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Water deficit induces changes in H⁺-ATPase activity and its gene expression in *Zea mays* L. roots

(Presented by Corresponding Member of Ukraine E.L. Kordyum)

*We investigated the functioning and the gene expression of H⁺-ATPase in the microsomal fraction enriched with plasmalemma vesicles isolated from roots of two *Zea mays* cultivars: Dostatok (drought resistant) and Pereyaslivskiy (moderately tolerant to drought). Both cultivars grew during 21 days under the optimal water supply (soil moisture – 70 %, control) and during 10 days under the water deficit conditions (soil moisture – 30 %, experiment). An increase in the H⁺-ATPase hydrolytic activity in both cultivars under the water stress has been detected under drought. The enzyme hydrolytic activity is increased by twice in Dostatok and by 1.3 times in Pereyaslivskiy. The H⁺-ATPase gene expression levels in both cultivars also increased under water deficit. It is shown that, in roots of both maize cultivars, the increase of four isoforms of H⁺-ATPase gene expression correlates with the increase of the hydrolytic enzyme activity under water stress conditions. However, these parameters are more sustainable in the drought-resistant cultivar Dostatok, by allowing one value to use the hydrolytic activity of H⁺-ATPase as a biomarker of drought resistance for corn varieties.*

Keywords: *H⁺-ATPase, microsomal fraction enriched with plasmalemma vesicles, gene expression, water regime, *Zea mays* L.*

Water deficit appears to be the most limiting factor for the crop production. Flowering plants affected by drought usually survive the harsh environmental conditions, by promptly activating the adaptive resistance mechanisms. The plant adaptation to water deficit depends on various traits and mechanisms that are involved in such conditions. The plant plasmalemma H⁺-ATPase is an important functional protein, which plays a central role in the plant physiology both under normal growth conditions and under abiotic stresses. H⁺-ATPase is involved in many different physiological processes. What is more, its activity can be changed by many of physiological factors [1]. A nonspecific response of plants to stress includes several interrelated mechanisms. We investigated the H⁺-ATPase functioning and its gene expression in the microsomal fractions enriched with vesicles of plasmalemma (PM) isolated from roots of maize plants of two cultivars: Dostatok (drought-resistant) and Pereyaslivskiy (moderately tolerant to drought). Plants grew in containers with sand substrate for 21 days. The plants grew under conditions of a sufficient water supply (moisture content of the substrate was 70 %) and under conditions of water stress when the substrate moisture was 30 % for 10 days. Plasmalemma fractions were obtained using the two-phase aqueous polymer technique [2]. The H⁺-ATPase hydrolytic activity was determined by the quantity of released inorganic phosphorus in nmol PO₄³⁻/mg protein / min. H⁺-ATPase gene expression was assessed on the basis of the mRNA accumulation, by using Rt-PCR method.

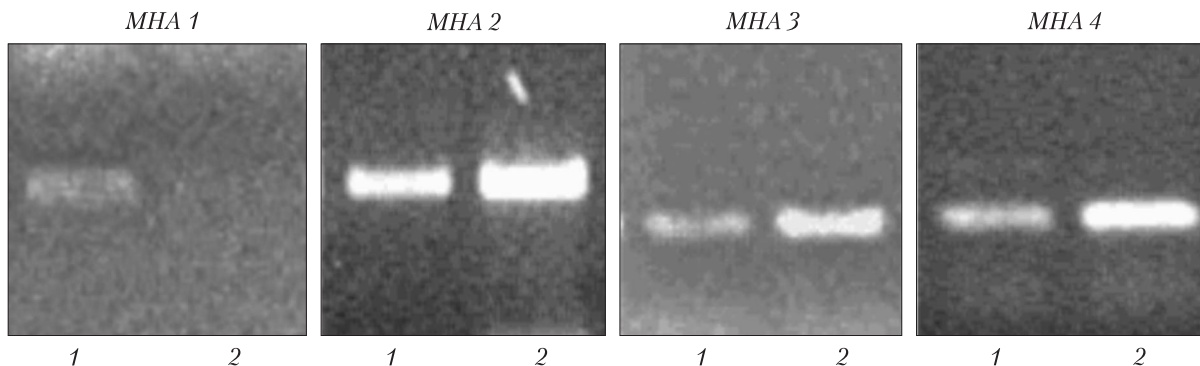


Fig. 1. RT-PCR analysis of gene expression of four H⁺-ATPase isoforms in roots of Dostatok cultivar under different soil moisture; 1 – 70 %; 2 – 30 %

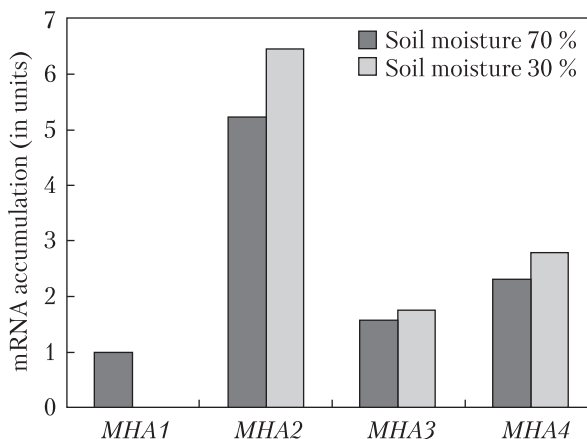


Fig. 2. mRNA accumulation levels of four H⁺-ATPase isoforms in roots of Dostatok cultivar under different soil moisture

An increase in the H⁺-ATPase hydrolytic activity of a plasmalemma root under drought stress was observed (Dostatok showed increase in 2 times and Pereyaslivskiy – increase by 1.3 times) (Table 1). The increase of the enzyme activity indicates that plants are under the stress caused by a water deficit in soil. The obtained data show that drought-resistant cultivar Dostatok is characterized by a more stable reaction to water deficit as compared to drought-sensitive cultivar Pereyaslivskiy. Abiotic stress conditions cause an increase of the root plasmalemma H⁺-ATPase hydrolytic activity of both investigated plants.

The H⁺-ATPase plasmalemma activity is regulated not only via the protein molecular activity, but also through the regulation of its own and regulatory protein genes expression. Plant H⁺-ATPase is coded by the multigene family. 4 isoforms of the enzyme (*MHA1*, *MHA2*, *MHA3*, and *MHA4*) have been found in *Zea mays* [3]. Changes of H⁺-ATPase activity in plasmalemma may result from H⁺-ATPase gene expression changes [4, 5]. We showed the presence of four H⁺-ATPase isoforms (*MHA1*, *MHA2*, *MHA3*, *MHA4*) in Dostatok and Pereyaslivskiy cultivars root cells and investigated their gene expression patterns. It was revealed that the H⁺-ATPase gene expression level in Dostatok root cells increased under the water deficit conditions. *MHA2* and *MHA4* isoform genes demonstrated the highest expression level under water deficit (6.44 and 2.78 relative units, respectively). Whereas isoform *MHA3* demonstrated a slightly lower gene expression level (1.78 in relative units), and the *MHA1* gene expression level was undetectable. Under the control condi-

Table 1. H⁺-ATPase hydrolytic activity in plasmalemma isolated from roots of *Zea mays* plants grown under different soil moistures

Soil moisture, %	H ⁺ -ATPase hydrolytic activity (nmol PO ₄ ³⁻ /mg protein/min)	
	Cultivars	
	Dostatok	Pereyaslivskiy
70	225.67 ± 52.8	117.93 ± 27.05
30	449.0 ± 59.59	156.88 ± 64.65

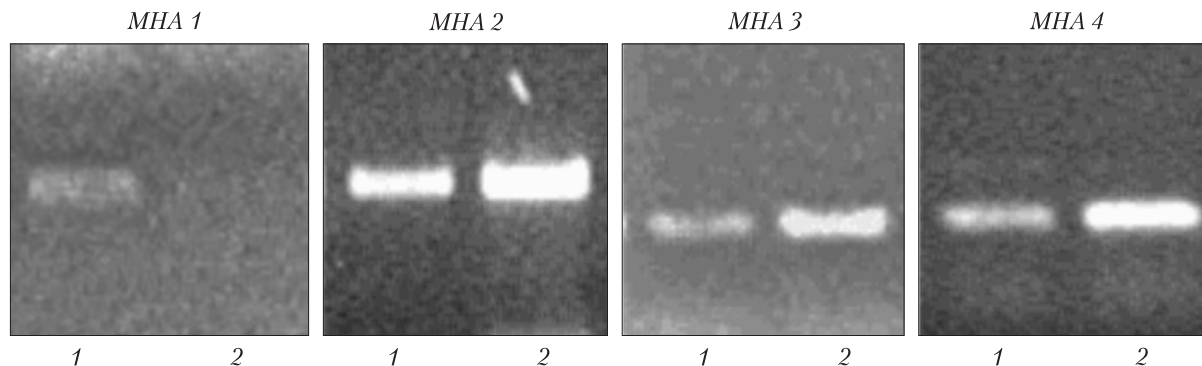


Fig. 3. RT-PCR analysis of gene expression of four H⁺-ATPase isoforms in roots of Pereyaslivskiy cultivar under soil moisture of 70 % (1) and 30 % (2)

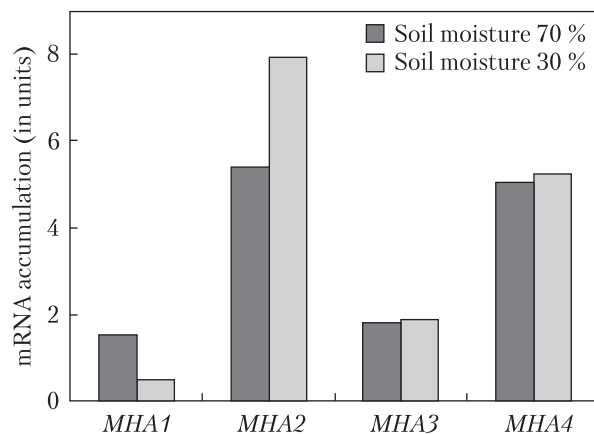


Fig. 4. mRNA accumulation levels of four H⁺-ATPase isoforms in root of Pereyaslivskiy cultivar under different soil moisture

tions (soil moisture – 70 %), *MHA2* and *MHA4* isoforms also demonstrated the higher gene expression level (5.22 and 2.33 in relative units, respectively) as compared to *MHA3* and *MHA1* (1.56 and 0.98 in relative units, respectively) (Fig. 1, 2).

Under the water stress *MHA2* and *MHA4* isoform genes in Pereyaslivskiy cultivar also demonstrated a higher expression level (7.91 and 5.24 in relative units, respectively) comparing to *MHA3* and *MHA1* isoforms (1.9 and 0.5 relative units, respectively). Under the control conditions (soil moisture – 70 %), *MHA2* and *MHA4* isoforms demonstrated gene expression levels of 5.38 and 5.03 relative units, *MHA3* and *MHA1* – 1.82 and 1.53 in relative units, respectively (Figs 3, 4).

Thus, the gene expression of all four isoforms of H⁺-ATPase in root cells of *Zea mays* increased, when plants were exposed to a water deficit. The data correlated with our results obtained for the H⁺-ATPase hydrolytic activity in roots of both cultivars. The results demonstrate that the H⁺-ATPase hydrolytic activity increases under water stress. It is known that *MHA1* gene expresses at the very low levels in all types of maize tissue [6]. Our results confirmed low level of the *MHA1* gene expression in root cells and indicate that *MHA1* gene plays an insignificant role in the electrochemical potential formation in plasmalemma. The highest expression level was observed for *MHA2* gene in both plant cultivars. This gene is considered to be the basic common H⁺-ATPase isoform with high expression level in root cells [7]. *MHA4* gene also demonstrated the high expression level in studies. Similar results were demonstrated in the study of the influence of nitrates on the H⁺-ATPase gene expression in *Zea mays* root cells [3]. It is believed that there are four paths of H⁺-ATPase activity regulation: at the transcriptional level, covalent regulation, allosteric regulation, and lipid regulation [8]. We suppose that the plasmalemma H⁺-ATPase resistant mechanisms, which are activated by different stresses in root cells of both investigated cultivars, are regulated by the gene expression. *MHA2* and *MHA4* isoform genes showed the highest expression level, and they obviously play a crucial role in stress responses. It is shown that, in roots of both maize cultivars, the increase of four

isoforms of H⁺-ATPase gene expression correlates with an increase of the hydrolytic enzyme activity under water stress conditions. However, these parameters are more sustainable in the drought-resistant cultivar Dostatok, by allowing us to use the hydrolytic activity of H⁺-ATPase as a biomarker of the drought resistance for corn varieties.

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ЗМІНИ АКТИВНОСТІ H⁺-АТФази ТА ЕКСПРЕСІЇ ЇЇ ГЕНІВ У КОРЕНЯХ ZEA MAYS L., ІНДУКОВАНІ ВОДНИМ ДЕФІЦИТОМ

Досліджено функціонування H⁺-АТФази в мікосомальній фракції, збагаченій фрагментами плазмалеми (ПМ) з коренів 21-добових рослин кукурудзи двох сортів: Достаток (посухостійкий) та Переяслівський (не посухостійкий), які зростали за умов достатнього водозабезпечення, при вологості субстрату 70 % та за умов водного дефіциту, протягом 10 діб, при вологості субстрату 30 %, а також експресію генів цього ензиму. Виявлено підвищення гідролітичної активності H⁺-АТФази в ПМ з коренів кукурудзи за умов посухи вдвічі в сорту Достаток і в 1,3 рази — в сорту Переяслівський. Встановлено, що рівень експресії генів у обох сортів за умов водного дефіциту був вищим, ніж за умов достатнього водозабезпечення, що корелює з даними гідролітичної активності ензиму, яка також була вищою в умовах дефіциту води. Причому ці показники мали більш сталий характер у посухостійкого сорту Достаток, що дає підставу використовувати значення гідролітичної активності H⁺-АТФази як біомаркер посухостійкості сортів кукурудзи.

Ключові слова: H⁺-АТФаза, мікосомальна фракція, збагачена фрагментами плазмалеми, експресія генів, водний режим, *Zea mays* L.

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ИЗМЕНЕНИЯ АКТИВНОСТИ H⁺-АТФазы И ЭКСПРЕССИИ ЕЕ ГЕНОВ В КОРНЯХ ZEA MAYS L., ИНДУЦИРОВАННЫЕ ВОДНЫМ ДЕФИЦИТОМ

Проведены исследования функционирования H⁺-АТФазы в микросомальной фракции, обогащенной фрагментами плазмалеммы (ПМ) из корней 21-суточных растений кукурузы двух сортов: Достаток (засухоустойчивый) и Переясловский (не засухоустойчивый), которые росли в условиях достаточного водообеспечения, при влажности субстрата 70 % и в условиях водного дефицита, на протяжении 10 суток, при влажности субстрата 30 %, а также экспрессии генов этого энзима. Установлено повышение гидролитической активности H⁺-АТФазы в ПМ из корней кукурузы в условиях засухи в два раза у сорта Достаток и в 1,3 раза у сорта Переясловский. Показано, что уровень экспрессии генов у обоих сортов в условиях водного дефицита был более высоким, чем в условиях достаточного водообеспечения, что коррелирует с данными по определению гидролитической активности энзима, которая также была более высокой в условиях дефицита воды. Причём эти показатели имели более устойчивый характер у засухоустойчивого сорта Достаток, что позволяет использовать значения гидролитической активности H⁺-АТФазы в качестве биомаркера засухоустойчивости сортов кукурузы.

Ключевые слова: H⁺-АТФаза, микросомальная фракция, обогащенная фрагментами плазмалеммы, экспрессия генов, водный режим, *Zea mays* L.