
Σ	44.6	43.5	45.0	45.7	29.9	28.2	34.8	35.8
Unsaturated fatty acids								
Oleic acid 18:1	4.5	5.0	6.6	6.0	6.3	3.7	4.2	4.9
Linoleic acid 18:2	34.1	23.8	40.5	39.3	32.4	45.5	34.1	23.8
Linolenic acid 18:3	1.9	1.7	2.2	2.8	2.6	2.8	1.9	1.7
Σ	40.9	30.4	49.3	48.1	41.3	52.0	40.9	30.4
saturated_coefficient	1.2	0.8	1.1	1.0	1.4	1.8	1.2	0.8

In all hybrids (except 'Yachta'), the unsaturated coefficient was close to 1, although the amount of some acids changed (Table 2). In the moderately drought-resistant 'Yachta' hybrid, the unsaturated coefficient was close to 2, what indicates an increase of root membrane fluidity, as a reaction to a reduction in substrate moisture. It is worth noting that the hybrid 'Yachta' was also characterized by the greatest water deficiency in the roots (Fig. 5), which is characteristic of reactions to water stress of unadapted plants.

Conclusions

Reduced expression of *PIP2;1* is a hallmark of moderately drought-resistant hybrids of corn 'Pereiaslavskyi' and 'Yachta' and indicates the reaction of plants to a decrease of moisture by up to 30% in substrate. This may be due to the reduced amount of *PIP2;1* protein, and the resulting resistance to water loss through the membrane. The reaction of the roots to these conditions is also indicated by an increase in their length, root water deficiency and a slight increase in the rigidity of the cytoplasmic membrane.

However, with substrate 30% moisture content expression of *PIP2;1* in drought-tolerant hybrids of corn 'Dostatok' and 'Flahman' on the contrary increased. At the same time, the water deficit of the roots was less than that of moderately drought-resistant hybrids. This suggests that such a sign as the expression is *PIP2*; 1 can be one of the defining signs of drought resistance of these corn hybrids. It is possible that the isoform *PIP2;1* performs a

specific function in ensuring the drought tolerance in plants. It is clear that the drought resistance of corn hybrids is a complex mechanism and is also mediated by many other molecular factors.

The observed molecular signs of drought tolerance can be used to improve the selection of maize aimed at adapting to drought.

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Мета. Дослідити експресію аквапорину *PIP2;1* у гібридів кукурудзи 'Достаток' і 'Флагман' (посухостійкі), а також у 'Переяславський' та 'Яхта' (помірно посухостійкі), які 10 діб зростали в умовах зниженої вологості субстрату (30%). Оцінити можливий вплив ліпідів та жирних кислот цитоплазматичної мембрани на функціонування *PIP2;1* за таких умов. **Методи.** Біохімічні: виявлення складу ліпідів та жирних кислот фракції цитоплазматичної мембрани коренів рослин (рідинна хроматографія); молекулярно-біологічні: дослідження відносної експресії аквапорину *PIP2;1* (полімеразна ланцюгова реакція, ПЛР); морфометричні виміри та статистичні методи обробки результатів. **Результати.** Показано, що в гібридів кукурудзи 'Переяславський' та 'Яхта' експресія *PIP2;1* знижувалася, а в 'Достаток' та 'Флагман', навпаки, підвищувалася. У коренях 'Достаток' і 'Флагман' в умовах зниженої вологості субстрату фіксували менший дефіцит води порівняно з 'Переяславський' та 'Яхта'. Крім того, у цитоплазматичній мембрані всіх гібридів збільшувалася кількість стеринів і фосфоліпідів. **Висновки.** Зниження експресії *PIP2;1*, відзначене в 'Переяславський' та 'Яхта', є характерним для нестійких рослин і свідчить про їхню реакцію на зниження вологості субстрату та протидію зневодненню, оскільки менша кількість аквапоринів забезпечує утримання води в клітинах. Водночас, за вологості субстрату 30% експресія *PIP2;1* у посухостійких гібридів 'Достаток' та 'Флагман', навпаки, підвищувалася на фоні меншого водного дефіциту коренів (порівняно з 'Переяславський' та 'Яхта'). Цілком імовірно, що посилена експресія ізоформи *PIP2;1* саме в 'Достаток' та 'Флагман' є специфічним індикатором посухостійкості гібридів. Отримані дані є важливими для вдосконалення селекції посухостійких гібридів кукурудзи.

Ключові слова: *кукурудза; аквапорин PIP2;1; стерини; фосфоліпіди; ненасичені жирні кислоти; корені; водний дефіцит; посухостійкість.*

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Цель. Исследовать экспрессию аквапорина *PIP2;1* у гибридов кукурузы 'Достаток' и 'Флагман' (засухоустойчивые), 'Переяславський' и 'Яхта' (умеренно засухоустойчивые), которые 10 суток росли при пониженной влажности субстрата (30%). Оценить возможное влияние состава липидов и жирных кислот на функциональную активность *PIP2;1* в данных условиях. **Методы.** Биохимические: исследование состава липидов и жирных кислот фракции цитоплазматической мембраны корней (жидкостная хроматография); молекулярно-биологические: выявление относительной экспрессии аквапорина *PIP2;1* в корнях (полимеразная цепная реакция, ПЦР); морфометрические измерения и статистические методы обработки результатов. **Результаты.** Показано, что у гибридов кукурузы 'Переяславський' и 'Яхта' экспрессия *PIP2;1* снижалась, а у гибридов 'Достаток' и 'Флагман', наоборот, повышалась. В корнях 'Достаток' и 'Флагман' в условиях пониженной влажности субстрата отмечали сниженный дефицит воды по сравнению с 'Переяславський' и 'Яхта'. Кроме того, в цитоплазматической мембране всех гибридов увеличивалось количество стеринов и фосфолипидов. **Выводы.** Снижение экспрессии *PIP2;1*, отмеченное у гибридов 'Переяславський' и 'Яхта' является характерным для нестойких к засухе растений и свидетельствует об их реакции на снижение влажности субстрата, а также, противодействие обезвоживанию, поскольку меньшее количество аквапоринов обеспечивает задержку воды в клетках. В то же время, при влажности субстрата 30%

экспрессия *PIP2;1* у засухоустойчивых гибридов 'Достаток' и 'Флагман', наоборот, усиливалась на фоне меньшего водного дефицита корней (по сравнению с гибридами 'Переяславський' и 'Яхта'). Не исключено, что усиленная экспрессия *PIP2;1* именно у данных гибридов 'Достаток' и 'Флагман' является специфическим индикатором засухо-устойчивости. Полученные данные вносят существенный вклад в совершенствование селекции засухоустойчивых гибридов кукурузы.