Application of antisense oligonucleotide method in cucumber.

Zastosowanie metody z użyciem antysensowych oligonuklotydów u ogórka.

Anna Wdowikowska
Department of Plant Molecular Physiology, Faculty of Biological Sciences, Wroclaw University, Kanonia 6/8, 50-328 Wroclaw, Poland;
anna.wdowikowska@uwr.edu.pl

An **A**nti**S**ense **O**ligonucleotides (ASO) method was used to change the expression of *CsHA2* gene encoding one of the major and better characterized isoform of the cucumber plasma membrane (PM) proton pump. PM H^+-ATPase plays a crucial role in the regulation of many fundamental for growth and development of plants physiological processes but also plays a special role in adaptation of plants to environmental stresses.

In order to obtain active ASOs, the introns and exons in *CsHA2* sequence (Gene Bank EU735752.2) were identified using the bioinformatics programs. ASOs were designed in the antisense orientation for different part of the *CsHA2* gene (5'UTR, exons, introns, 3'UTR) using Sfold software (sfold.wadsworth.org). Two delivery strategies were used to ASO introduction into 5 days old cucumber seedlings: in 100 mM sugar solution and vacuum infiltration. In both analyzed delivery methods 30 μM of ASO were used.

In the case of sucrose solution, treatment of cucumber seedlings with 200 mM sucrose was causing wilting however, the use of 100 mM sucrose was sufficient for effective ASO delivery and proper condition of the plants. In the case of ASO delivery by vacuum infiltration, whole cucumber seedling were incubated in a water with ASOs in a vacuum chamber for 15 minutes. After this time, the plants were transferred to the medium for 24 h.

The expression changes of *CsHA2* gene were analyzed in cucumber roots via Real-Time PCR performed using Real-Time 2xPCR Master Mix SYBR B (A&A Biotechnology). Primers used for reactions (For 5'-ACCCGAGTCGACAAACATCT-3' and Rev 5'-CTTGGCACAGCAAAGTGAAA-3') come from Santi et al. 2005. The reference gene was clatryne (CACS Clathrin adaptor complex subunit – Gene Bank GW881874.1; For 5'-TGGGAAGATTCTTATGAAGTGC-3' and Rev 5'-CTCGTCAAATTTACACATTGGT-3').

The treatment of cucumber seedlings with ASOs homologous to the different part of *CsHA2* gene and saccharose solution revealed changes in *CsHA2* expression. The greatest effect was observed in the case of ASO directed to the 3'UTR region (5' TTTTCTTTCCTTGGCACAGC-3'): this ASO activated *CsHA2* gene expression tree times in cucumber roots (Figure 1A). The vacuum infiltration method did not give satisfactory results, what is evidenced by only mild changes of *CsHA2* expression level after ASO treatment (Figure 1B).

These results indicated that ASO application with sugar solution is effective and can be used to study *CsHA2* gene function in cucumber and analysis of signal transduction pathways leading to plant adaptation to environmental stresses, in which the proton plasma membrane pump plays an important role.

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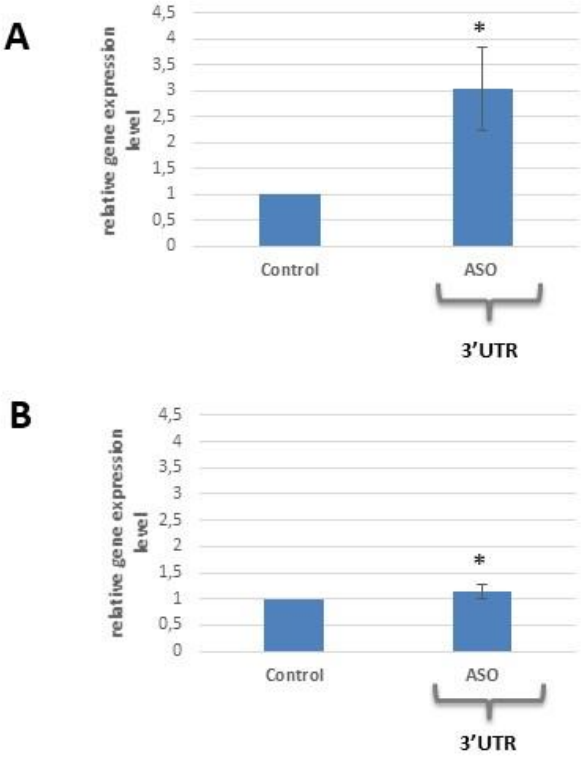

Figure 1. Expression level of *CsHA2* gene in the roots of cucumber seedlings treated with 100 mM sucrose and 30 µM of ASO for 24 h (**A**) (control plants were grown in 100 mM sucrose for 24 h) or were incubated with 30 µM of ASO in vacuum chamber by 15 min. (B). The data are the results of the real-time PCR reaction. The clatryne gene (CACS) was used as reference. Transcript levels of *CsHA2* were normalized to the levels observed in the control plants (set as 1). Each value is presented as relative gene expression level. Data constitute the mean value ±SD from at least two technical repeats of four independent biological samples. The significance of the differences between each mean and control was determined by Student's t-test. Asterisk indicates $p < 0,05$.

Santi S, Cesco S, Varanini Z, Pinton R (2005) Two plasma membrane H^+ATPase genes are differentially expressed in iron-deficient cucumber plants. Plant Physiol and Biochem 43: 287-292