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PIP2;1 aquaporin gene expression in maize hybrids different for drought tolerance to water deficit

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At the mRNA level, different PIP2;1 aquaporin gene expressions in roots of drought-tolerant and drought-sensitive maize genotypes with sufficient substrate moisture, as well as their distinct responses towards water deficit, are shown. An analysis of the obtained data allow us to consider PIP2;1 expression as a possible molecular marker in maize breeding for drought tolerance.

Keywords*: Zea mays L., drought tolerance, PIP2;1-aquaporin expression, RT-PCR.*

To date, it is well known that aquaporins, which are membrane proteins forming the water channels, ensure the transport of water through membranes and/or facilitate the transport of small neutral solutes (urea, boric acid, silicic acid) or gases (ammonia, carbon dioxide), and thus they are involved in many basic vital important processes such as cell signalling, stress responses and nutrient acquisition [1–3]. The multiple mechanisms, including the regulation of transcript or protein abundance, subcellular trafficking, or gating by phosphorylation or cytosolic protons, can regulate the aquaporin transport activity [2].

Aquaporins of the plasmalemma (plasma membrane intrinsic proteins, PIPs) are the most numerous family in plants, which is divided in 2 subfamilies: PIP1 and PIP2, the latter is characterized with a significantly more capability of the water tranport in comparison with PIP1[4]. In the *Arabidopsis thaliana* L. genome, there are 35 genes coding aquaproteins [5, 6], 36 genes — in *Zea mays* L. [7], and 33 genes in *Oryza sativa* L. [8]. Different responses of PIP aquaporin genes to water stress — enhancement or weakening of the expression or absence of any response — have been established that supposes their unequal role in the regulation of the water transport through a membrane. It is suggested that aquaporins play distinct roles in facilitating a water flux and maintaining the water potential in cells along with various water transport activities. In addition, aquaporins are involved in the plant adaptation to water deficiency [3, 9]. Therefore, an aim of our work was to study the PIP2;1 aquaporin gene expression in four, different for drought resistant hybrids of *Z. mays* grown under conditions of low soil moisture.

Material and Methods. Four corn hybrids were selected, namely Dostatok (drought resistant) and Pereiaslavskii (moderately drought resistant) kindly provided by the Institute of Physiology and Genetics of the National Academy of Sciences of Ukraine (Kyiv), and Flagman (drought resistant) and Yacht (moderately drought resistant) kindly provided by the Breeding and Genetic Institute – a National Center for Seed Research and Variety Research (Odessa). 120 plants were grown in vegetation vessels filled with sandy substrate of optimum 70 % moisture under the tent during 10 days. Then 60 plants continued to grow under these conditions and were kept as controls, 60 plants continued to grow in the substrate of 30 % moisture for 10 days as this period of drought is critical for plant growth. Experiments were performed in three replicates. Substrate moisture was controlled every two days according to [10, pp. 75-77].

The relative amounts of expression of mRNA encoding *PIP2;1* aquaporin were determined in corn roots by the reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using TRI-REAGENT (Sigma) according to the manufacturer protocol. Yield was determined using the absorbance of RNA at 260 nm. RT-PCR was carried on the FERMENTAS protocol (Lithuana) with an amplificator Terzik (DNA-technology, Russia). PCR was carried using the obtained cDNA and primers with 19-21 nucleotides:

Forward 5′- GTT CCA GAG CGC CTA CTT C -3′

Reverse 5′- GGG CTT GTC CTT GTT GTA GAT -3′.

The PCR products were separated on 1.5 % agarous gel, visualized in ultraviolet light and photographed using the system of gel visualization Bio-Vision (VILBER LOURMAT). Actin gene expression was used as an endogenic control on passing the reaction with primers of 18–20 nucleotides.

Forward 5′- GTT CCA ACC ATC CCT TGT-3′

Reverse 5′- CGT GAT CTC CTT GCT CAT AC-3′.

The level of gene expression was measured using GelAnalyzer2010a (www.GelAnalyzer.exe). Software Image Mater Total Lab™ was used to estimate the quantity of products. The obtained data are processed statistically using Microsoft Excel 2013.

Results and Discussion. At the mRNA level, different expressions of *PIP2;1* in roots of drought-tolerant and drought-sensitive genotypes in normal conditions (substrate moisture 70 %), as well as their distinct responses towards water deficit (substrate moisture 30 %) (Fig. 1, 2), are shown.

Expression of *PIP2;1* increased in Dostatok and Flagman growing on the substrate with 30 % moisture, while expression of this gene lowered or remained at about the same level in Pereiaslavskii and Yacht under these conditions. In addition, these data indicate up-regulation of *PIP2;1* transcripts in drought-resistance hybrid and down-regulation of transcripts in drought-sensitive upon water deficit, which supposes the genotype-specific aquaporin transcription in investigated maize genotypes.

The obtained results are consistent with available literature data on the participation of PIP aquaporins in plant responses to drought stress. So, distinct responses of PIP aquaporin genes to water stress by treatment with 20 % PEG have been revealed in *Oryza sativa* L. cultivars: drought-resistance upland Zhonghan 3 (spp. Indica) and drought-sensitive lowland Xiushui 63 (spp. Japonica) [11]. PIP aquaporin gene expression was significantly up-regulated in upland rice, whereas it remained unchanged or down-regulated in lowland rice. Up- or down-regulation of

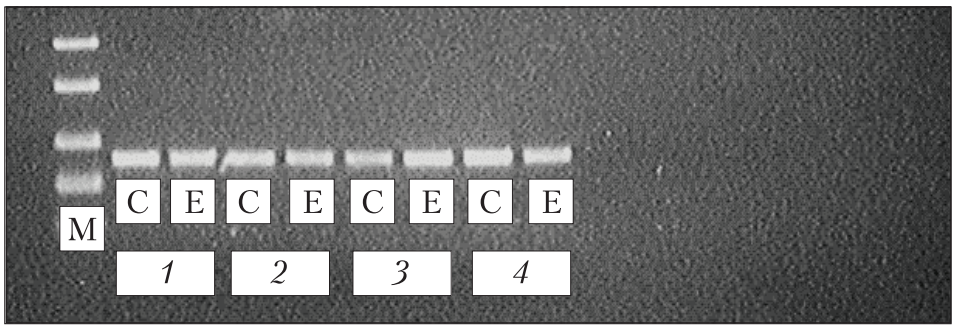

Fig. 1. Agarous gel-electrophoresis with the products of RT–PCR, *PIP2;1* gene. *1* – Pereiaslavskii; *2* – Dostatok; *3* – Yacht; *4* – Flagman. M — marker, C — control (substrate moisture 70 %), E — experiment (substrate moisture 30 %)

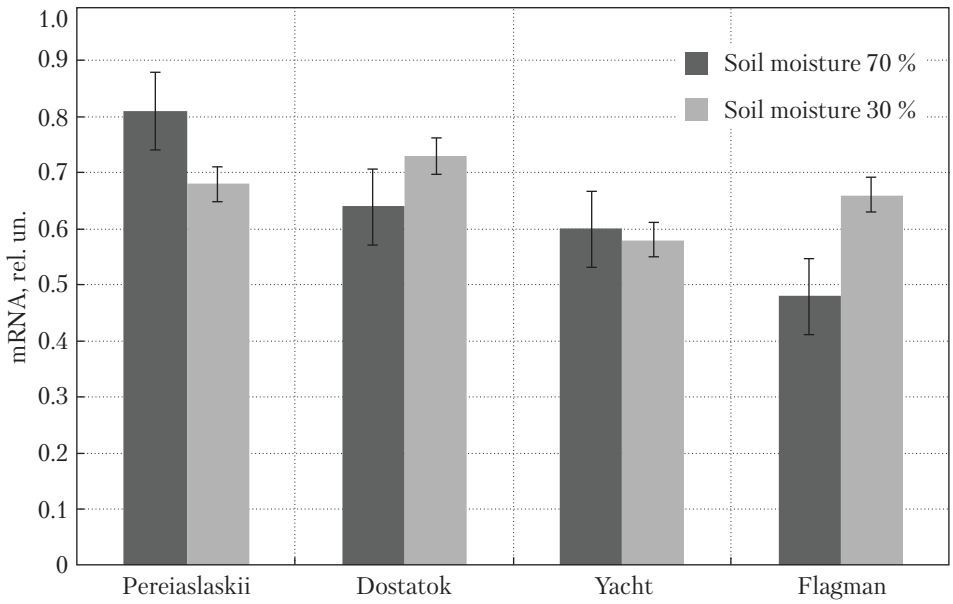

Fig. 2. *PIP2;1* aquaporin gene expression in roots of maize hybrids different for drought tolerance grown in substrates with 70 % and 30 % moisture

PIP genes was also described in *A. thaliana* under water deficit after the treatment with a 250-mM mannitol solution [6, 7]. Aquaporin gene expression varied in a *Vitis* hybrid (*V. berlandieri* × *V. rupestris*) Richter-110 depending on the type of aquaporin and the water stress intensity [12].

The essential differences in PIP2 aquaporin gene expression has been revealed in closely related, but ecologically different *Sium latifolium* L. and *Sium sisaroideum* L. The level of mRNA of *PIP2* aquaporin was higher in *S. sisaroideum* terrestrial plants growing under the conditions of moderate water deficit in comparison with that in aerial-aquatic *S. latifolium* growing in a river near the bank, and it increased when the temperature rose to 35—40 °C [13]. PIP aquaporin gene divergent expressions were shown in tolerant and sensitive genotypes of *Saccharum officinarum* L. under normal and drought stress conditions, pointing to the aquaporin transcription

in this species to be potentially genotype-specific [14], that is especially important for scanning molecular markers for plant breeding.

In our experiments, the genotype-specific regulation of PIP aquaporin gene expression was also revealed in responses of investigated maize hybrids different for drought tolerance to water deficiency. In addition, up-regulation of *PIP2;1* aquaporin gene in maize drought-resistance hybrid Dostatok under 30 % substrate moisture correlated with the essential enhancement in the H^+-ATPase hydrolytic activity, which was higher about three times in comparison with that in more drought-sensitive hybrid Pereiaslavskii [15]. The obtained data allow us to consider the *PIP2;1* aquaporin gene expression as a molecular marker in maize breeding for drought tolerance.

REFERENCES

1. Tyerman, S. D., Niemietz, C. M. & Bramley, H. (2002). Plant aquaporins: multi functional water and solute channels with expanding roles. Plant Cell Environ., 25, No. 2, pp. 173-194. PMID: 11841662
2. Maurel, C., Verdoucq, L., Luu, D. T. & Santoni, V. (2008). Plant aquaporins: membrane channels with multiple integrated functions. Annu. Rev. Plant. Biol., 59. pp. 595-624. doi: https://doi.org/10.1146/annurev.arplant.59.032607.092734
3. Li, G., Santoni, V. & Maurel, C. (2014). Plant aquaporins: roles in plant physiology. Biochim. Biophys. Acta.,1840, pp.1574-1582. doi: https://doi.org/10.1016/j.bbagen.2013.11.004
4. Chaumont, F., Barrieu, F., Jung, R. & Chrispeels, M. (2000). Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. Plant Physiol., 122. pp. 1025-1034. doi: https://doi.org/10.1104/pp.122.4.1025
5. Jang, J. Y., Kim, D. G., Kim, Y. O., Kim, J. S. & Kang, H. (2004). An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in *Arabidopsis thaliana*. Plant Mol. Biol., 54, No. 5, pp. 713-725. doi: https://doi.org/10.1023/B:PLAN.0000040900.61345.a6
6. Alexandersson, E., Fraysse, L., Sjövall-Larsen, S., Gustavsson, S., Fellert, M., Karlsson, M., Johanson, U. & Kjellbom, P. (2005). Whole gene family expression and drought stress regulation of aquaporins. Plant Mol. Biol., 59. No. 3, pp. 469-484. doi: https://doi.org/10.1007/s11103-005-0352-1
7. Chaumont, F., Barrieu, F., Wojcik, E., Chrispeels, M. J. & Jung, R. (2001). Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol., 125, pp. 1206-1215. PMCID: PMC65601
8. Sakurai, J., Ishikawa, F., Yamaguchi, T., Uemura, M. & Maeshima, M. (2005). Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol., 46, No. 1, pp. 1568-1577. doi: https://doi.org/10.1093/pcp/pci172
9. Kapilan, R., Vaziri, M. & Zwiazek, J. J.(2018). Regulation of aquaporins in plants under stress. Biol Res., 51, No. 4. doi: https://doi.org/10.1186/s40659-018-0152-0
10. Voizechivska, O. V., Kapustian, A. V., Kosyk, O. I., Musienko, M. M., Olchovich, O. P., Paniuta, O. O., Parshikova, T. V. & Slavnii, P. S. (2010). Plant Physiology: Practicum. Lutsk: Teren (in Ukrainian).
11. Lian, H. L., Yu, X., Lane, D., Sun, W-N., Tang, Z-C. & Su, W-A. (2006). Upland rice and lowland rice exhibited different PIP expression under water deficit and ABA treatment. Cell Res., 16, pp. 651-660. doi: https://doi.org/10.1038/sj.cr.7310068
12. Galmes, J., Pou, A., Alsina, M. M., Tomas, M., Medrano, H. & Flexas, J. (2007). Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): relationship with ecophysiological status. Planta, 226, No. 3, pp. 671-681. doi: https://doi.org/10.1007/s00425-007-0515-1
13. Bliuma, D. (2010). PIP2 — aquaporin gene expression of *Sium latifolium* L. under different water supply. Nauk. zap. Ternop. nac. ped. un-tu., Ser. Biol., 45, No. 4, pp. 3-8 (in Ukrainian). doi: https://doi.org/10.15407/ukrbotj74.01.080
14. Da Silva, M. D., de Oliveira Silva, R. L., Neto Ferreira, J. R. C., Guimarães, A. C. R., Veiga, D. T., Chabregas, S. M., Burnquist, W. L., Kahl, G., Benko-Iseppon, A. M. & Ederson, A. K. (2013). Expression analysis of sugarcane aquaporin genes under water deficit. J. Nucleic Acids, 763945, doi: https://doi.org/10.1155/2013/763945

15. Ovrutska, I. I. & Bluma, D. A. (2016). Water deficit induces changes in H^+-ATPase activity and its gene expression in *Zea mays* L. roots. Dopov. Nac. acad. nauk Ukr., 10, pp. 84-87. doi: https://doi.org/10.15407/dopovidi2016.10.084

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ЕКСПРЕСІЯ ГЕНА АКВАПОРИНУ *PIP2;1* У РІЗНИХ ЗА СТІЙКІСТЮ ДО ПОСУХИ ГІБРИДІВ *ZEA MAYS* L.

На рівні mRNA показано відмінності в експресії гена *PIP2;1* аквапорину в коренях стійких і чутливих до посухи гібридів кукурудзи при достатній вологості субстрату, а також різні реакції генотипів на водний дефіцит. На підставі одержаних даних експресію гена *PIP2;1* можна розглядати як можливий молекулярний маркер в селекції кукурудзи на посухостійкість.

***Ключові слова**: Zea mays L., посухостійкість, експресія гена PIP2;1-аквапорин, ПЛР зі зворотною транскрипцією.*

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ЭКСПРЕССИЯ ГЕНА АКВАПОРИНА *PIP2;1* У РАЗЛИЧНЫХ ПО СТОЙКОСТИ К ЗАСУХЕ ГИБРИДОВ *ZEA MAYS* L.

На уровне mRNA показаны отличия в експрессии гена *PIP2;1* аквапорина в корнях стойких и чувствительных к засухе гибридов кукурузы при достаточной влажности субстрата, а также различные реакции генотипов на водный дефицит. Анализ полученных данных позволяет рассматривать экспрессию гена *PIP2;1* в качестве возможного молекулярного маркера в селекции кукурузы на засухоустойчивость.

***Ключевые слова**: Zea mays L., засухоустойчивость, экспрессия гена PIP2;1-аквапорин, ПЦР с обратной транскрипцией.*