New insights into the evolution and functional divergence of the *CIPK* gene family in *Saccharum*

Weihua Su[1,2], Yongjuan Ren[1,2], Dongjiao Wang[1,2], Long Huang[1,2], Xueqin Fu[3], Hui Ling[1], Yachun Su[1,2], Ning Huang[1,2], Hanchen Tang[1,2], Liping Xu[1,2], Youxiong Que[1,2]*

[1]Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China

[2]Key Laboratory of Genetics, Breeding and Multiple Utilization of Crops, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou 350002, China

[3] Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Plant Immunity Center, Fujian Agriculture and Forestry University, Fuzhou 350002, China.

***Correspondence should be addressed to quevouxiong@126.com**

The full postal address of the submitting author Youxiong Que is as follows: Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China

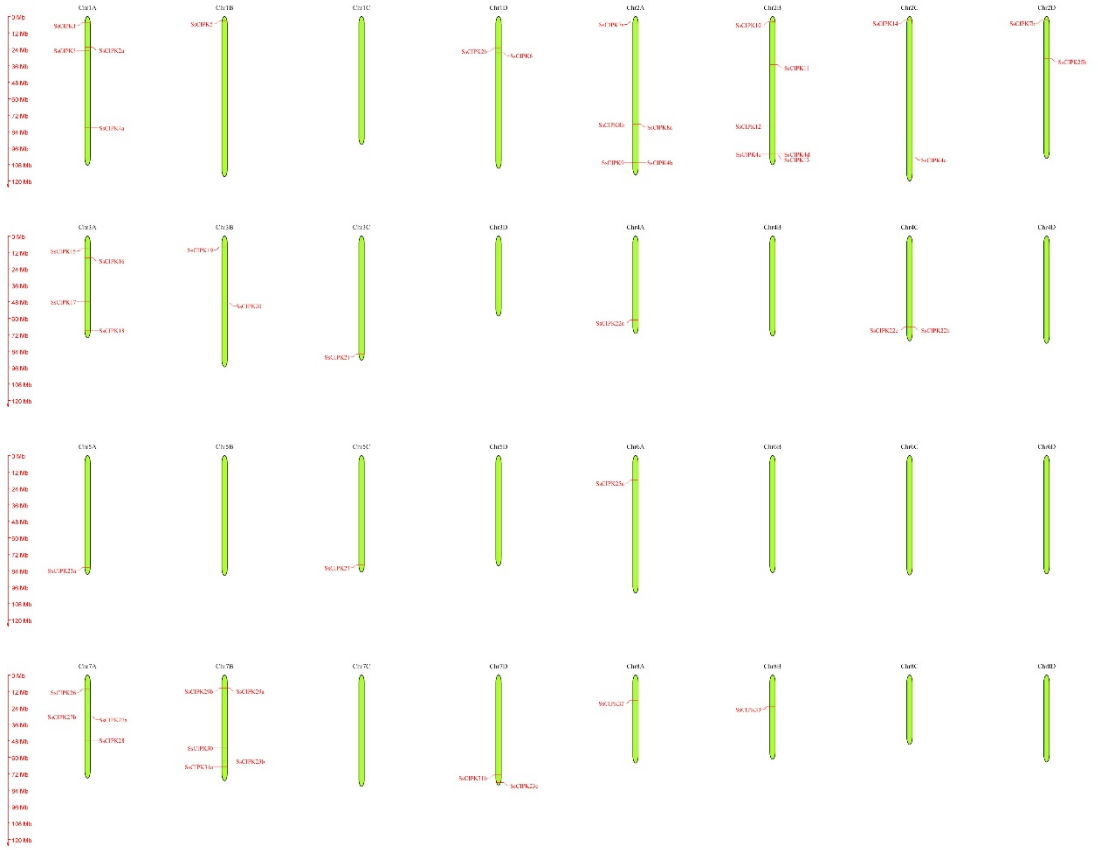

Fig. S1 Chromosomal distribution of *SsCIPKs*. The scale bar on the left indicated the length (Mb) of sugarcane chromosomes.

Fig. S2 Analysis and distribution of conserved motifs in SsCIPK proteins.

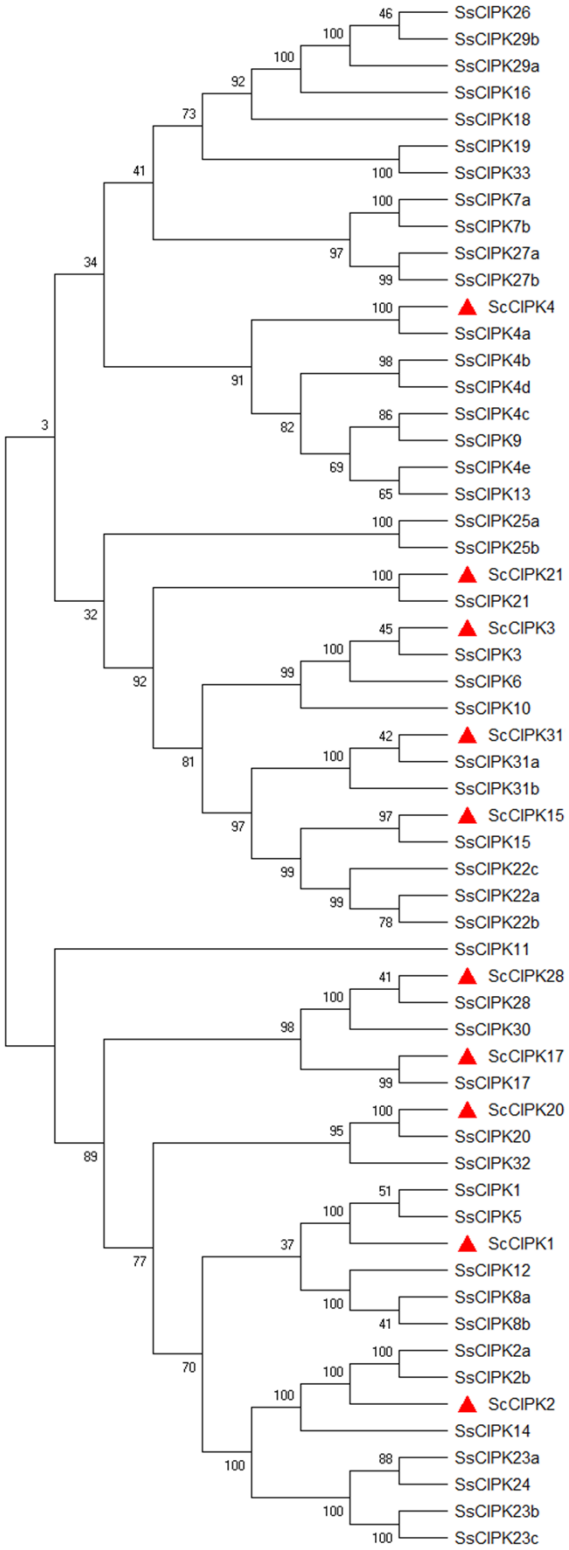

Fig. S3 Phylogenetic analysis of CIPK proteins from *S.spontaneum* and *Saccharum* spp. hybrid (ROC22). Red triangles represent ScCIPK proteins.

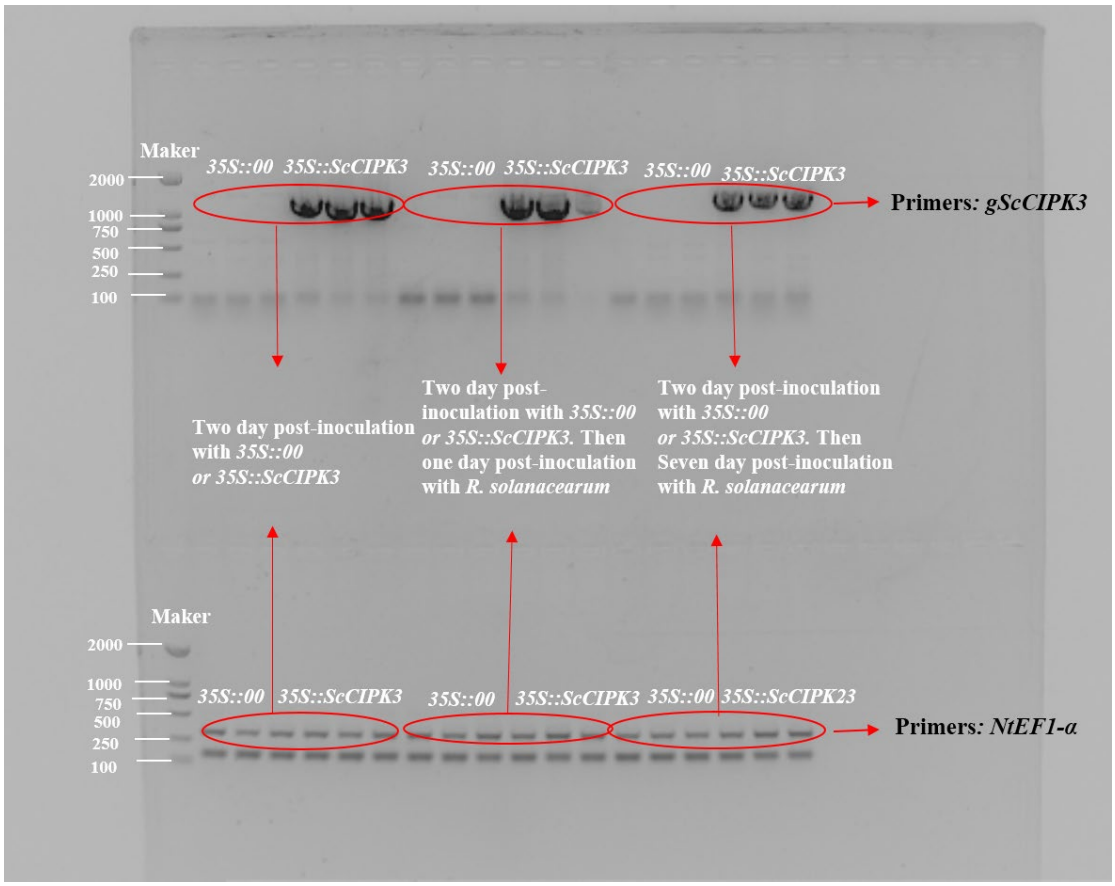

Fig. S4 RT-PCR analysis of *ScCIPK3* in the *N. benthamiana* leaves 2 days after infiltration with *Agrobacterium* strain GV3101 that carried the vector *35S::00* or *35S::ScCIPK3*, in the *ScCIPK3* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for one day, and in the *ScCIPK3* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for seven days.
Note: *35S::00* stands for the control templet, *35S::ScCIPK3* stands for the template of instantaneous expression *ScCIPK3*. *gScCIPK3*and *NtEF1-α* represented the primers used in the RT-PCR.

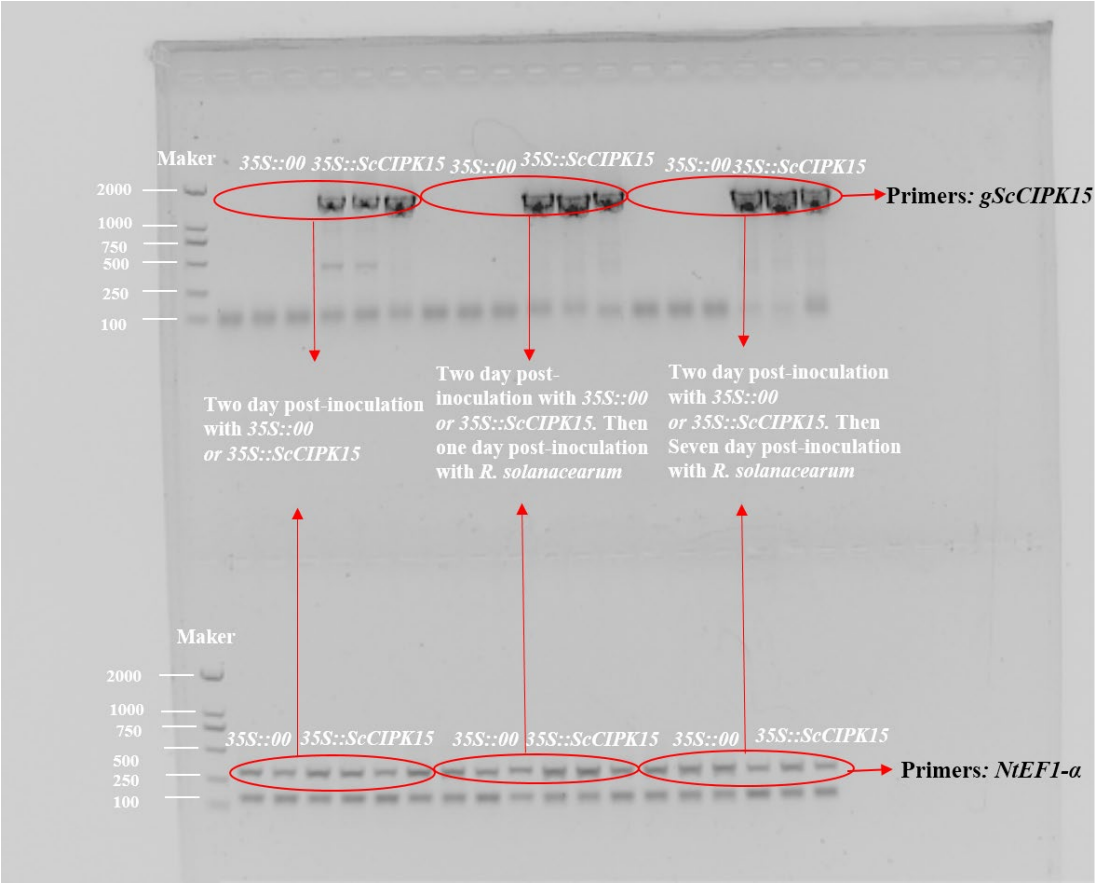

Fig. S5 RT-PCR analysis of *ScCIPK15* in the *N. benthamiana* leaves 2 days after infiltration with *Agrobacterium* strain GV3101 that carried the vector *35S::00* or *35S::ScCIPK15*, in the *ScCIPK15* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for one day, and in the *ScCIPK15* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for seven days.

Note: *35S::00* stands for the control templet, *35S::ScCIPK15* stands for the template of instantaneous expression *ScCIPK15*. *gScCIPK15*and *NtEF1-α* represented the primers used in the RT-PCR.

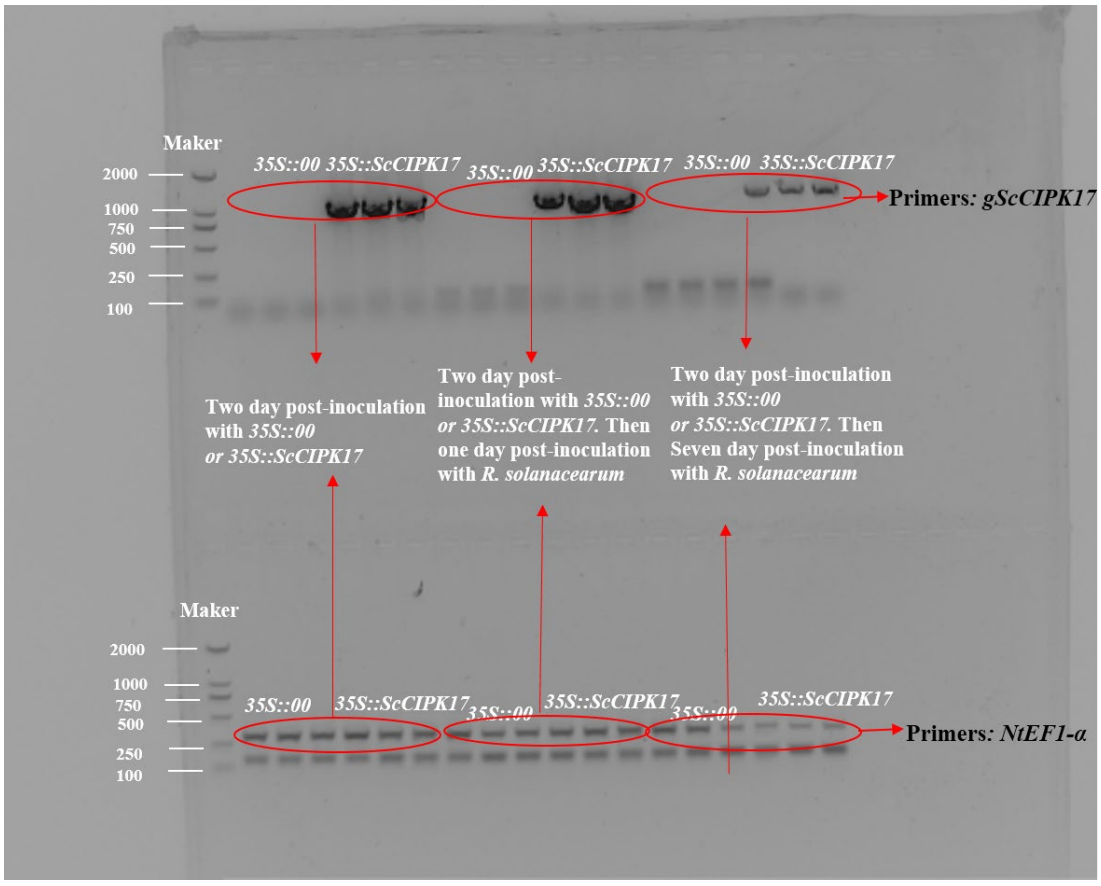

Fig. S6 RT-PCR analysis of *ScCIPK17* in the *N. benthamiana* leaves 2 days after infiltration with *Agrobacterium* strain GV3101 that carried the vector *35S::00* or *35S::ScCIPK17*, in *the ScCIPK17* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for one day, and in *the ScCIPK17* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for seven days.

Note: *35S::00* stands for the control templet, *35S::ScCIPK17* stands for the template of instantaneous expression *ScCIPK17*. *gScCIPK17*and *NtEF1-α* represented the primers used in the RT-PCR.

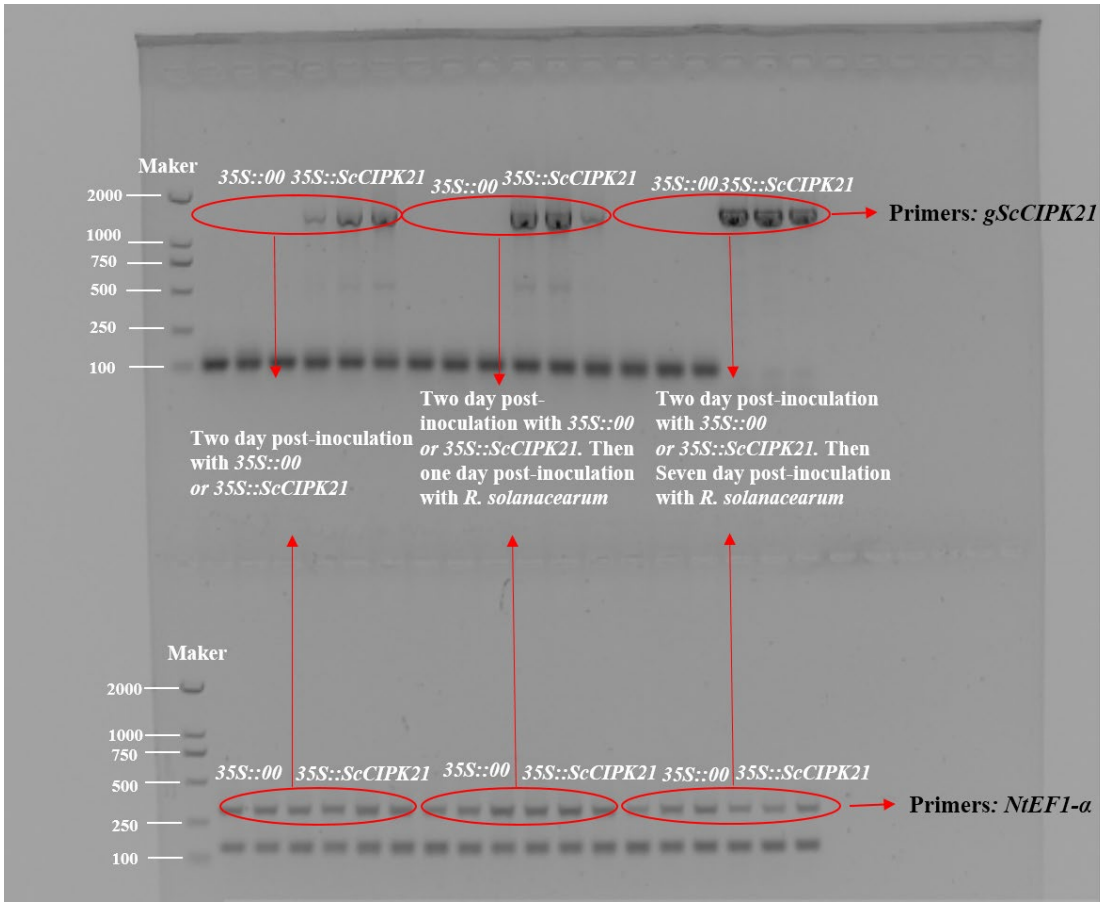

Fig. S7 RT-PCR analysis of *ScCIPK21* in the *N. benthamiana* leaves 2 days after infiltration with *Agrobacterium* strain GV3101 that carried the vector *35S::00* or *35S::ScCIPKs*, in the *ScCIPK21* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for one day, and in the *ScCIPK21* transiently expressed *N. benthamiana* challenged by *R. solanacearum* for seven days.
Note: *35S::00* stands for the control templet, *35S::ScCIPK21* stands for the template of instantaneous expression *ScCIPK21*. *gScCIPK21*and *NtEF1-α* represented the primers used in the RT-PCR.