A CBL-interacting protein kinase *AdCIPK5* confers salt and osmotic stress tolerance in transgenic tobacco

Naveen Kumar Singh[1, 2*], Pawan Shukla1 [1, 3], P. B. Kirti[1, 4]

***Corresponding author at:** naveenks11@gmail.com (Naveen Kumar Singh)

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad-500046, India

[2] Agricultural Research Organization-the Volcani Center, 68 HaMaccabim Road P.O.B 15159 Rishon LeZion 7505101, Israel

[3] Central Sericultural Research and Training Institute, Central Silk Board, NH-1A, Gallandar, Pampore - 192 121, J & K, India

4 Agri Biotech Foundation, Rajendranagar, Hyderabad-500030, India

Journal: Scientific Reports

Figure S1. Nucleotide sequence showing 2,031 bp long *AdCIPK5* c-DNA sequence with 5' and 3' UTR sequences in lower case. Start and stop codons are mentioned in bold letters.

agcccaatcccaatcccaatcccaatctcaatatattccccctcactccattaataaattccatcacaacacaaacttcttcaactttcactcaacctaacccaaaaacggaaa
aaccataccctttatttttttcccttctttttcttctttctctcttgcacgtacatacacaccggagttccaatctggtgtatcacctatgcaccatgaagatcttcaacccttctgaatcct
ttctccgaaacgttgtgtgtccctcggacactatattcctcaaaggaatgaacctctccaatcttcaccactccacattctccctcctctcttggccatacaattagccttcccaa
ccactaatcttaactaactttctttctttttcctttttctctctctggaatcttcctccc**<u>ATG</u>**GATCTGAAAAACGAGATGGAGATCAAGTTGTCG
AACAGCGCTAGGACGGTGATATTCAACAAGTACGAGATGGGACGCGTGTTAGGTCAGGGGAATTTTG
CCAAGGTATACTACAGCAGGAACCTAGCGACGAACGAAAGTGTGGCGATTAAGGTGATAAAGAAGGA
TAAGCTGAGGAAGGAGAGGCTGATAGAGCAGATTAAGAGAGAGGTTTCGGTTATGAGGCTGGTGAGG
CATCCGAACATAGTGGAGCTGAAGGAAGTCATGGCCACGAAGGGGAAGATATTCTTGGTTATGGAGT
ATGTTAAGGGAGGCGAATTGTTCAAGAAAGTCGAGAAAGGGAAGCTCAAGGAAGATGTAGCTAGAAA
GTATTTTCAGCAGTTGATCTCGGCCGTTGATTTCTGCCATAGCCGCGGCGTCACTCACAGAGATCTCAA
GCCGGAGAATCTGCTGCTTGATGAGAATGAAGAGCTTAAGGTCTCCGATTTTGGCCTCTCTGCTTTGCC
GGAACAACGCCGTGCAGATGGTATGCTGATAACACCGTGTGGAACACCTGCATATGTGGCACCAGAA
GTGTTGAAGAAGAAAGGGTATGATGGATCCAAAGCAGATATATGGTCTTGTGGAGTGATTCTTTATGC
TCTGGTTTGTGGCTATCTACCCTTCCAAGGCGAAAACGTGATGAGAATCTACAGAAAAGCCTTCAAGG
CTCAGTATGAATTCCCTGATTGGGTTCCGGAAGGAGCAAAGAACTTGATCTCAAACCTACTTGTAGCT
GATCCTGAAAAGAGGTTCTCAATTGCAGATATCATGAAGGATCCTTGGTTCCAAGTCGGCTTCATGCG
TCCGATTGCATTCTCCTTTAAGGAGTCTGCCATTGGGGACAATGTGGATGACTTCGATGAGGATGATA
GTGATAGTAATAACAATCAAGAGTTTAACAAGGATGATTTGAAGCAGAGTAGTTCTGCTAAGCCTGCG
CGGCCGTTTTACAATGCTTTCGAGATAATTTCCTCGCTGTCGCATGGTTTTGATCTGAGGAGTTTGTTTG
AGACAAGGAAGAGGTCACCTTCAGTGTTTATATCAAAGCTTTCGGCTCAAGCAGTGGTGGGGAAGCTA
GAGGCTATGGCGAAGAAATTGAATTTCAGAGTGGCCGGGAAGAAGAAGAAGGAGTTTGTTGTGAGGA
TGGAAGGGGAGAGGGAAGGGAGGAAAGGGAGGCTGGCCATGACGGTGGAAGTGTTCGAGGTGGCGC
CGGAGGTGGCCGTGGTGGAGTTCTCGAAGTCTTCTGGGGATACCTTGGAGTATATCAAATTCTGTGAG
GAAGAAGTTAGGCCTTCATTGAATGACATTGTTTGGAGTTGGCAGGGAGATGGTAACAAC**<u>TAA</u>**taatacta
gtcatgccattgggattattttgcctatgttgttcttccttttactgtttgatgcagctatataataatatagtttgatttgcataataagtaaaagaaaaggaagatgctaggaaag
aaataatgctgtattttgattttagttacaggatttcatttcatttgtgtaaaaaatttgagtttagtttctgatgatgtttgaagttaatactgaaaaaaaaaaaaaaa

Figure S2. Alignment of deduced amino acid sequences of AdCIPK5 with closely related CIPKs from other plant species. Adu: *Arachis duranensis*; Gm: *Glycine max*; At: *Arabidopsis thaliana*.

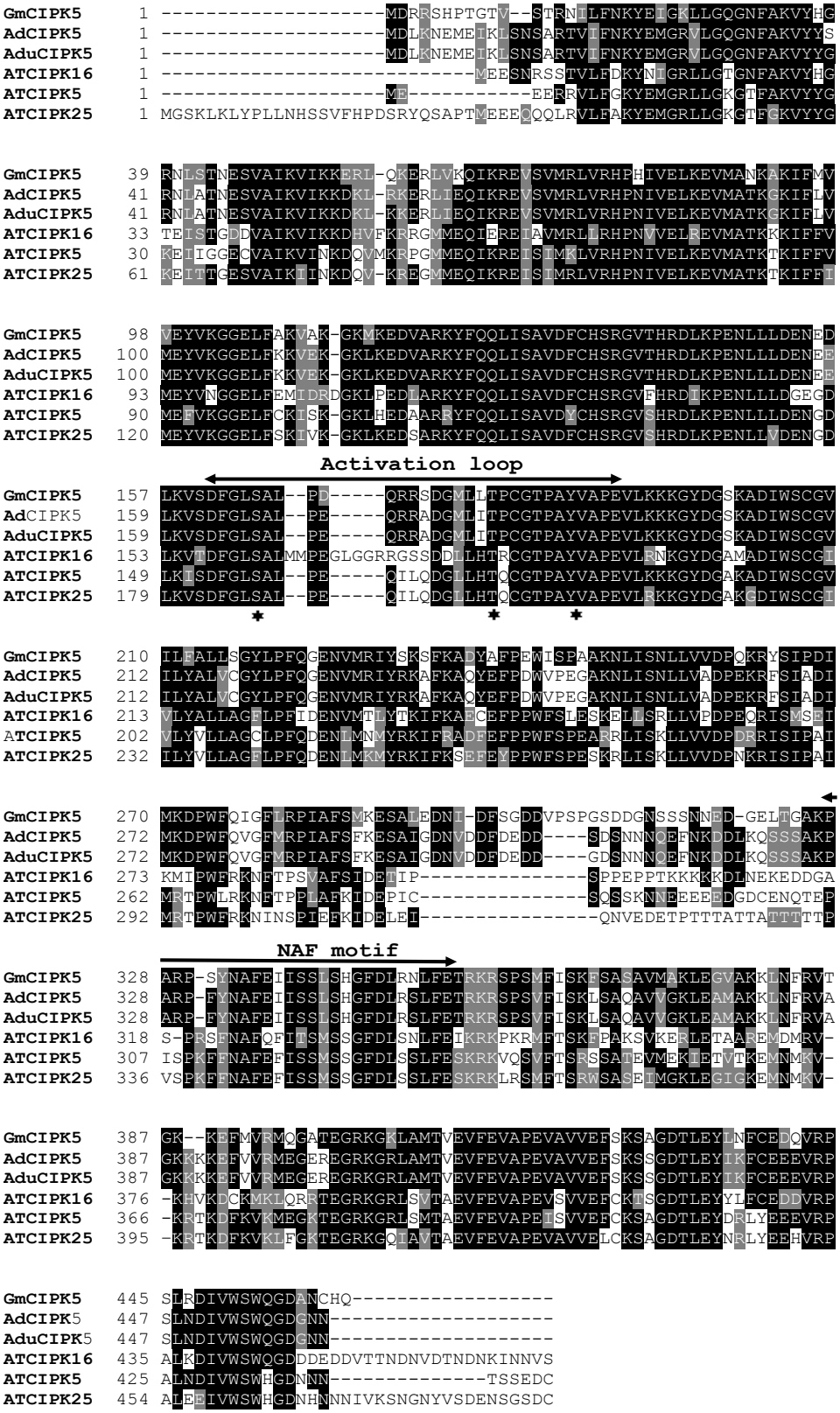


Figure S3. Phylogenetic tree of AdCIPK5 and related protein kinases: AduCIPK5 of *Arachis duranensis*; GmCIPK5 of *Glycine max*; AtCIPK 1–26 of *Arabidopsis thaliana.*

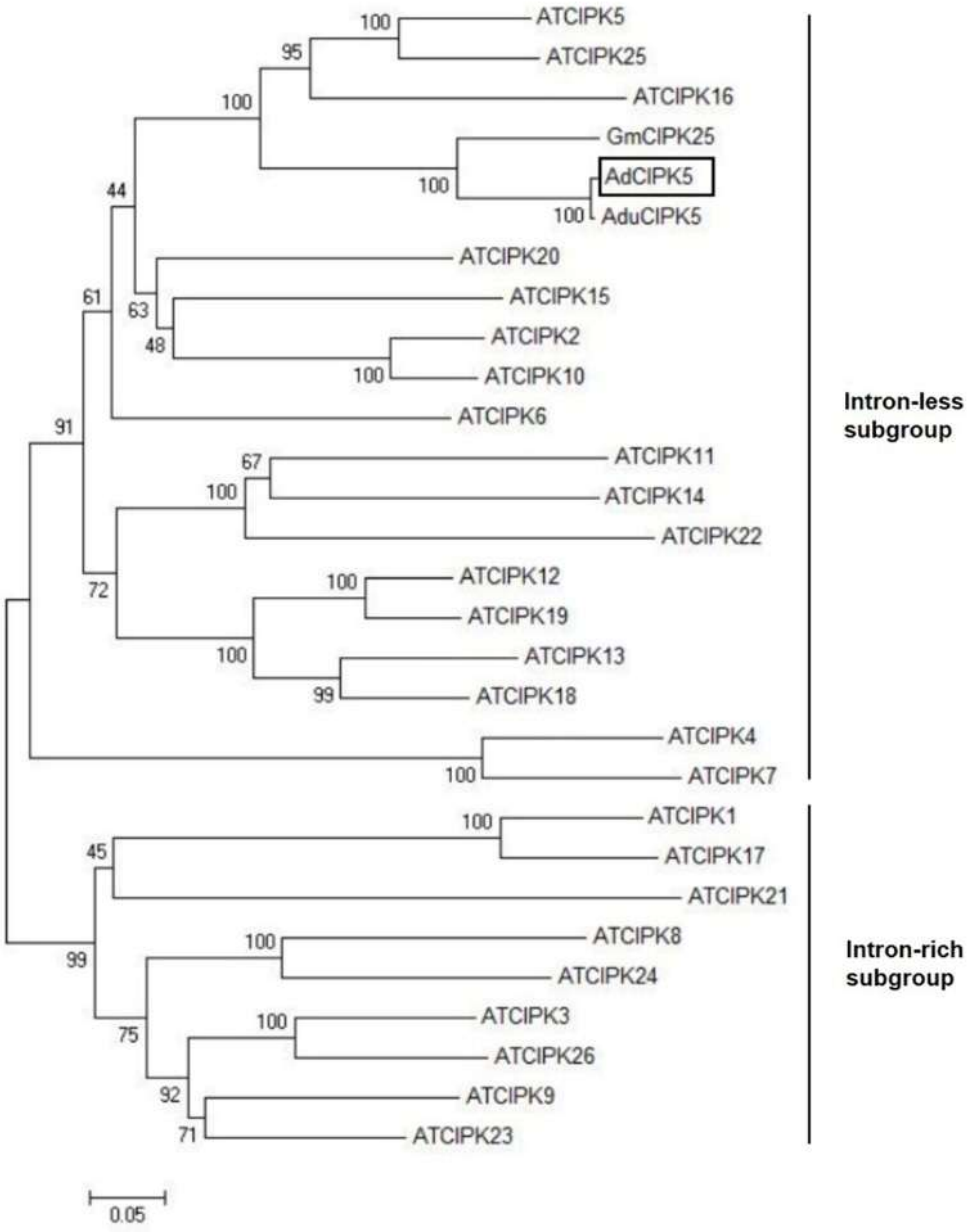


Figure S4. Gel pictures showing 739 bp amplified PCR product of *nptII* (A) and 1386 bp of *AdCIPK*5 ORF (B) from genomic DNA of nine different putative T_0 transgenic lines and WT plants. Letter M represents λ *Eco*RI/*Hind*III DNA ladder.

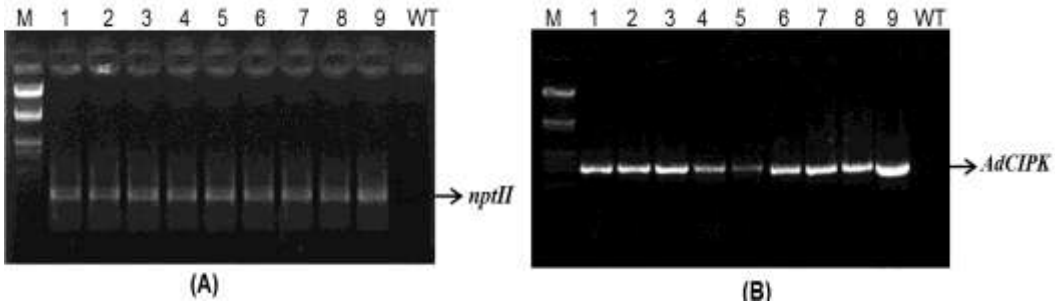


Figure S5. Semi-quantitative RT-PCR analysis of *AdCIPK5* gene in putative T_0 **(A)** and T_2 **(B)** transgenic plants. Lane 1 and 6 represent high expression lines and lane 2 represent the low expression line. *Actin* served as internal control to demonstrate equal loading

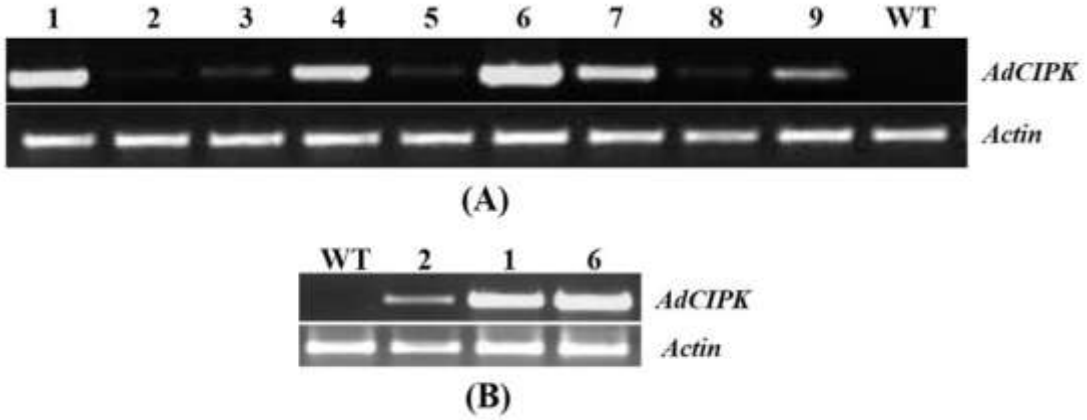
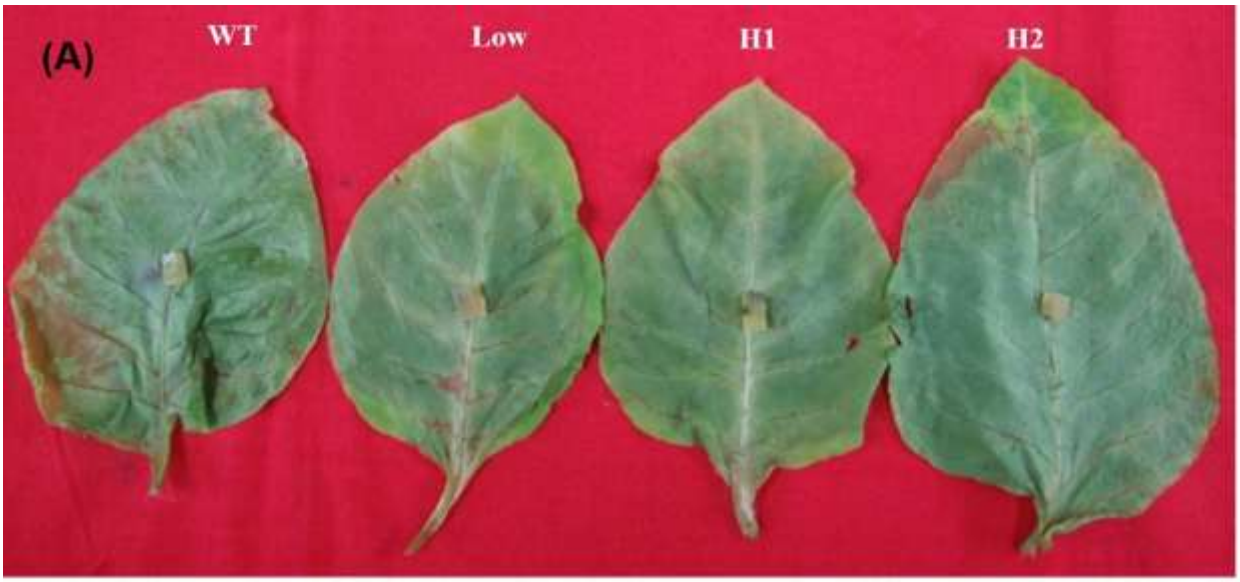
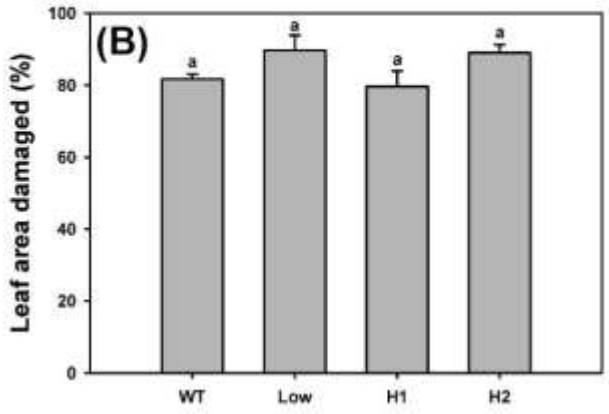


Figure S6. Resistance to fungal infection was checked in mature leaves of WT and T_2 transgenic plants against *Phytophthora parasitica* var. nicotianae after 5 d of treatment **(a)**. No differences were observed between wild type and transgenic lines in terms of damaged leaf area and tissue cell death **(b and c)**. Data represent means ±SE of three replicates and same letters indicate no significant difference among the lines.





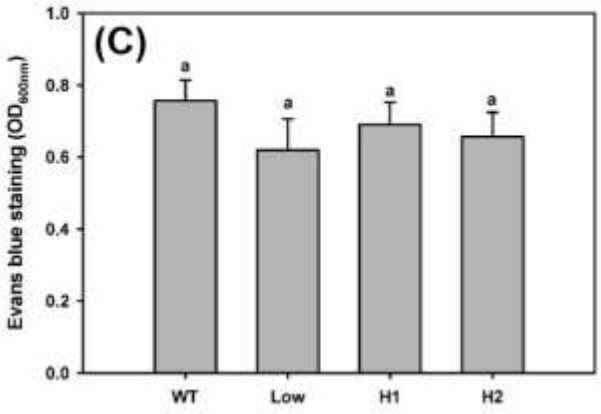


Table S1. Sequences of the oligonucleotides used for RACE, cloning and PCR analysis (see text for details).

Name of the primers	**Primer sequences (5′-3′)**
SMARTer II A oligo	AAG CAG TGG TAT CAA CGC AGA GTA CGC GGG
5′ CDS	5′-$(T)_{25}$ V N-3′ (N=A, C, G or T; V= A, G or C)
3′ CDS	AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}$ V N (N=A, C, G or T; V=A, G or C)
Universal Primer A Mix	Long 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3′ Short 5'-CTAATACGACTCACTATAGGGC-3'
Nested Primer	AAGCAGTGGTATCAACGCAGAGT
5′GSP-1	AGAACTACTCTGCTTCAA
5′GSP-2	CATCCTCATCGAAGTCATCCA
5′GSP-3	AAAGGAGAATGCAATCGGACG
3′ GSP1	TGGATGACTTCGATGAGGATG
ORF-F1	GCCATGGATCTGAAAAACGAGATGGA
ORF-R1	CCACTAGTGTTGTTACCATCTCCCT
ORF-F2	CCGGGCCCATGGATCTGAAAAACGAGATGGA
ORF-R2	CCGGTACCTTAGTTGTTACCATCTCCCTGC

Table S2. Sequences of the oligonucleotides used in qRT-PCR (see text for details). Nt: *Nicotiana tabacum*; Ad: *Arachis diogoi*.

Oligo name	**Forward (5′-3′)**	**Reverse (5′-3′)**
NtCAT	GGCCGCTACAACTCTCTCTTT	ACAGGACCTCTTGCACCAAC
NtERD10C	AAAGCCAACTCATGCCCAAG	AGAGCTGCTACTTGATCGATGG
NtERD10D	GCACGAGGGAAGAAGAGAAGG	TGGAGGCGCCACTTCCTC
NtNCED1	TGTCTGAAATGATCCGGGGC	AGTTTCCGGCTCTTCCCAAG
NtSUS1	CACGGATATTTCGCCCAGGA	GCAGCAGCCGAGTAGCAATA
NtSOS1	CAAATGTTATCCCCCGAAAGC	CGGAGAACCTGAGGAAATGTGA
NtUbq	GAGTCAACCCGTCACCTTGT	ACATCTTTGAGACCTCAGTAGACA
Adadh3	GACGCTTGGCGAGATCAACA	AACCGGACAACCACCACATG
Adcipk	CCATGACGGTGGAAGTGTTC	TACCATCTCCCTGCCAACTC