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ВЛИЯНИЕ МУТАЦИИ АУТОИНГИБИТОРНОГО ДОМЕНА CDPK1 *ARABIDOPSIS THALIANA* НА ИНДУЦИРОВАННОЕ ТЕПЛОВЫМ СТРЕССОМ СТАРЕНИЕ В ТРАНСГЕННЫХ РАСТЕНИЯХ ТАБАКА

Effect of mutation in autoinhibitory domain of CDPK1 *Arabidopsis thaliana* on heat-induced stress senescence in transgenic tobacco plants

EFFECT OF MUTATION IN AUTOINHIBITORY DOMAIN OF CDPK1 *ARABIDOPSIS THALIANA* ON HEAT-INDUCED STRESS SENESCENCE IN TRANSGENIC TOBACCO PLANTS

O.A. Tikhonova[1], G.N. Veremeichik[1], V.P. Grigorchuk[1]

1- Federal Scientific Center of East Asian Terrestrial Biodiversity FEB RAS

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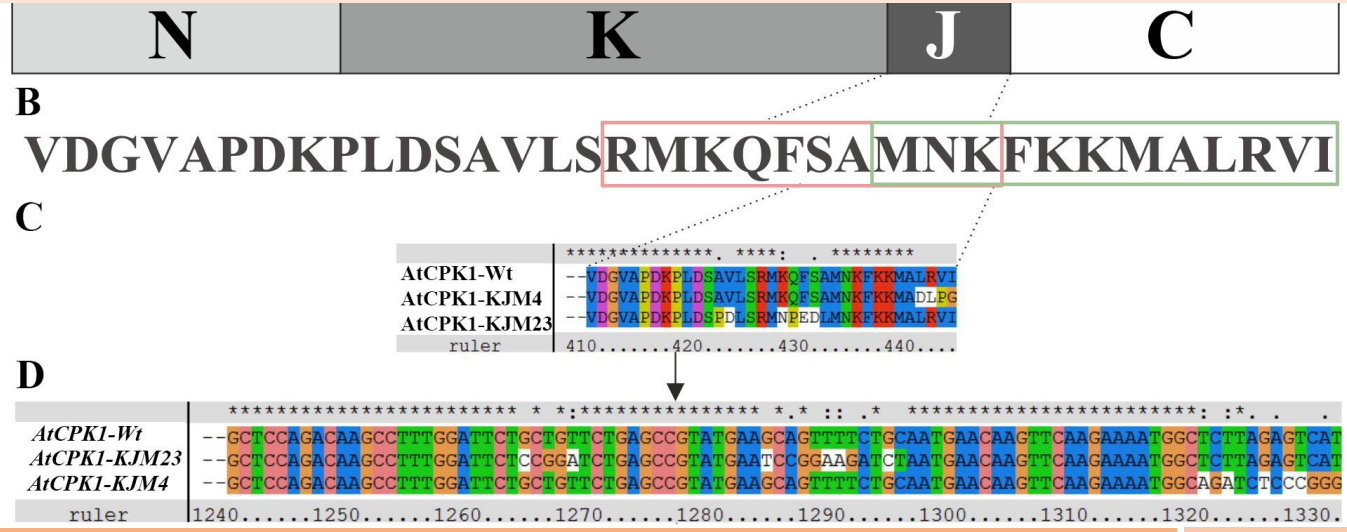
This study aimed to investigate whether a mutation in the junction domain of the CDPK1 in *Arabidopsis thaliana*, which renders it independent of fluctuating cytoplasmic calcium ion levels, affects the tolerance of *Nicotiana tabacum* L. plants to high temperatures and heat-stress-induced senescence.

Domain structure of CDPK: **N**-terminal variable domain, responsible for localization and specific recognition; **C**-terminal Ca^{2+}-binding domain; **K**, kinase and **J** junction, domain, which inhibits kinase activity in the absence of calcium ions



The object of the study was tobacco plants into which several variants of the Arabidopsis CPK1 gene were transferred by agrobacterial transformation. They correspond to the name of the mutant forms that were obtained in the 90s by Harper and Huang while studying the mechanism of pseudosubstrate inhibition [1].

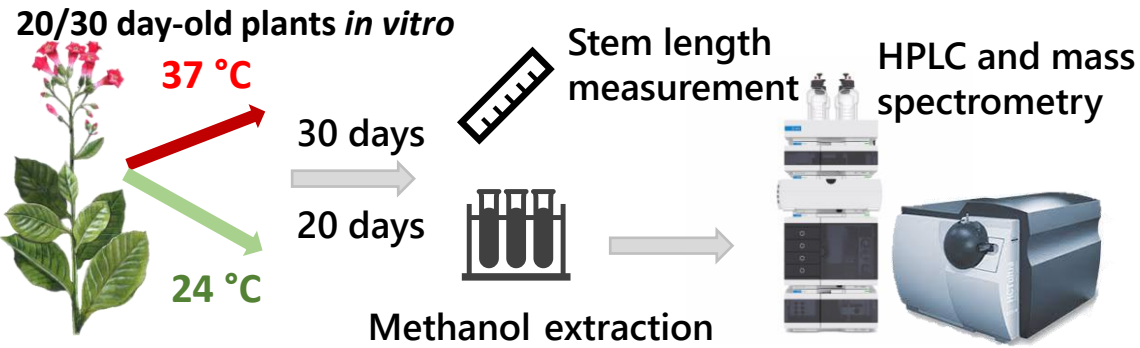
AtCPK1-Wt
- Ca^{2+} activity 1.5%
+ Ca^{2+} activity 100%

AtCPK1-KJM4
- Ca^{2+} activity 0.6%
+ Ca^{2+} activity 1.2%

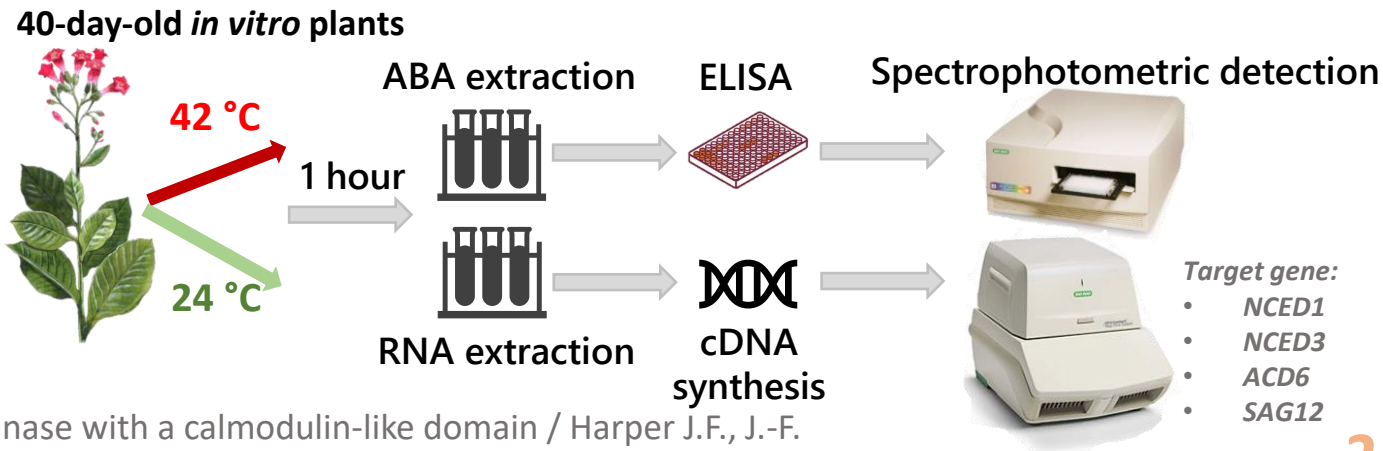
AtCPK1-KJM23
- Ca^{2+} activity 86%
+ Ca^{2+} activity 97%

The native form **WT** of CPK1 functions only in the presence of calcium;
The mutation **KJM4** has been shown to lead to a reduction in enzyme activity to almost zero, irrespective of calcium stimulus.
The **KJM23** mutation, a 6-amino acid substitution in the central part, had the opposite effect and made the enzyme activity close to 100%, regardless of the calcium stimulus.

Modeling the effects of prolonged heat stress

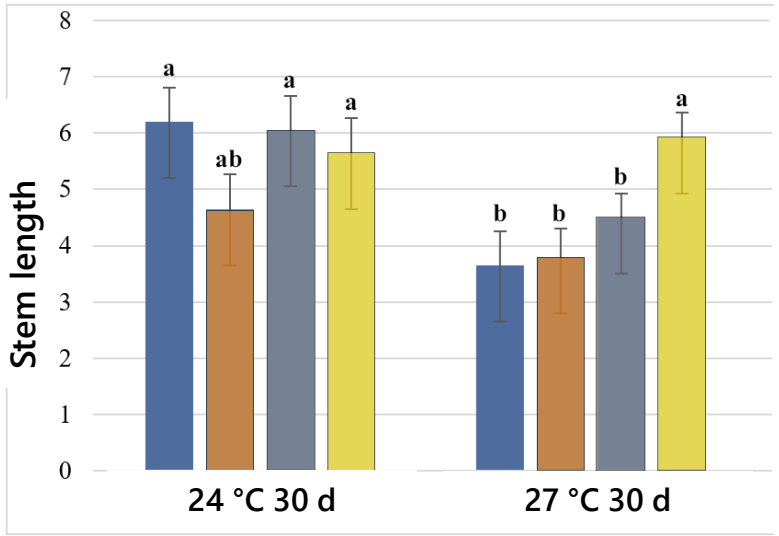


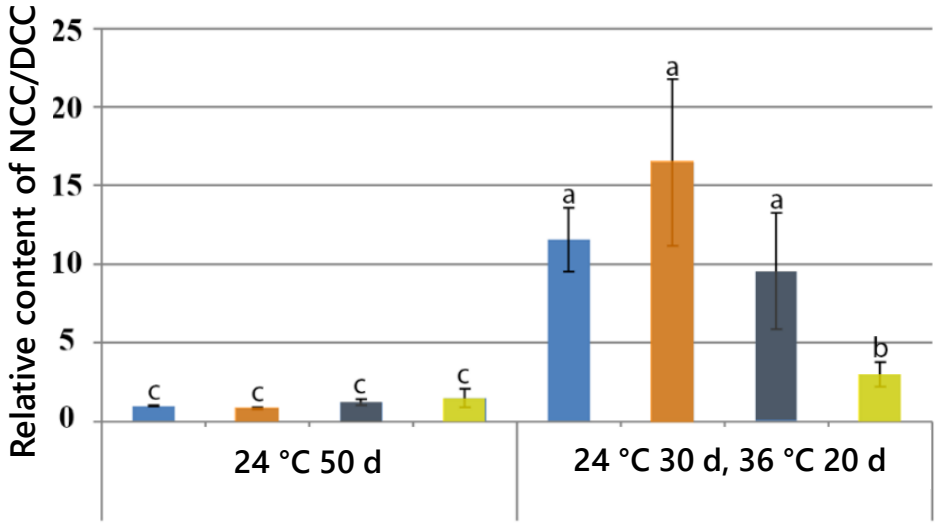
Modeling the effects of short-term heat stress



[1]Harper, J.F. Genetic identification of an autoinhibitor in CDPK, a protein kinase with a calmodulin-like domain / Harper J.F., J.-F. Huang, S.J. Lloyd // Biochemistry. – 1994. – № 33. –P. 7267–7277.

Results



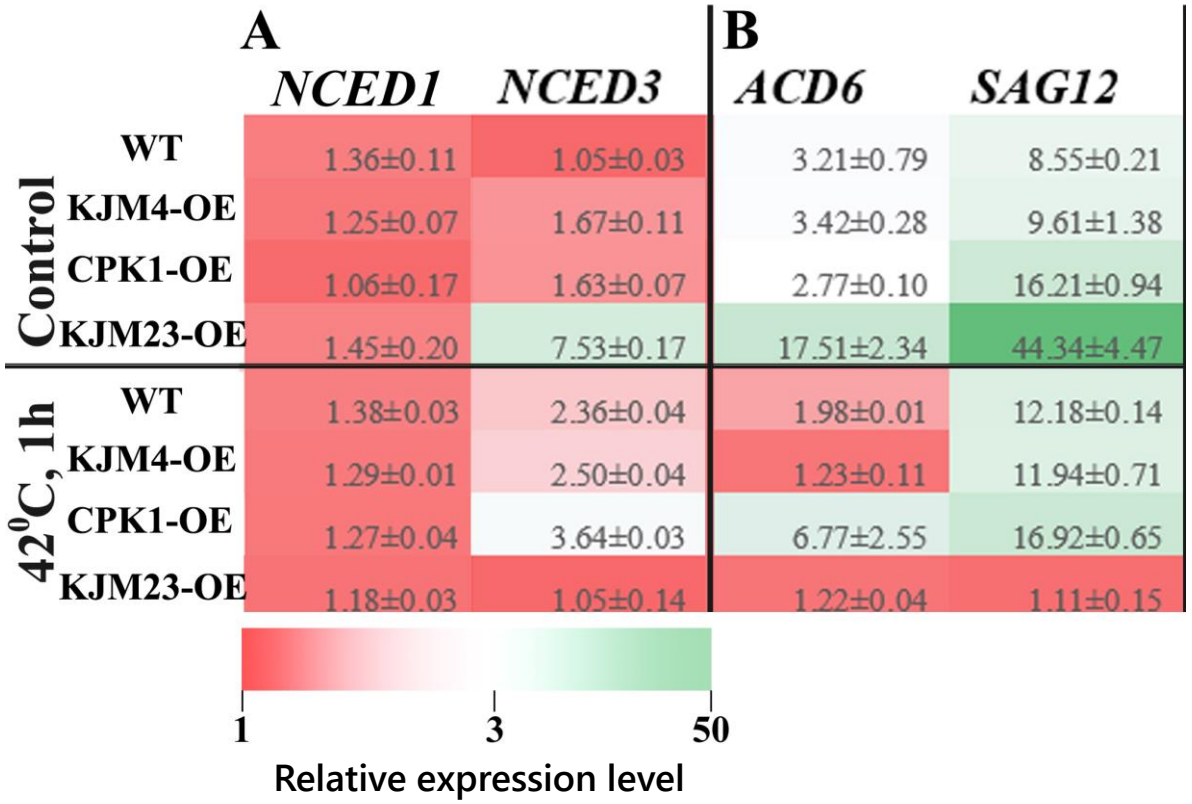


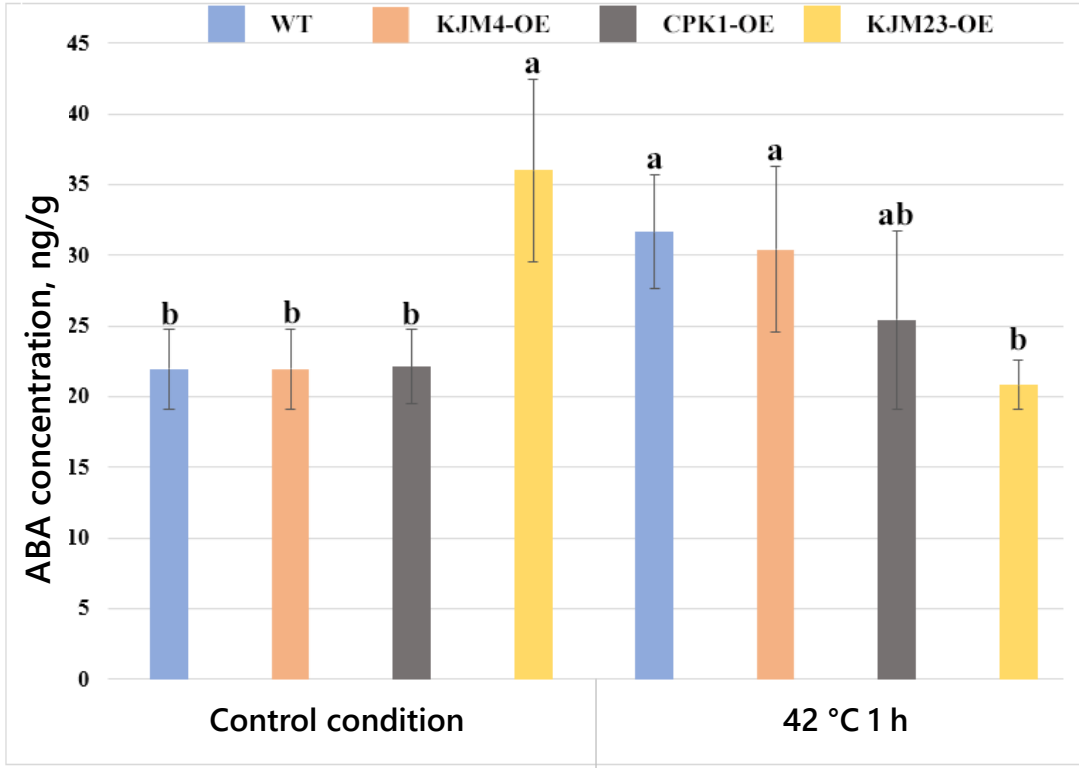
The color of the squares is indicative of the variant under study: **wild-type (WT)** plants, transgenic *N. tabacum* plants transformed with the native (**CPK1-OE**) and mutant constantly active (**KJM23-OE**) and inactivated (**KJM4-OE**) forms of *AtCPK1.*

Prolonged exposure to heat stress was observed to result in a decrease in growth rate for the plants in this study. However, the KJM-23 transgenes exhibited no such change, maintaining a growth rate that was equivalent to that of the plants under control conditions.

Externally, KJM23-transgenic plants exhibited a reduced susceptibility to the negative effects of heat stress, as evidenced by a decrease in leaf yellowing. When analysing the content of chlorophyll catabolites, no differences were observed between the studied forms and the wild type under control conditions. However, under prolonged heat stress, the content of chlorophyll catabolites increased 12-fold for the two studied forms and the wild type. Only a two-fold increase in degradation products was observed for the transgenes with the permanently active form, indicating strong defence mechanisms activated in these plants.

Results





Abscisic acid (ABA) is directly related to the aging process, so it is important to study the expression of its biosynthesis genes and the expression of proteases gene involved in cell death. In KJM-23 transgenic plants, an increased expression of the NCED3 gene under control conditions was observed. The transformation of KJM23 also activated the expression of SAG12 and ACD6 genes. However, under heat stress conditions, there was a complete blockage of the expression of both biosynthesis genes and aging-related proteases. The native form of the gene didnt have this effect, and its expression pattern was similar to the wild type.

It was necessary to confirm the expression of data by analyzing abscisic acid content. As a result of this analysis, it was possible to reliably confirm an increase in the content of abscisic acids in KJM-23 transgenic plants under control conditions, as well as a dramatic decrease in concentration of abscisin to wild-type levels under control conditions in response to stress.

Conclusions

- In the presented work we showed that the transformation of tobacco plants to a constitutively active modified form of the AtCPK1 gene (KJM23-OE plant) allows to significantly mitigate such negative heat stress effect as degradation of chlorophyll.

- Detailed molecular-biochemical analysis showed that KJM23-OE plant resistance to long-term heat stress is accompanied by a decrease in the level of ABA biosynthesis and a decrease in ABA-dependent processes of stress-induced aging, presumably due to CPK-dependent on the activation of the SA signal system, which is the antagonist of the ABA signal system.

- The overexpression of the native form of the gene had no effect on resistance to long-term intense heat stress.

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Thank you for your attention