

Expression in potato plants of phosphomimetically mutated gene *AteIF2 α* , coding for alpha subunit of translation initiation factor 2 from *Arabidopsis thaliana*, provides resistance to drought

Karpova O.*, Alexandrova A., Nargilova R., Beisenov D., Stanbekova G., Kryldakov R., Yeriskina E., Nizkorodova A., Polimbetova N., Zhigailov A., Iskakov B.

M. Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan

* e-mail: oxkarpova@mail.ru

The cDNA-gene *AteIF2 α* from *A. thaliana* was amplified by RT-PCR and cloned in pUC19 vector. *In vitro* mutagenesis was carried out replacing the 56th serine codon with triplet encoding phosphomimetic aspartic acid. *AteIF2 α (S56D)*-gene was cloned in binary agrobacterial vector pCambia2300 under the control of either constitutive (*35SCaMV*) or stress-inducible (*rd29A*) promoter. Translational enhancer Ω (5'UTR of TMV) was inserted downstream of *rd29A* promoter.

The resulting DNA constructs [*35SCaMV*-(*His-tag*)-*AteIF2 α (S56D)*-*nos_pCambia*] and [*rd29A*- Ω -(*His-tag*)-*AteIF2 α (S56D)*-*nos_pCambia*] were transfected into agrobacteria cells, which were then used for vacuum infiltration of tobacco leaves for transient expression. Using RT-PCR, the transcription of (*His-tag*)-*AteIF2 α (S56D)*-transgene is shown in leaves on the third (from *35S*) and fifth day (from *rd29A*). On the same days, the synthesis of (*His-tag*)-*AteIF2 α (S56D)*-protein was confirmed by immunoblotting using anti(*His-tag*) antibodies.

Stable transformation of virus-free potato plants of 'Milena' variety was carried out. Regenerated plants were tested by PCR for presence of transgenic inserts. Total RNA preparations were isolated from transgenic plants and analyzed by RT-PCR to assess the levels of transgene mRNA synthesis. Synthesis of mRNA was confirmed only in plant lines that were genetically modified (GM) by DNA-construct [*rd29A*- Ω -(*His-tag*)-*AteIF2 α (S56D)*-*nos_pCambia*]. These lines were propagated and tested for resistance to elevated temperatures and to water deficiency.

In particular, the tested GM-potato line No. 91 expressing the *AteIF2 α (S56D)* transgene showed significant resistance to drought. Similarly, all control potato plants of 'Milena' variety died after 14 days without watering, whereas all GM-plants of No. 91-line survived after 21 days of drought.

This technology for improving plant resistance to abiotic stresses can apply not only to potatoes, but also to other economically important crops.