Вестник Пермского университета. Серия Биология. 2024. Вып. 2. С. 212–220.
Bulletin of Perm University. Biology. 2024. Iss. 2. P. 212–220.

ГЕНЕТИКА

Научная статья
УДК 575.2
RITUFV
doi: 10.17072/1994-9952-2024-2-212-220

Сравнительная оценка профилей экспрессии гена *GhCIPK6* при различных концентрациях NaCl в проростках хлопчатника (*Gossypium hirsutum* L.)

Шадер Айдын Ализаде
Бакинский Государственный Университет, Баку, Азербайджан, shader622@mail.ru

Аннотация. Накопление Ca^{2+} у растений в условиях солевого стресса улучшает передачу сигнала и защищает их от фатальных последствий. Кальцинейрин B-подобные белки (CBL) представляют собой уникальную группу Ca^{2+}-сенсоров, которые декодируют Ca^{2+}-сигналы путем активации семейства растительно-специфичных протеинкиназ, известных как CBL-интерактивные протеинкиназы (CIPK). Семейство генов CIPK участвует в реакциях на абиотические стрессоры, такие как соль, засуха, высокие и низкие температуры. В работе изучена относительная экспрессия *GhCIPK6* в условиях стресса при концентрации NaCl 100 и 200 мМ у 31 географически удаленного сорта хлопчатника, относящегося к виду *Gossypium hirsutum* L. У сортов наблюдалась различная динамика относительной экспрессии, которые отличаются своей солеустойчивостью. Увеличение транскриптов *GhCIPK6* наблюдалось как у устойчивых, так и у восприимчивых сортов. При этом снижение уровня экспрессии определялось как в резистентных, так и в чувствительных генотипах. Полученные результаты показали, что *GhCIPK6* в разной степени индуцируется солевым стрессом и механизмы, обеспечивающие солеустойчивость у растений, различны.

Ключевые слова: хлопчатник, солевой стресс, Кальцинейрин B-подобный белок (CBL), CBL-взаимодействующая протеинкиназа (CIPK)

Для цитирования Ализаде Ш. А. Сравнительная оценка профилей экспрессии гена *GhCIPK6* при различной концентрации NaCl в проростках хлопчатника (*Gossypium hirsutum* L.) // Вестник Пермского университета. Сер. Биология. 2024. Вып. 2. С. 212–220. http://dx.doi.org/10.17072/1994-9952-2024-2-212-220.

GENETICS

Original article

Comparative evaluation of *GhCIPK6* gene expression profiles under different concentrations of NaCl in cotton (*Gossypium hirsutum* L.) seedlings

Shader A. Alizade
Baku State University, Baku, Azerbaijan, shader622@mail.ru

Abstract. Ca^{2+} accumulation in plants under salt stress improves signal transduction and protect them from fatal consequences. Calcineurin B-like proteins (CBLs) are a unique group of Ca^{2+} sensors that decode Ca^{2+} signals by activating a family of plant-specific protein kinases known as CBLinteracting protein kinases (CIPKs). The CIPK gene family is involved in responses to abiotic stressors such as salt, drought, high and low temperatures. In this investigation, the relative expression of *GhCIPK6* was studied under stress conditions of 100 mM and 200 mM concentration of NaCl in 31 geographically distant cotton cultivars belonging to the species *Gossypium hirsutum* L. Different dynamics of relative expression patterns were observed in cultivars that differ in their salt tolerance. An increase in *GhCIPK6* transcripts was observed in both resistant and susceptible cultivars. At the same time, a decrease in the expression level was determined in both resistant and sensitive genotypes. The obtained results showed that the *GhCIPK6* is induced to different degrees by salt stress and the mechanisms that ensure the salt tolerance in plants are different.

Keywords: cotton, salt stress, Calcineurin B-like protein (CBL), CBL-interacting protein kinase (CIPK)

For citation: Alizade S. A. Comparative evaluation of *GhCIPK6* gene expression profiles under different concentrations of NaCl in cotton (*Gossypium hirsutum* L.) seedlings. *Bulletin of the Perm University. Biology*. Iss. 2 (2024): pp. 212-220. http://dx.doi.org/10.17072/1994-9952-2024-2-212-220.

Introduction

Soil salinity is one of the serious abiotic stresses caused by high concentration of salt ions in soil that affect plant growth and development, and reduce plant productivity and crop quality. More than 800 million hectares of land and 20% of the arable land throughout the world are affected by salt stress [Lin et al., 2021].

Plants have evolved complex signaling mechanisms to respond to harsh environmental conditions [Alizada et al., 2020]. Calcium signaling is a crucial mechanism that allows plants to respond to environmental stimuli. In plants, have been identified several classes of calcium-sensing proteins. Ca^{2+}-binding proteins include calmodulin (CaM), CaM-like proteins (CMLs), calcineurin B-like proteins (CBLs), and calcium-dependent protein kinases (CDPKs) [Deng et al., 2013]. Calcineurin B-like protein (CBL)-interacting protein kinase complex (CIPK) is a major component of calcium sensors in the perception of various biotic and abiotic stress signals. CBL proteins contain four elongation factor motifs capable of binding four Ca^{2+} ions [Shu et al., 2020].

CIPKs are plant-specific protein kinases and belong to the SnRK3 subfamily of plant protein kinases. As major regulators of several ion channel proteins this proteins are regulating plant growth and development [Bai et al., 2022].

CIPK consists of two structural domains: an N-terminal and a C-terminal domain. This domain are connected by a junction domain. The N-terminal domain, the site of phosphorylation and comprises three conserved amino acids that are crucial for the proper CIPK functioning and activity. The C-terminal is also the regulatory domain of CIPK and further comprises NAF/FISL and PPI. Each member of this gene family generates unique proteins that helps in plant adaptation to a variety of stressors by interacting with calcium ion signals. In plants, the CIPK-CBL interaction plays several roles reacting to abiotic stress, ion homeostasis, and biotic stress factors [Yang et al., 2022].

Under salt stress, the mechanism of regulation of ion homeostasis and balance of Na^+ and K^+ ions inside the cell was studied through the CBL-CIPK complex. High Na+ concentration activates the formation of CIPK24/SOS2 and CBL4/SOS3 complex. This complex, in turn, activates SOS1, which allows Na^+ to flow out of the cell through plasma membrane anti-porters. The flow of Na^+ ions into the vacuole is regulated through the CBL10-CIPK24/SOS2 complex [Yin et al., 2020]. The importance of the Ca^{2+} complex in regulating the amount of K ions inside the cell through the AKTI channel was noted [Li et al., 2006].

The overexpression of *SICIPK24* increased salt tolerance in tomato [Huertas et al., 2012]. In Arabidopsis expression of *AtCIPK3* modulate abscisic acid and low temperature signal transduction and increase tolerance to high salt, low temperature and drought stress. [Kim et al., 2003]. In grapevine genome (*Vitis vinifera*) eight *CBL* and 20 *CIPK* genes were identified and diverse expression patterns of *VvCBLs* and *VvCIPKs* determined in response to salt stress [Xi et al., 2017].

The expression levels of *AlCBL* and *AlCIPK* genes were investigated under 600 mM NaCl stress conditions in a halophyte model *Aeluropus littoralis.* Among the studied genes one AlCBL gene was not expressed under the tested conditions. After 24 h. of salt treatment three genes were differentially induced: they were upregulated in the leaf, while they were downregulated in the root. Only one *AlCIPK* gene was downregulated in both root and leaf. Other *AlCIPK* genes showed different dynamics in different time intervals [Arab et al., 2023].

Chen et al. [2014] studied transgenic *Arabidopsis* plants under two different concentration of NaCl. Over-expressing *ZmCIPK21* markedly increased resistance of transgenic plants to salt than wild-type plants under salt stress. Therefore, the content of H_2O_2 in wild-type plants was higher than in transgenic plants. These findings showed that over-expression of *ZmCIPK21* might improve membrane integrity and keep ROS levels low during salt stress.

The expression patterns of 42 *AvCIPKs* were investigated in the salt-tolerant ZMH kiwifruit variety at four time points after salt stress and results showed the expression profile of *AvCIPK* genes had distinct profiles after salt stress [Gu et al., 2023]. Overexpressing of different *OsCIPK* genes in transgenic rice significantly improved tolerance to different abiotic stress factors including salt stress [Yong et al., 2007]. It is determined that by modulating ion homeostasis, the CBL4-CIPK5 pathway promotes salt tolerance but not chilling or drought tolerance [Huanga et al., 2020]. In transgenic *Arabidopsis* plant overexpression of *NtCIPK11* approximately twice improved seed germination under 100 mM or 150 mM of NaCl treatment. Furthermore, in transgenic plant the proline significantly accumulated compared with WT and transgenic plants grew more vigorously under salt stress conditions [Lu et al., 2021]. Under high-salinity conditions, *TaCIPK25* expression in transgenic wheat did not decrease and remained much higher than in wild-type. Furthermore, transmembrane Na^+/H^+ exchange was hindered in transgenic wheat root cells, implying that *TaCIPK25* negatively controlled salt response in wheat [Jin et al., 2016].

Cotton is an important commercial crop and a major source of raw material for a wide range of consumer goods [Mammadova et al., 2021; Akparov et al., 2021]. It accounts for approximately 35% of total fiber production worldwide. This plant is cultivated in more than 80 countries and it is a leading plant in more than 30

countries [Billah et al. 2021; Ализаде, 2022]. Cotton is a relatively salt tolerant crop with a salinity threshold of 7.7 dSm^{-1}. In order to create salt-tolerant cotton cultivars researchers have concentrated their efforts on identifying the major molecular components that involved in the response to salt stress [Wei et al., 2017].

The cotton samples stored in the National Genbank of Azerbaijan were evaluated mainly on the basis of morphometric descriptors, and the durability of the samples was not studied at the molecular-genetic level. The main goal of the research work is to evaluate the expression level of the *GhCIPK6* gene under salt stress conditions based on a gene-specific marker, and to compare the change in the expression level in salt-resistant and sensitive genotypes.

Materials and Methods

The research was carried out in the Department of Industrial and Forage Crops of the Institute of Genetic Resources of the Ministry of Science and Education of Azerbaijan. 31 cotton varieties belonging to the species *Gossypium hirsutum* L. were used as research material. The seeds of the cultivars were received from National Genebank. The used varieties and their used and their origin are presented in Table 1.

Table 1

Research material used for analysis

Genbank ID	Genotype	Origin	Genbank ID	Genotype	Origin
AzGR-10139	Aghdash-3	Azerbaijan	-	Select	Greece
AzGR-3601	AP-317	Azerbaijan	AzGR-3590	Kırqızıstan-174	Kyrgyzstan
AzGR-10202	Bayraqdar	Azerbaijan	AzGR-13638	Beyaz altun-440	Turkiye
AzGR-11836	Barakat	Azerbaijan	AzGR-13637	Edessa	Turkiye
AzGR-5852	Ganja-110	Azerbaijan	-	CSN-12	Turkiye
AzGR-7733	Ganja-114	Azerbaijan	AzGR-13640	Carisma	Turkiye
-	Ganja-160	Azerbaijan	AzGR-13636	Lima	Turkiye
AzGR-11468	Ganja-182	Azerbaijan	-	May-344	Turkiye
AzGR-12215	Ganja-195	Azerbaijan	AzGR-13641	PG	Turkiye
AzGR-12216	Ganja-200	Azerbaijan	-	Sezener-76	Turkiye
AzGR-11839	Zafar	Azerbaijan	AzGR-13639	Flash	Turkiye
AzGR-835	Kharabakh-11	Azerbaijan	AzGR-3591	Navai-9	Uzbekistan
-	Kharabakh-12	Azerbaijan	AzGR-5396	Tashkent - 1	Uzbekistan
-	Assos	Greece	-	Tashkent - 2	Uzbekistan
-	Cristina	Greece	-	Tashkent - 3	Uzbekistan
-	Prime	Greece			

Growth Conditions and Salt Treatments: For the control and salt variants 10 pre-fumigated seeds of each variety were planted in plastic containers. Plants were irrigated of Hoagland's solution [Hoagland, Arnon, 1950] without sodium chloride until the first true leaf stage. From the transition phase to the first true leaf, 100 mM and 200 mM of NaCl were added to the solution until the final concentration reached [Basal, 2010]. 72 h after the application of salt stress the leaf, root and stem samples of control and salt treated variants were collected and stored at -80°C until RNA extraction. The experiments were performed in three biological replications.

Molecular genetic analysis: The extraction of total RNA was carried out using a RNX Plus (Cat. No: EX6101). SinaClon First Strand cDNA Kit (Cat. No. RT5201) was used to perform first strand cDNA according to manufacturer's instructions. The primers designed by using online tool https://primer3.ut.ee/. Beta tubulin encoding gene (*GhTUB1*) was used as an endogenous stabilizing factor (Table 2). The RT² SYBR Green qPCR Mastermix (Qiagen, Cat. No: 330502) was used to evaluate the relative expression, in Rotor Gene Q 5plex (Qiagen, Cat. No. / ID: 9001570). The quantitative PCR reaction was carried out in the following steps: activation stage at 95^0C for 5 min, 35 cycles (at 95^0C for 15 s, at 58^0C for 30 s, at 72^0C for 1 min), melting curve (at 72^0C for 1 min). The relative expression level of *GhCIPK6* gene was calculated using the $2^{-\Delta\Delta C}$T method [Pfaffl, 2001]. The least significant difference test was performed using SPSS (IBM, SPSS v.25) software.

Table 2

Characteristics of *GhTUB1* and *GhCIPK6* primers

Gene	GenBank ID	Primer	Sequence	Tm (°C)
GhTUB1	AF487511.1	Forward	ATGGATCTGGAACCCGGTAC	59.35
		Reverse	AATCGCAATTCTCGGCTTCC	57.30
		Reverse	GCAGCTTCGGGATGGTAATG	59.35
GhCIPK6	HM002633.1	Forward	CCAAATACCCGAATCACCAC	58
		Reverse	CAAACAACGGTGACAAATCG	56

Results and Discussion

In previous studies, the salt resistance of studied cultivars was evaluated based on the germination index (which includes various germination parameters), total chlorophyll content, dry and wet parameters of the root and shoot, and sensitive and resistant cultivars were determined [Alizade, 2022; Alizade, Mammadova, 2023a; Alizade et al., 2023b].

Significant differences between group were found in control and both stress variants in stem and root for *GhCIPK6* gene expression (Table 3). However, in leaves, significant differences were determined in the control and 200 mM concentration of stress variant.

Table 3

One-way ANOVA analysis of relative expressions of *GhCIPK6* gene

Multiple Comparisons

Dependent variable	(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. Error.	Sig.	95% confidence interval	
						Lower Bound	Upper bound
GhCIPK6 Leaf	Control	100 mM	-1,83	4,81	,704	-11,40	7,73
		200 mM	-9,65*	4,81	,048	-19,22	-0,09
GhCIPK6 Stem	Control	100 mM	-27,57*	7,63	,000	-42,73	-12,42
		200 mM	-22,93*	7,63	,003	-38,08	-7,77
GhCIPK6 Root	Control	100 mM	-19,47*	8,56	,025	-36,47	-2,47
		200 mM	-45,26*	8,56	,000	-62,26	-28,26

Note: * - the mean difference is significant at the 0.05 level.

The studied genotypes showed significant different expression changes under NaCl treatment in leaves (Table 4). Although a change in the expression level was detected in the leaves of all studied cultivars, this situation was not recorded in the root and stem. At the same time, an increase or decrease in the expression profiles of all the three investigated vegetative organs was not determined. *GhCIPK6* gene was upregulated in 16 cultivars and downregulated in 15 cultivars under 100 mM and 200 mM of salt concentrations. Expression patterns of this gene were downregulated in only one tolerant sample at 100 mM concentration. At this concentration, the expression level decreased in sensitive varieties.

Table 4

***GhCIPK6* gene expression under salt stress in different vegetative organs**

Genotype	Salt tolerance	Leaf		Stem		Root	
		100 mM	200 mM	100 mM	200 mM	100 mM	200 mM
Aghdash-3	moderate	up	up	up	down	up	down
AP-317	tolerant	up	up	down	up	up	up
Bayraqdar	moderate	up	down	up	down	-	up
Barakat	moderate	down	down	down	up	up	up
Ganja-110	moderate	down	down	up	down	up	down
Ganja-114	moderate	down	down	up	up	up	down
Ganja-160	moderate	down	down	up	-	down	-
Ganja-182	sensitive	down	up	up	up	down	down
Ganja-195	moderate	up	up	down	down	up	up
Ganja-200	moderate	down	down	-	down	down	up
Kharabakh -11	moderate	down	up	up	up	down	down
Kharabakh -12	moderate	up	up	up	up	-	up
Zafar	moderate	up	up	-	down	down	up
Kırqızıstan-174	tolerant	up	down	up	-	up	up
Tashkent-1	moderate	down	down	up	up	up	-
Tashkent-2	tolerant	up	up	up	down	up	down
Tashkent-3	tolerant	up	down	-	up	down	up
Navai-9	tolerant	up	up	up	up	down	up
Edessa	moderate	down	down	down	up	down	down
Sezener-76	moderate	up	down	up	down	up	up
May-344	moderate	up	up	up	up	up	-
Beyaz altun-440	tolerant	down	up	down	-	up	up
CSN-12	moderate	up	up	up	up	down	down
PG	moderate	down	down	-	down	up	-

End of the table

Genotype	Salt tolerance	Leaf		Stem		Root	
		100 mM	200 mM	100 mM	200 mM	100 mM	200 mM
Flash	moderate	down	down	up	-	up	up
Lima	moderate	up	up	down	up	-	up
Carisma	sensitive	down	down	up	up	up	up
Cristina	moderate	down	down	down	down	up	up
Assos	moderate	up	up	down	-	down	down
Prime	moderate	down	up	up	down	up	up
Select	moderate	up	up	down	-	up	up

Moreover, the cotton genotypes showed a wide spectrum of *GhCIPK6* gene expression in stem and root. In stem, the expression of *GhCIPK6* was upregulated in 18 samples and downregulated in 9 samples, while in 200 mM concentration, it was upregulated in 14 samples, and downregulated in 11 samples. Although the expression level of *GhCIPK6* gene increased in sensitive cultivars at both concentrations of salt, a wide diversity was determined in the change of expression patterns in resistant and moderately resistant cultivars.

In root, under 100 mM concentration of NaCl, expression patterns of *GhCIPK6* were upregulated in 18 samples and downregulated in 10 samples, while at 200 mM concentration of NaCl, it was upregulated in 18 samples, and downregulated in 9 samples. The expression level decreased in both concentrations in 1 sensitive varity.

The analysis of mean realtive expression values of *GhCIPK6* gene showed an increasing at 100 mM salt concentration in all vegetative organs, however under 200 mM salt concentration.increasing were detected in leaf (Fig. 1.) and root (Fig. 3.) and decreasing were detected in stem (Fig. 2.).

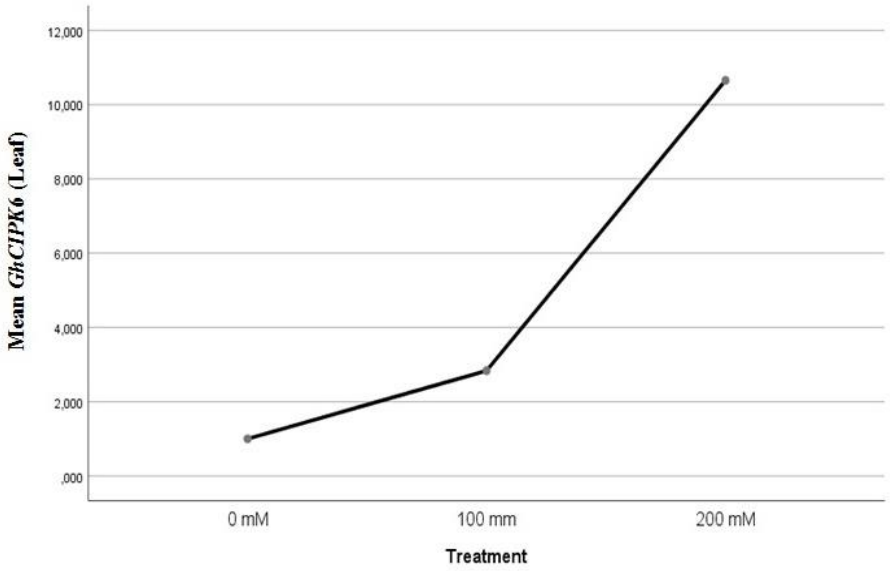

Fig. 1. Mean value of *GhCIPK6* in leaf

In maize under 250 mmol/L NaCl treatment *ZmCIPK* genes expression in leaf and root showed similar or different expression dynamics in salt-tolerant variety. Also there were different expression patterns of *ZmCIPKs* between cold-sensitive and cold-tolerant genotypes under cold stress [Chen et al., 2011]. Moreover, in *Marchantia polymorpha* plants under three concentration of NaCl two CIPKs and three CBLs genes showed different expression dynamics [Tansley et al., 2023]. Tripathi et al. [2009] studied CIPK6 for development and salt tolerance in plants. Overexpression of *CaCIPK6* promoted salt tolerance in transgenic tobacco, whereas Arabidopsis mutants were more sensitive to salt stress compared to wild-type. Morever, tobacco mutants showed a developed root system and increased basipetal auxin transport. Four CIPK6 genes were upregulated in roots of salt tolerant wild diploid cotton species *Gossypium klotzschianum* under 300 mM of NaCl treatment [Wei et al., 2017]. *GhCIPK6a* overexpression lines revealed increased salt tolerance through involvement in MAPK pathways and ROS scavenging [Billah et al., 2021]. In addition, transgenic Upland cotton lines with high expression of *GhCIPK6* showed significantly higher seed germination, seedling field emergence percentages, fiber quality under saline conditions than wild type [Su et al., 2020]. Taghizadeh et al. [2018] evaluated *GhCIPK6* gene expression level under two different concentration of NaCl. The results showed that relative expression of *GhCIPK6* was increased after 14 days under salt concentration than 7 day in leaf stem and root. Moreover relative expression of this gene was higher in tolerant cultivar than sensitive cultivar.

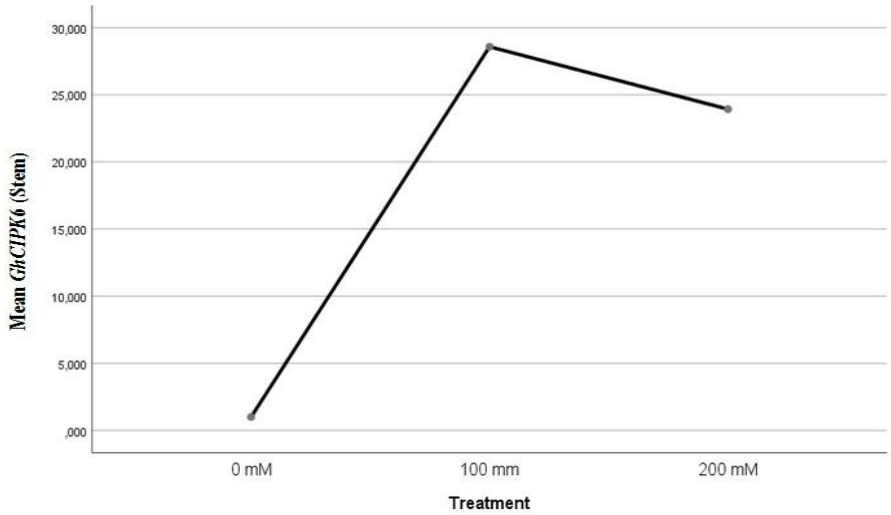

Fig. 2. Mean value of *GhCIPK6* in stem

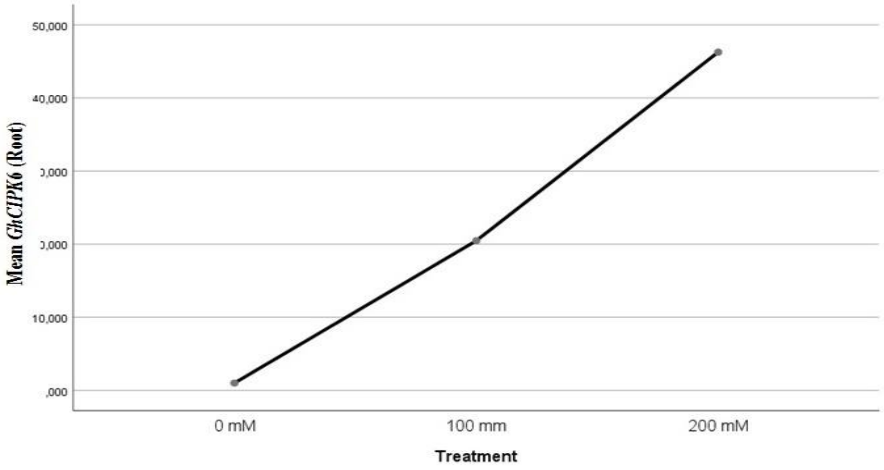

Fig. 3. Mean value of *GhCIPK6* in root

Conclusion

In this study, the relative expression level of *GhCIPK6* gene was evaluated in 3 different vegetative organs under two different salt conditions in 31 geographically distant cotton genotypes that differing in salt tolerance. The average value of the relative expression level of *GhCIPK6* increased in all vegetative organs at low concentration of salt, although, the increase was observed in leaves and roots at 200 mM concentration of salt. In addition, LSD (least significiantdifference) means test results for relative gene expression of *GhCIPK6* gene in different vegetative organs showed significant differences between group.

Similar results were obtained in the assessment of the expression level of mitogen-activated protein kinase (*GhMAPK*) [Ализаде, 2023] and antiporter encoding (*GhNHX1*) [Alizade, Aliyeva, 2024] genes in 31 studied cotton varieties at 100 and 200 mM concentration of NaCl and the differences between the susceptible and resistant groups allow us to talk about the stability samples are controlled by individual dominant genes.

Based on the obtained data, similar and different changes in the levels of transcripts belonging to different geographical groups and differing in salt tolerance indicate that salt tolerance has a complex genetic structure. At the same time, the differences between the susceptible and resistant groups suggest that the resistance of the samples is controlled by individual dominant genes. At the same time, there is a need to study more *CIPK* genes in cotton to evaluate salt tolerance in cotton.

The obtained results can be useful in the research works conducted in the direction of salt resistance in cotton.

Список источников

1. Ализаде Шадер. Роль миРНК в ответах на солевой стресс хлопчатника // Достижения в области биологии и наук о Земле. 2022. Т. 7, № 1. С. 80–84.

2. Ализаде Ш.А. Оценка уровня экспрессии гена *GhMAPK* в условиях солевого стресса у сортов хлопчатника // Биотехнология и селекция растений. 2023. Т. 6, № 4. С. 1–8.

3. Akparov Z.I. et al. Competitive evaluation of perspective cotton lines in variety development nursery // Advances in Current Natural Sciences. 2021. Vol. 10. P. 7–12.

4. Alizada S. et al. System Perspective Analysis for Molecular and Genetic Source of Salt Tolerance in Cotton // Khazar Journal of Science and Technology. 2020. Vol. 4, № 1. P. 70–83.

5. Alizada Sh., Aliyeva K. Comparative analysis of expression profiles of antiporter encoding gene (*GhNHX1*) under different concentrations of NaCl in cotton (*Gossypium hirsutum* L.) // Advances in Biology & Earth Sciences. 2024. Vol. 9, № 1. P. 168–174.

6. Alizade S. Comparative study of SPAD values in cotton plant under salt stress // Proceedings of Genetic Resources Institute of ANAS. 2022. Vol. 11, № 1. P. 139–146.

7. Alizade S., Mammadova R. Assessment of salt stress resistance of cotton varieties based on different parameters // Advances in Biology & Earth Sciences. 2023a. Vol. 8, № 1. P. 58–66.

8. Alizade S., Mammadova R., Sirajli N., Evaluation of morphometric traits of upland cotton genotypes under different concentration of NaCl // Advances in Biology & Earth Sciences. 2023b. Vol. 8, № 3. P. 301–307.

9. Arab M. et al. Comprehensive Analysis of Calcium Sensor Families, CBL and CIPK, in *Aeluropus littoralis* and Their Expression Profile in Response to Salinity // Genes. 2023. Vol. 14. P. 1–14.

10. Bai X. et al. Characterization of CBL-Interacting Protein Kinases Gene Family and Expression Pattern Reveal Their Important Roles in Response to Salt Stress in Poplar // Forests. 2022. Vol. 13. P. 1–13.

11. Basal H. Response of cotton (*Gossypium hirsutum* L.) genotypes to salt stress // Pak. J. Bot. 2010. Vol. 42, № 1. P. 505–511.

12. Billah M, Li F., Yang Z. Regulatory Network of Cotton Genes in Response to Salt, Drought and Wilt Diseases (*Verticillium* and *Fusarium*): Progress and Perspective // Front. Plant Sci. 2021. Vol. 12. P. 1–19.

13. Chen X. et al. Identification and characterization of putative CIPK genes in maize // Journal of Genetics and Genomics. 2011. Vol. 38. P. 77–87.

14. Chen X. et al. *ZmCIPK21*, A Maize CBL-Interacting Kinase, Enhances Salt Stress Tolerance in *Arabidopsis thaliana* // Int. J. Mol. Sci. 2014. Vol. 15. P. 14819–14834.

15. Deng X. et al. TaCIPK29, a CBL-Interacting Protein Kinase Gene from Wheat, Confers Salt Stress Tolerance in Transgenic Tobacco // PLoS ONE. 2013. Vol. 8, № 7. P. 1–13.

16. Gu S. et al. Transcriptome-Wide Identification and Functional Characterization of CIPK Gene Family Members in *Actinidia valvata* under Salt Stress // Int. J. Mol. Sci. 2023. Vol. 24, № 1. P. 1–15.

17. Hoagland D.R., Arnon D.I. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station, 1950. 347 p.

18. Huanga S. et al. CBL4-CIPK5 pathway confers salt but not drought and chilling tolerance by regulating ion homeostasis // Environmental and Experimental Botany. 2020. Vol. 179. P. 1–30.

19. Huertas R. et al. Overexpression of SlSOS2 (SlCIPK24) confers salt tolerance to transgenic tomato // Plant Cell Environ. 2012. Vol. 35. P. 1467–1482.

20. Jin X. et al. Wheat CBL-interacting protein kinase 25 negatively regulates salt tolerance in transgenic wheat // Scientific Reports. 2016. Vol. 6. P. 1–16.

21. Kim K.N. et al. CIPK3, a calcium sensor-associated protein kinase that regulates abscisic acid and cold signal transduction in Arabidopsis // Plant Cell. 2003. Vol. 15. P. 411–423.

22. Li L. et al. A Ca^{2+} signaling pathway regulates a K^{+} channel for low-K response in Arabidopsis // Proc. Natl. Acad. Sci. 2006. Vol. 103. P. 12625–12630.

23. Lin C. et al. Integrated transcriptome and proteome analysis reveals complex regulatory mechanism of cotton in response to salt stress // Journal of Cotton Research. 2021. Vol. 4. P. 1–13.

24. Lu L. et al. *CIPK11*: a calcineurin B-like proteininteracting protein kinase from *Nitraria tangutorum*, confers tolerance to salt and drought in *Arabidopsis* // BMC Plant Biology. 2021. Vol. 21. P. 1–16.

25. Mammadova R.B. et al. Prospects of the remote hybridization on improvement of the main economical traits of cotton genotypes with naturally colored fibre // East European Scientific Journal. 2021. Vol. 6, № 70. P. 4–7.

26. Pffafl M.W. A new mathematical model for relative quantification in real-time RT-PCR // Nucleic Acids Research. 2001. Vol. 29, № 9. P. 1–6.

27. Shu B. et al. Identifying citrus *CBL* and *CIPK* gene families and their expressions in response to drought and arbuscular mycorrhizal fungi colonization // Biologia Plantarum. 2020. Vol. 64. P. 773–783.

28. Su Y. et al. *GhCIPK6a* increases salt tolerance in transgenic upland cotton by involving in ROS scavenging and MAPK signaling pathways // BMC Plant Biology. 2020. Vol. 20. P. 1–19.

29. Taghizadeh N. et al. Salt-related Genes Expression Pattern in Salt-Tolerant and Salt Sensitive Cultivars of Cotton (*Gossypium* sp.) under NaCl Stress // J. Plant Mol. Breed. 2018. Vol. 6, № 1. P. 1–15.

30. Tansley C. et al. CIPK-B is essential for salt stress signalling in *Marchantia polymorpha* // New Phytologist. 2023. Vol. 237. P. 2210–2223.

31. Tripathi V. et al. CIPK6, a CBL-interacting protein kinase is required for development and salt tolerance in plants // The Plant Journal. 2009. Vol. 58. P. 778–790.

32. Wei Y. et al. Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis // PLoS ONE. 2017. Vol. 12, № 5. P. 1–25.

33. Xi Y. et al. The CBL and CIPK Gene Family in Grapevine (*Vitis vinifera*): Genome-Wide Analysis and Expression Profiles in Response to Various Abiotic Stresses // Front. Plant Sci. 2017. Vol. 8. P. 1–15.

34. Yang C. et al. Diverse roles of the CIPK gene family in transcription regulation and various biotic and abiotic stresses: A literature review and bibliometric study // Front. Genet. 2022. Vol. 13. P. 1–17.

35. Yin X. et al. The protein kinase complex CBL10–CIPK8–SOS1 functions in Arabidopsis to regulate salt tolerance // J. Exp. Bot. 2020. Vol. 71. P. 1801–1814.

36. Yong X., Yuemin H., Lizhong X. Characterization of Stress-Responsive CIPK Genes in Rice for Stress Tolerance Improvement // Plant Physiology. 2007. Vol. 144. P. 1416–1428.

References

1. Alizade S. [Role of miRNAs in cotton salt stress responses]. *Dostiženija v oblasti biologii i nauk o Zemle.* V. 7, No.1 (2022): pp. 80-84. (In Russ.).

2. Alizade Sh.A. [Evaluation of the *GhMAPK* gene expression level under salt stress in cotton cultivars]. *Biotechnologija i selekcija rastenij.* V. 6, No. 4 (2023): pp. 1-8. (In Russ.).

3. Akparov Z.I., Mamedova R.B., Guseynova L.A., Abdulalieva G.S., Yunusova F.M., Alizade Sh.A. Competitive evaluation of perspective cotton lines in variety development nursery. *Advances in Current Natural Sciences*. V.10 (2021): pp. 7-12.

4. Alizada S, Guliev R, Mammadova R, Zaefizadeh M. System Perspective Analysis for Molecular and Genetic Source of Salt Tolerance in Cotton. *Khazar Journal of Science and Technology.* V. 4, No. 1 (2020): pp. 70-83.

5. Alizada Sh., Aliyeva K. Comparative analysis of expression profiles of antiporter encoding gene (*GhNHX1*) under different concentrations of NaCl in cotton (*Gossypium hirsutum* L.). *Advances in Biology & Earth Sciences*. V. 9, No. 1 (2024): pp. 168-174.

6. Alizade S. Comparative study of SPAD values in cotton plant under salt stress. P*roceedings of Genetic Resources Institute of ANAS.* V. 11, No. 1 (2022): pp. 139-146.

7. Alizade S., Mammadova R. Assessment of salt stress resistance of cotton varieties based on different parameters. *Advances in Biology Earth Sciences.* V. 8, No. 1 (2023a): pp. 58-66.

8. Alizade S., Mammadova R., Sirajli N. Evaluation of morphometric traits of upland cotton genotypes under different concentration of NaCl. *Advances in Biology & Earth Sciences* V. 8, No. 3 (2023b): pp. 301-307.

9. Arab M., Najafi Z.H., Nematzadeh G., Heidari P., Hashemipetroudi S.H., Kuhlmann M. Comprehensive Analysis of Calcium Sensor Families, CBL and CIPK, in *Aeluropus littoralis* and Their Expression Profile in Response to Salinity. *Genes.* V. 14 (2023): pp. 1-14.

10. Bai X., Ji J., Wang W., Gu C., Yu Q., Jiang J., Yang C., Liu G. Characterization of CBL-Interacting Protein Kinases Gene Family and Expression Pattern Reveal Their Important Roles in Response to Salt Stress in Poplar. *Forests.* V. 13 (2022): pp. 1-13.

11. Basal H. Response of cotton (*Gossypium hirsutum* L.) genotypes to salt stress. *Pak. J. Bot.* V. 42, No. 1 (2010): pp. 505-511.

12. Billah M., Li F., Yang Z. Regulatory Network of Cotton Genes in Response to Salt, Drought and Wilt Diseases (*Verticillium* and *Fusarium*): Progress and Perspective. *Front. Plant Sci.* V. 12 (2021): pp. 1-19.

13. Chen X., Gu Z., Xin D., Hao L., Liu C., Huang J., Ma B., Zhang H. Identification and characterization of putative CIPK genes in maize. *Journal of Genetics and Genomics.* V. 38 (2011): pp. 77-87.

14. Chen X., Huang Q., Zhang F., Wang B., Jianhua W., Jun Z., *ZmCIPK21*, A Maize CBL-Interacting Kinase, Enhances Salt Stress Tolerance in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* V. 15 (2014): pp. 14819-14834.

15. Deng X., Hu W., Wei S., Zhou S., Zhang F. TaCIPK29, a CBL-Interacting Protein Kinase Gene from Wheat, Confers Salt Stress Tolerance in Transgenic Tobacco. *PLoS ONE*. V. 8, No. 7 (2013) pp. 1-13.

16. Gu S., Abid M., Bai D., Chen C., Sun L., Qi X., Zhong Y., Fang J. Transcriptome-Wide Identification and Functional Characterization of CIPK Gene Family Members in *Actinidia valvata* under Salt Stress. *Int. J. Mol. Sci.* V. 24, No. 1 (2023): pp. 1-15.

17. Hoagland D.R., Arnon D.I. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station, 1950. 347 p.

18. Huanga S., Chena M., Zhaoa Y., Wena X., Guob Z., Lua S. CBL4-CIPK5 pathway confers salt but not drought and chilling tolerance by regulating ion homeostasis, *Environmental and Experimental Botany*. V. 179 (2020): pp. 1-30.

19. Huertas R., Olias R., Eljakaoui Z., Galvez F.J., Li J., De Morales, P.A., Overexpression of SlSOS2 (SlCIPK24) confers salt tolerance to transgenic tomato. *Plant Cell Environ.* V. 35 (2012): pp. 1467–1482.

20. Jin X., Sun T., Wang X., Su P., Ma J., He G., Yang G. Wheat CBL-interacting protein kinase 25 negatively regulates salt tolerance in transgenic wheat. *Scientific Reports* V. 6 (2016): pp. 1-16.

21. Kim K.N., Cheong Y.H., Grant J.J., Pandey G.K., Luan S. CIPK3, a calcium sensor-associated protein kinase that regulates abscisic acid and cold signal transduction in Arabidopsis. *Plant Cell.* V. 15 (2003): pp. 411–423.

22. Li L., Kim B.G., Cheong Y.H., Pandey G.K., Luan S. A Ca^{2+} signaling pathway regulates a K^+ channel for low-K response in Arabidopsis. *Proc. Natl. Acad. Sci.* V. 103 (2006): pp. 12625–12630.

23. Lin C., Heng S., Jie K., Haijiang X., Xiyan Y. Integrated transcriptome and proteome analysis reveals complex regulatory mechanism of cotton in response to salt stress. *Journal of Cotton Research.* V. 4 (2021): pp. 1-13.

24. Lu L., Chen X., Wang P., Lu Y., Zhang J., Yang X., Cheng T., Shi J., Chen J. *CIPK11*: a calcineurin B-like proteininteracting protein kinase from *Nitraria tangutorum*, confers tolerance to salt and drought in *Arabidopsis*. BMC Plant Biology.V. 21 (2021): pp. 1-16.

25. Mammadova R.B., Huseynova L.A., Abdulaliyeva G.S., Yunusova F.M., Alizade Sh.A. Prospects of the remote hybridization on improvement of the main economical traits of cotton genotypes with naturally colored fibre. *East European Scientific Journal.* V. 6, No. 70 (2021): pp. 4-7.

26. Pffafl M.W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*. V. 29, No. 9 (2001): pp. 1-6.

27. Shu B., Cai D., Zhang F., Zhang D.J., Liu C.Y., Wu Q.S., Luo C. Identifying citrus *CBL* and *CIPK* gene families and their expressions in response to drought and arbuscular mycorrhizal fungi colonization. *Biologia Plantarum.* V. 64 (2020): pp. 773-783.

28. Su Y., Guo A., Huang Y., Wang Y., Hua J. *GhCIPK6a* increases salt tolerance in transgenic upland cotton by involving in ROS scavenging and MAPK signaling pathways. *BMC Plant Biology.* V. 20 (2020): pp. 1-19.

29. Taghizadeh N., Ranjbar G., Nematzadeh G., Ramazani-Moghaddam M., Salt-related Genes Expression Pattern in Salt-Tolerant and Salt Sensitive Cultivars of Cotton (*Gossypium* sp.) under NaCl Stress, *J. Plant Mol. Breed.* V. 6, No.1 (2018): pp. 1-15.

30. Tansley C., Houghton J., Rose A.M.E., Witek B., Payet R.D., Wu T., Miller B.J., CIPK-B is essential for salt stress signalling in *Marchantia polymorpha*. *New Phytologist*. V. 237 (2023): pp. 2210-2223.

31. Tripathi V., Parasuraman B., Laxmi A., Chattopadhyay D. CIPK6, a CBL-interacting protein kinase is required for development and salt tolerance in plants. *The Plant Journal.* V. 58 (2009): pp. 778-790.

32. Wei Y., Xu Y., Lu P., Wang X., Li Z., Cai X., Zhou Z., Wang Y., Zhang Z., Lin Z., Liu F., Wang K. Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis. *PLoS ONE.* V. 12, No. 5 (2017): pp. 1-25.

33. Xi Y., Liu J., Dong C., Cheng Z.M. The CBL and CIPK Gene Family in Grapevine (*Vitis vinifera*): Genome-Wide Analysis and Expression Profiles in Response to Various Abiotic Stresses. *Front. Plant Sci.* V. 8 (2017): pp. 1-15.

34. Yang C., Yi-feng J., Yushu W., Yansong G., Qi W., Xue Y. Diverse roles of the CIPK gene family in transcription regulation and various biotic and abiotic stresses: A literature review and bibliometric study. *Front. Genet.* V. 13 (2022): pp. 1-17.

35. Yin X., Xia Y., Xie Q., Cao Y., Wang Z., Hao G. The protein kinase complex CBL10–CIPK8–SOS1 functions in Arabidopsis to regulate salt tolerance. *J. Exp. Bot.* V. 71 (2020): pp. 1801-1814.

36. Yong X, Yuemin H., Lizhong X. Characterization of Stress-Responsive CIPK Genes in Rice for Stress Tolerance Improvement. *Plant Physiology.* V. 144 (2007): pp. 1416-1428.

Статья поступила в редакцию 26.02.2024; одобрена после рецензирования 26.04.2024; принята к публикации 10.06.2024.

The article was submitted 26.02.2024; approved after reviewing 26.04.2024; accepted for publication 10.06.2024.

Информация об авторе

Ш. А. Ализаде – докторант, научный сотрудник.

Information about the author

S. A. Alizade – PhD student, researcher.