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Original article

Genome-wide investigation of CIPK gene family in *Aeluropus littoralis* L.

M. Arab[1], H. Najafi Zarrini[2], Gh. Nematzadeh[3], S.H. Hashemipetroudi[4*]

1. *M.Sc. Student in Plant Breeding, Sari Agricultural Sciences and Natural Resources University (SANRU), Iran*
2. *Associated Professor, Department of Biotechnology, Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran*
3. *Professors, Department of Genetic Engineering and Biology, Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University (SANRU). Iran*
4. *Assistant Professors, Department of Genetic Engineering and Biology, Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran*

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Extended abstract

Introduction

The CBL-CIPK signaling network, which decodes calcium signals triggered by environmental stresses, is one of the most crucial signal transduction systems in plants. Proteins bound to calcium ions serve as sensor molecules, receiving cellular calcium ion signals and transmitting messages to the downstream gene cascade. Because of its tolerance to abiotic stresses, especially salinity stress, and its relationship to cereals, many researchers are interested in the molecular mechanisms of the halophyte grass *Aeluropus littoralis*. The in-silico discovery of the AlCIPK gene family and their expression profile in responses to salinity stress were considered in this analysis due to this plant's genome sequence's availability.

Materials and methods

Using local TblastN program, the CIPK protein sequences of *Arabidopsis thaliana* gene families were blasted against *A. littoralis* genomic sequences. BlastP was used to verify all sequences after redundant sequences were removed. The detected proteins were analyzed in various protein domain databases such as Pfam, PROSITE, and InterProScan to identify, annotate, and interpret domain structures. In all of AlCIPK, the SALAD approach was used to perform similarity clustering based on motifs patterns. The exon and intron arrangement were determined by comparing the predicted CDS against AlCIPK genomic sequences in the gene structure display server (GSDS). Expasy-Prosite was used to determine the domain structure. A signal-dependent software based on SignalP 5.0 was used to identify signal peptides in proteins. Exploring the expression pattern of AtCIPK genes at various growth and developmental stages using Genevestigator (https://www.genevestigator.com/gv/plant.jsp) and the EFP browser (http://bar.utoronto.ca/). Transcriptome research was used to examine the expression patterns of AlCIPK genes in leaf and root tissues under salinity stress and recovery conditions.

Results and discussion

Based on sequence homology with *Arabidopsis* genes, 20 CIPK genes were discovered in the *A. littoralis* genome. The Arabidopsis AtCIPK homologous proteins were used to name the Aeluropus CIPK genes.

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[*] Corresponding author: Seyyed Hamidreza Hashemipetroudi; E-Mail: shr.hashemi@sanru.ac.ir

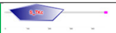
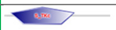
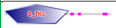
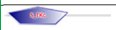
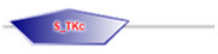
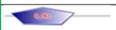
According to subcellular localization analysis, these proteins are active in a specific cellular compartment. The phylogenetic tree of 20 AlCIPK, 26 AtCIPK, and 33 OsCIPK showed that these 79 CIPKs are closely related. Exon/intron structure analysis was used to separate all AlCIPK into intron-poor and intron-rich classes. The expression of 25 AtCBL gene family members in 68 samples under salinity stress was compared using Genevestigator tools, which revealed that all 25 genes tested in different developmental/ environmental stages, including control and stress, had different expression patterns. A tissue-specific expression pattern was discovered after analyzing these AtCBL genes' expression pattern in both root and shoot tissues. In salinity stress and recovery conditions, the expression profile pattern of AlCIPK genes in leaf and root tissues was distinct. The distinct expression profiles of the AlCIPK gene family confirmed their functional and structural convergence.

Conclusion

Systematic study of members of this gene family revealed that CIPKs in Halophyte grass, i.e., *A. littoralis*, share main CIPK family characteristics with other monocotyledonous and dicotyledonous plants, which are likely important factors in this species' adaptation and stress tolerance. The lack of homologous AtCIPK24 genes in the Aeluropus genome is a key finding in this study, suggesting that the CBL-CIPK gene network in this plant has a distinct regulatory function, necessitating further studies. Future studies using the RT-qPCR method to examine the expression of AlCBL and AlCIPK gene family genes under different abiotic stresses could aid in understanding the mechanism of SOS-related gene expression regulation. This study's findings reveal the functional characteristics of the calcium gene family and provide essential information for future research on their functional roles.

***Keywords*:** Calcium sensor, CBL-CIPK, CBL-interacting protein kinase, Salt stress, Signaling network

Table 1. Characteristics of domains identified in the *AlCIPK* gene family.

	Homology parameter to *Arabidopsis thaliana*					Domain organization				
						Pfam			SMART (SM00220)	PROSITEE
Gene Name	**Accession no.**	**Accession no.**	**Identity %**	**Coverage %**	**Total Score**	**PF02149 KA**	**PF03822 Naf**	**PF00069 PKinase**	**Ps50816**	**(PS50011)**
AlCIPK1.1	**Alg4127**	**AT3G17510.1**	**56.39%**	**94%**	**507**	🟦	🟥	🟩		
AlCIPK1.2	**Alg7902**	**AT3G17510.1**	**55.53%**	**90%**	**473**		🟥	🟩		
AlCIPK3.1	**Alg7566**	**AT2G26980.4**	**75.51%**	**98%**	**701**	🟦	🟥	🟩		
ALCIPK3.2	**Alg12052**	**AT2G26980.4**	**70.59%**	**94%**	**655**		🟥	🟩		
AlCIPK4	**Alg15044**	**AT4G14580.1**	**50.47%**	**97%**	**404**		🟥	🟩		
AlCIPK5	**Alg5583**	**AT5G10930.1**	**54.88%**	**95%**	**507**		🟥	🟩		
AlCIPK10.1	**Alg12300**	**AT5G58380.1**	**61.14%**	**%84**	**594**		🟥	🟩		
ALCIPK10.2	**Alg9524**	**AT5G58380.1**	**59.87%**	**100%**	**572**		🟥	🟩		
AlCIPK10.3	**Alg4701**	**AT5G58380.1**	**58.76%**	**%97**	**545**		🟥	🟩		
AlCIPK10.4	**Alg3308**	**AT5G58380.1**	**58.72%**	**%91**	**581**		🟥	🟩		
AlCIPK10.5	**Alg13906**	**AT5G58380.1**	**56.26%**	**%95**	**499**		🟥	🟩		
AlCIPK10.6	**ALg9805**	**AT5G58380.1**	**49.45%**	**%98**	**432**		🟥	🟩		
AlCIPK11	**Alg2698**	**AT2G30360.1**	**45.65%**	**95%**	**365**		🟥	🟩		
AlCIPK12.1	**Alg8115**	**AT4G18700.1**	**65.48%**	**92%**	**652**		🟥	🟩		
AlCIPK12.2	**Alg10559**	**AT4G18700.1**	**59.02%**	**94%**	**583**		🟥	🟩		
AlCIPK12.3	**Alg11449**	**AT4G18700.1**	**51.27%**	**96%**	**478**		🟥	🟩		
AlCIPK20	**Alg11347**	**AT5G45820.1**	**64.79%**	**96%**	**599**		🟥	🟩		
AlCIPK21	**Alg8711**	**AT5G57630.1**	**53.63%**	**95%**	**434**		🟥	🟩		
AlCIPK23	**Alg1003**	**AT1G30270.1**	**81.46%**	**94%**	**740**		🟥	🟩		
AlICIPK26	**Alg7179**	**AT5G21326.1**	**71.66%**	**96%**	**662**		🟥	🟩		

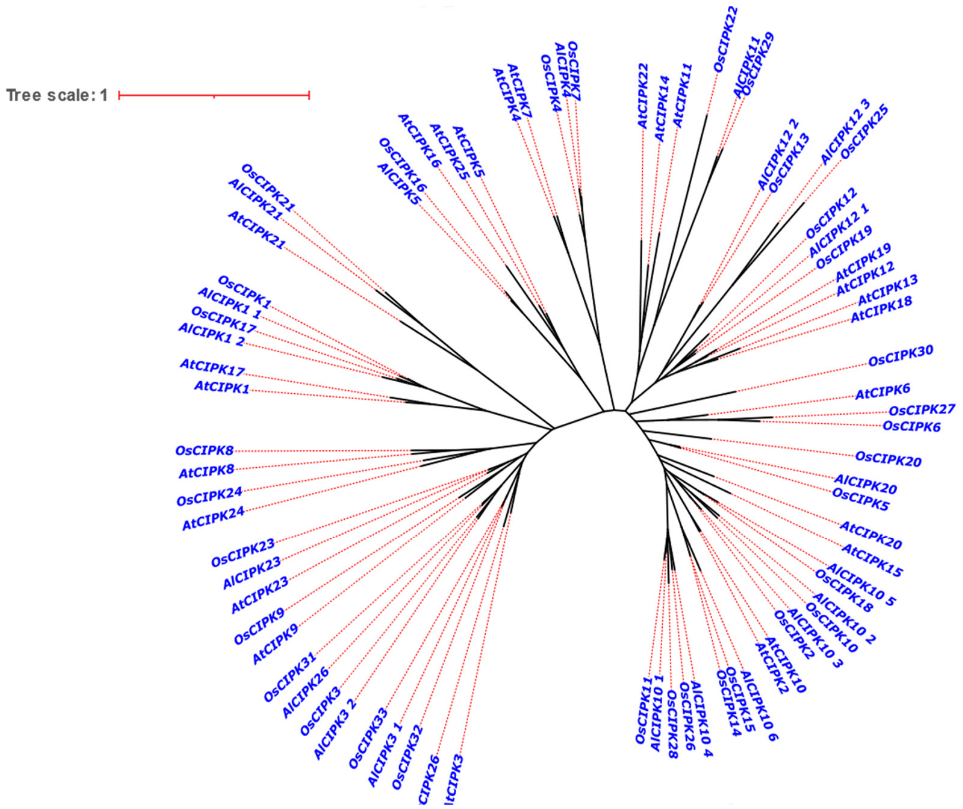


Fig. 1. Investigation of phylogenetic relationships of CIPK proteins (79 protein sequences) in Aeluropus, Arabidopsis and rice

Table 2. Physicochemical properties of CIPK proteins in *A. littoralis*.

Gene Name	Protein length (aa)	Exon no.	Intron no.	Molecular weight (kDa)	instability index	Aliphatic index	Isoelectric point	GRAVY	N-Myristoylation	palmitoylation
AlCIPK10.2	438	1	0	49.716	33.20	95.71	9.28	-0.260	5 MVDKGNILMQKY	-----
AlCIPK12.1	516	1	0	57.364	44.07	79.19	8.64	-0.341	-----	446 RRKDWRVCLEGTREG
AlCIPK26	448	14	13	50.437	29.56	86.43	8.40	-0.395	-----	-----
AlCIPK10.6	67	2	1	42.040X	3.42	6.35	8.99X	0.480	5 MECRGKILMERY	3 MECRGKILM
AlCIPK5	444	1	0	48.192X	2.98	7.21	.52X	0.054	9 ARGREADGGEPPRKL	449 PAAAILGCA
AlCIPK3.1	442	14	13	50.760	38.66	83.55	7.64	-0.460	-----	-----
AlCIPK23	449	14	13	50.510	36.84	86.19	9.16	-0.371	6 MSVAVGRTRVGRY	233 IFKADFSCPSWFSTS
AlCIPK11	433	1	0	47.398	39.14	89.24	8.95	-0.151	-----	-----
AlCIPK10.4	478	1	0	54.659	42.80	84.46	9.13	-0.514	10 DRRTILMGRYEIGRQ	-----
AlCIPK10.1	523	1	0	58.971	48.45	85.22	9.03	-0.503	3 MDGRRTILMD	-----
AlCIPK10.3	421	1	0	47.985X	2.48	4.73	9.03X	0.400	-----	207 IGKAEFKCPGWFSTD 425 KQQQQQSC
AlCIPK1.1	473	13	12	53.481X	46.07	9.62	6.52X	0.372	4 MVKGEAEAECT	10 KGEAEAECTRSSLLG 396 KLKVMKNCKSFKNPK
AlCIPK3.2	448	14	13	50.638	33.40	85.29	8.23	-0.407	-----	-----
AlCIPK10.5	410	2	1	45.994	35.30	88.68	8.93	-0.307	-----	317 KLEDVATCSKLTVKK
AlCIPK12.3	490	1	0	54.065	45.71	85.98	8.84	-0.254	-----	255 RLIRGLLCPDPARRI
AlCIPK1.2	454	12	11	50.693	36.53	93.19	6.62	-0.320	4 MVSGGKGELAL 5 MVSGGKGELALE 7 MVSGGKGELALEAA	-----
AlCIPK4	427	1	0	46.344	49.80	93.72	8.59	-0.115	-----	-----
AlCIPK21	430	14	13	48.541	35.57	89.09	6.21	-0.303	2 MGFTESVGK	-----
AlCIPK20	456	1	0	51.639	36.45	85.50	9.08	-0.422	-----	-----
AlCIPK12.2	515	1	0	57.476	44.04	79.57	8.06	-0.374	5 MASKGASNGGGR 9 ASKGASNGGGREAKK 10 SKGASNGGGREAKKP	-----

	E-value	Sites	Width
1.	7.0e-654	19	50
2.	1.3e-630	19	50
3.	1.0e-384	18	41
4.	8.7e-369	18	50
5.	3.5e-351	19	41
6.	3.3e-248	19	41
7.	5.3e-118	19	31
8.	8.7e-076	19	11
9.	4.7e-073	20	19
10.	1.4e-060	13	15

Sequence	E-value
AlCIPK20	2.3e-301
AlCIPK26	6.8e-298
AlCIPK23	1.3e-295
AlCIPK3.1	4.1e-292
AlCIPK3.2	1.5e-290
AlCIPK10.1	8.0e-287
AlCIPK10.2	1.5e-286
AlCIPK10.4	2.8e-285
AlCIPK12.1	2.4e-267
AlCIPK10.3	3.4e-264
AlCIPK10.5	1.8e-248
AlCIPK12.2	4.9e-247
AlCIPK10.6	1.5e-230
AlCIPK1.1	7.3e-230
AlCIPK12.3	9.4e-220
AlCIPK5	5.5e-198
AlCIPK1.2	9.6e-195
AlCIPK21	6.6e-187
AlCIPK11	7.6e-177
AlCIPK4	2.2e-175

Fig. 2. Motif analysis and domain search in AlCIPK protein sequences. (A) Motif logo of AlCIPK protein in the MEME program. The size of each amino acid indicates its frequency in the respective domain. (B) the distribution of identified motifs in each protein sequence.

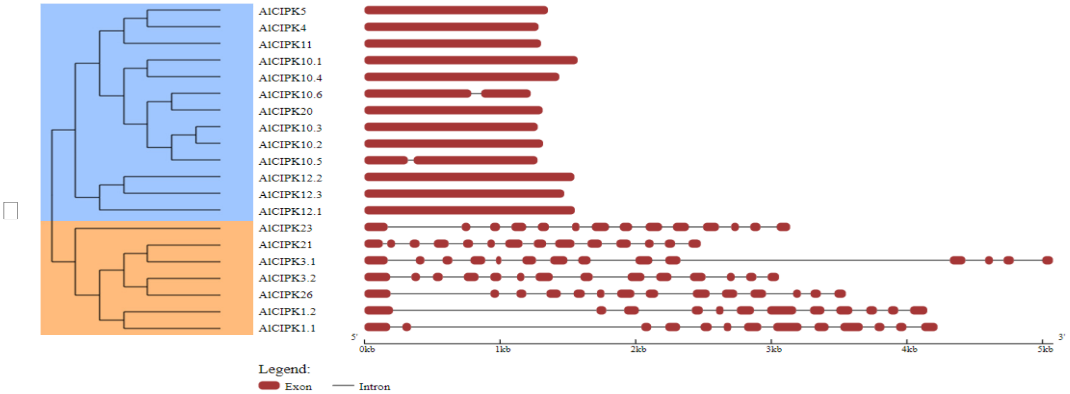


Fig. 3. Analysis of gene structure and phylogenetic tree of *AlCIPK* gene family

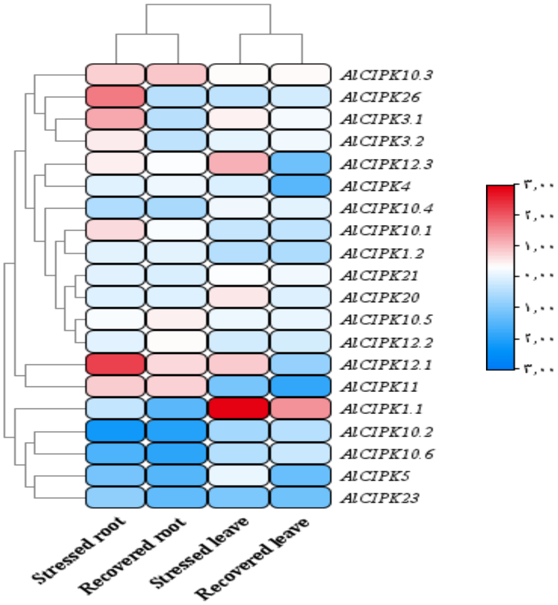
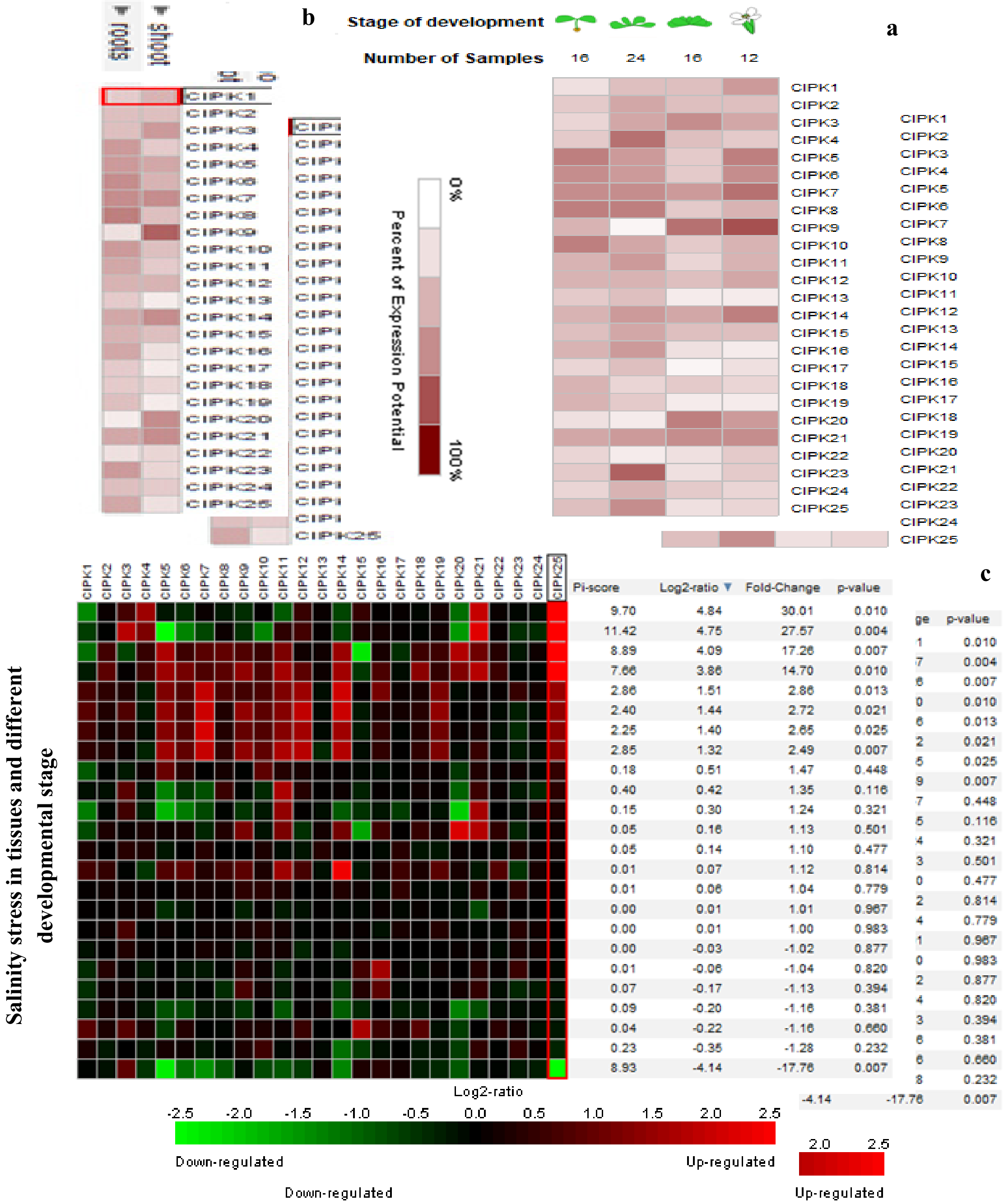


Fig. 4. Heatmap and hierarchical clustering of the *AlCIPK* gene family.



Pi-score	Log2-ratio ▼	Fold-Change	p-value
9.70	4.84	30.01	0.010
11.42	4.75	27.57	0.004
8.89	4.09	17.26	0.007
7.66	3.86	14.70	0.010
2.86	1.51	2.86	0.013
2.40	1.44	2.72	0.021
2.25	1.40	2.65	0.025
2.85	1.32	2.49	0.007
0.18	0.51	1.47	0.448
0.40	0.42	1.35	0.116
0.15	0.30	1.24	0.321
0.05	0.16	1.13	0.501
0.05	0.14	1.10	0.477
0.01	0.07	1.12	0.814
0.01	0.06	1.04	0.779
0.00	0.01	1.01	0.967
0.00	0.01	1.00	0.983
0.00	-0.03	-1.02	0.877
0.01	-0.06	-1.04	0.820
0.07	-0.17	-1.13	0.394
0.09	-0.20	-1.16	0.381
0.04	-0.22	-1.16	0.660
0.23	-0.35	-1.28	0.232
8.93	-4.14	-17.76	0.007

Fig. 5. Investigation of *AtCIPK* gene family expression profile in Arabidopsis model plant using Genevestigator. Microarray expression analysis of *AtCIPK1* to *AtCIPK25* genes (a) in 4 different developmental stages, (b) root and shoot tissues, (c) in different salinity stresses