RESEARCH ARTICLE

Genome-wide identification and characterization of the CKII gene family in the cultivated banana cultivar (Musa spp. cv Tianbaojiao) and the wild banana (Musa itinerans)

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Abstract

Plant casein kinase II (CKII) plays an essential role in regulating plant growth and development, and responses to biotic and abiotic stresses. Here, we report the identification and characterization of the CKII family genes in Musa spp. cv. ‘Tianbaojiao’ (AAA group) and the wild banana (Musa itinerans). The 13 cDNA sequences of the CKII family members were identified both in ‘Tianbaojiao’ and wild banana, respectively. The differences between CKII α and CKII β members are corroborated through the subcellular localizations, phosphorylation sites and gene structures. The cloning of CKII β-like-2 gDNA sequences in wild banana and ‘Tianbaojiao’ and the analysis of gene structures showed MiCKII β-like-2b and MaCKII β-like-2 are likely alternatively spliced transcripts, which were derived from the alternative splicing events that involved exon deletion. The qPCR validation showed differential expression CKII family members in response to cold stress and also in all tested tissues (leaf, pseudostem and root) of wild banana. In particular, the normal transcript MiCKII β-like-2a was highly expressed in response to cold stress in wild banana; oppositely, the alternatively spliced transcript MiCKII β-like-2b was quite lowly expressed. The complex origin and long-term evolution of Musa lineage might explain the alternative splicing events of CKII β-like-2.

Introduction

Casein kinase II (CKII or CK2) is a Ser/Thr kinase involved in the regulation of protein functions in eukaryotes. Plant CKII is a tetrameric protein composed of two catalytic (α) and two regulatory (β) subunits, and it is also a pleiotropic enzyme. It plays an essential role in regulating various cellular processes such as growth, development, circadian rhythms, light responses, hormone responses, transcription, translation, cell-cycle regulation, nuclear transport, Ca$^{2+}$ storage, seed storage, salicylic acid-mediated defenses, flowering time, DNA repair and responses to biotic and abiotic stresses in plants, such as maize, tobacco, wheat, mustard and...
Arabidopsis thaliana [1–13]. Salinas et al (2006) [14] presented a complete survey of the CKII gene family and found four α subunits and four β subunit genes, which were all expressed in the inflorescences, stems, leaves and roots in Arabidopsis. Mulekar et al (2012) [4] further reported that CKII α subunits affect multiple developmental and stress-responsive pathways in Arabidopsis. Portoles and Mas (2010) [15] found that the functional interplay between CKII and CCA1 (circadian clock associated 1) transcriptional activities is essential for clock temperature compensation in Arabidopsis. In plant cells, CKII is localized in the cytosol and the nucleus [16], and α subunits of the CKII family members are localized in the chloroplasts in mustard and Arabidopsis [8].

CKII is an extremely conserved pleiotropic protein kinase with more than 300 substrates [6,17]. The CKII phosphor acceptor sites are specified by multiple acidic residues, with the one at position +3 relative to the target residue being crucial. The CKII holoenzyme is composed of two catalytic subunits (αα, αα′ or αα′′), which act mainly as catalysts of phosphorylation, and a dimer of two non-catalytic β subunits, which act mainly as regulators of enzymatic activities [2,4,17]. Dennis and Browning (2009) reported the differential phosphorylation of plant translation initiation factors by Arabidopsis thaliana CKII holoenzymes [18]. Recent plant whole-genome sequencing projects will allow the precise structure and function of CKII to be fully characterized.

Banana belongs to the genus Musa, a member of the family Musaceae, and is the most popular fruit in the worldwide. It is thermophilic crop, and distribute in the warm tropical or subtropical regions. Fujian province, in the northern margin of China is one such region prominent for banana cultivation. 'Tianbaojiao', which is the famous traditional cultivar in Fujian, often suffered low temperature stress in winter and early spring (S1 Fig). The critical temperature of growth is thought to be around 13˚C for most banana cultivars in China [19]. The morphological changes of 'Tianbaojiao' leaves were quite different at low temperature stress (4˚C) and when the treatment time was increased. The changes such as slight waterlogging (3 h), wilting (5–7 h) or death (at 28˚C to recover) were observed (S2 Fig). The wild banana genetic resources are abundant in China, particularly in Fujian province. A novel wild banana line, which was found at Sanming city, Fujian province, is thought to be extremely cold resistant based on screening the wild banana genetic resources collected by our team for over 10 years [20]. It can grow well around 0˚C [21], and its semilethal temperature was lower than other nine Musa genus plants, reached as low as -1.776˚C [22]. So the wild banana (cold-resistant) and 'Tianbaojiao' (cold-sensitive) were used as materials to study the existence and expression of CKII family genes.

The banana genome has been published [23–24], allowing for the identification of CKII family genes in banana. In this study, the members of CKII family genes in banana genome A from Musa acuminata 'DH-Pahang' and in banana genome B from Musa balbisiana Pisang Klutuk Wulang (PKW) were analyzed using the banana genome’s data, and then the CKII gene family members in Musa spp. cv. 'Tianbaojiao' and the wild banana (Musa itinerans) were cloned and characterized, which is beneficial to understanding the structure and functions of the CKII family genes in Musa plant.

**Materials and methods**

2.1 Analysis of the CKII family genes in banana genomes A and B

Using banana genome A (Musa acuminata var. DH-Pahang, AA group, the wild banana from Malaysia) [23] and banana genome B (Musa balbisiana var. Pisang Klutuk Wulang, BB group) [24] databases, the CKII genes were obtained by searching for the term of 'casein kinase II', and then the known CKII sequences in NCBI were used as the probes to search for the CKII...
genes in banana genomes. They were further analyzed as the candidate CKII genes of banana genomes.

2.2 Isolation of the CKII family genes cDNA sequences and CKIIβ-like-2 gDNA sequences from wild banana and ‘Tianbaojiao’

The leaves of the wild banana (Musa itinerans) from Sanming City, China and the cultivated banana ‘Tianbaojiao’ (Musa spp., Cavendish, AAA group, the famous traditional cultivar in China, which originated from the wild banana Musa acuminata, AA group) collected from the Banana Germplasm Nursery of Institute of Horticultural Biotechnology of Fujian Agriculture and Forestry University were used as the materials for RNA and DNA extraction, according to the method of Feng et al. (2015) [25]. Total RNA was reverse transcribed using a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Fermentas, EU) for cDNA sequences cloning. Using candidate CKII genes in banana genomes combined with published CKII sequences in NCBI, all of the CKII family members cDNA sequences of wild banana and ‘Tianbaojiao’ were cloned by RT-PCR (reverse transcription PCR) technique. The CKIIβ-like-2 gDNA sequences of wild banana and ‘Tianbaojiao’ were obtained from DNA templates using PCR. Primer sequences were designed from known CKII sequences in NCBI and the banana genome databases, and are listed in S1 Table.

2.3 Bioinformatic analysis of CKII family genes in the A genome, the wild banana and ‘Tianbaojiao’

On the NCBI website, the nucleotide and protein sequences of CKII family members were identified by BLASTn and BLASTp, respectively. DNAMEN 6.0 was used to analyze CDSs (coding sequences) and protein sequences [23–24]. MEGA 6.0 was used to construct the phylogenetic trees of the CKII proteins, and the NJ (neighbor-joining) method was then applied to this analysis with 1,000 bootstrap replications. GSDS was used to analyze the gene structures. The conserved domains were analyzed on the NCBI website. NetPhos 3.1 was used to analyze the CKII phosphorylation sites. The conserved motifs of CKII protein sequences were analyzed on MEME server [26]. The protein subcellular localization prediction tool of ‘PSORT’ was used to predict the subcellular location of the CKII protein sequences.

2.4 Plant materials and treatments for qPCR

The wild banana (Musa itinerans) from Sanming City and the cultivated banana ‘Tianbaojiao’ (Musa spp., AAA group) were used in this study. The in-vitro plantlets were regenerated by tissue culture from the explants of suckers. After transplanting them to the pots and cultivating for 1 month at 28˚C under 2000 lx throughout lighting in a 12 h/12 h light-dark cycle, seedlings at the uniform growth stage were selected for treatments. After sufficiently watering for 2 d, the seedlings were put in the growth chambers set to 28˚C (the control), and at 13˚C, 4˚C and 0˚C, under 2000 lx fluorescent lighting in a 12 h/12 h light-dark cycle (synchronized with the natural light cycle) at a relative humidity of 70%-80% for 24 h. After 24 h treatments, the first young leaf was detached from 10 seedlings at each temperature point (28˚C, 13˚C, 4˚C and 0˚C) for each biological replicate. The leaf samples of each of the 10 seedlings were harvested and pooled for each temperature point. All of the treatments were performed with 3 biological replicates. Finally, they were frozen in liquid N2 and stored at -80˚C for total RNA extraction and used in the qPCR (real-time quantitative PCR) assay. The leaves, roots, and pseudo-stems were also to taken from the potted plants grown at 28˚C.
2.5 Real-time quantitative PCR and data analysis

The total RNA extracted from the leaves after cold treatments (including the control) using Column Plant RNAOUT 2.0 Kit (TIANDZ, China), and 0.5 ug total RNA was used for reverse transcription of qPCR analysis with PrimeScript™ RT Master Mix (Perfect Real Time) kit (Takara, Japan) according to the method of Feng et al. (2015) [25]. The expression detection of the CKII family genes was performed on a LightCycler 480 (Roche). The reaction system and procedures were those of Feng et al. (2015) [25]. The qPCR analyses were performed as described by Lin and Lai (2013) [27] and the CAC gene was used as the internal control [28]. The primer sequences were designed using Primer 3 input software and are listed in the S2 Table. The amplification efficiency for each primer pairs of the CKII family genes was determined in a qPCR assay using a five-fold dilution series from a pooled cDNA template (S3 Fig). The PCR efficiency values of all CKII family genes ranged from 1.853 to 2.040, and as listed in S2 Table. SPSS was used to assess the statistically significant differences of data, and all data are expressed as the means ± SDs of three independent replicates. Duncan’s multiple range test was used for the significant differences. *: significant difference (at p-value <0.05) identified by comparing with 28℃, **: very significant difference (at p-value <0.01) identified by comparing with 28℃.

Results

3.1 Analysis of the CKII family genes in banana genomes A and B

In total, there are 13 CKII family genes in banana genome A and 11 CKII family genes in banana genome B. The functional domains analyses indicated that all 13 members of the CKII family in banana genome A contained STKc_CK2_alpha or CK_II_beta functional domains, while the 11 CKII family genes of banana genome B had only three members (ITC1587_Bchr2_P04995, ITC1587_Bchr6_P16283 and ITC1587_Bchr6_P18330) that contained complete functional domains (STKc_CK2_alpha or CK_II_beta). The phylogenetic tree of the 13 CKII members from genome A and the 11 CKII members from genome B had two branches, one containing CKII α members and the other containing CKII β members and unclassified CKII subunits members. Additionally, ITC1587_Bchr9_P25856 and ITC1587_Bchr5_P13181, with PKc-like superfamily domains, were clustered to a clade (S4 Fig). Thus, the integrity and accuracy of CKII from genome A was greater than that from genome B, which was suggested to function as the reference genome for identification of the CKII gene family members in the genus Musa.

3.2 Cloning of the CKII gene family members in ‘Tianbaojiao’ and the wild banana

Using RT-PCR, 13 cDNA sequences of the CKII family members were obtained from the Musa spp. cv. ‘Tianbaojiao’ and wild banana (Musa itinerans), respectively (Table 1). The sequence lengths of 9 members were the same but those of the other 4 members were different between the ‘Tianbaojiao’ and the wild banana (Table 1). Between them, both CKIIβ-4-2 and CKIIβ-4-3 had sequences that differed by 3–6 bp (Fig 1A, Fig 1B and 1C), and CKIIβ-3-like (Fig 1D) and CKIIα-4 (Fig 1E) also had sequence differences, making them more similar to those of genome A, which resulted in different stop codons. In addition, there were 2 transcripts of CKIIβ-like-2 in wild banana, and one (MiCKIIβ-like-2a) was similar to that of genome A, while the other (MiCKIIβ-like-2b) was similar to that of the ‘Tianbaojiao’ banana (MaCKIIβ-like-2) (Fig 1F). The sequence analysis indicated that the MiCKIIβ-like-2a and MiCKIIβ-like-2b genes had a 216 bp sequence difference, which belonged to an exon region when compared with the A genome. Therefore, we inferred that an alternative splicing event during evolution resulted in an exon deletion in the transcripts of MiCKIIβ-like-2b and
MaCKIIβ-like-2. The sequence comparisons of CKII family members among wild banana, ‘Tianbaojiao’ and genome A were conducted (Table 2). Most of the members were the same or similar among these comparisons, except for the alternatively spliced transcripts of MiCKIIβ-like-2b and MaCKIIβ-like-2, which were both quite different from the transcripts of the genome A. In addition, the CKIIα subunit members were generally similar, while CKIIβ subunit members were relatively different.

The analysis of the functional domains indicated that all of the CKII family members contained the whole STKc_CK2_alpha or CK_II_beta conserved domains in wild banana and ‘Tianbaojiao’.

3.3 Cloning and analyses of the CKIIβ-like-2 gDNA sequences in wild banana and ‘Tianbaojiao’

The gDNA sequences of CKIIβ-like-2 in wild banana and ‘Tianbaojiao’ was cloned, and the gene structures of MiCKIIβ-like-2a, MiCKIIβ-like-2b and MaCKIIβ-like-2 were predicted to further validate the alternative splicing of CKIIβ-like-2 in wild banana and ‘Tianbaojiao’ (S5 Fig). The results showed that, 4176 bp and 4164 bp CKIIβ-like-2 gDNA sequences in wild banana and ‘Tianbaojiao’ were obtained, respectively. And the gene structures analysis showed 5 exons and 4 introns existed in MiCKIIβ-like-2a, while there was one deletion exons in MiCKIIβ-like-2b in wild banana. The MaCKIIβ-like-2 in ‘Tianbaojiao’ also has 4 exons and 3 introns, similar with MiCKIIβ-like-2b in wild banana. So, the MiCKIIβ-like-2b in wild banana and MaCKIIβ-like-2 in ‘Tianbaojiao’ might be the exon deletion alternative splicing transcript.

3.4 Predicted subcellular localizations of the CKII family members among wild banana, ‘Tianbaojiao’ and the A genome

The subcellular localization of the CKII family members among wild banana, ‘Tianbaojiao’ and the A genome were predicted (Table 3).

Comparisons of the subcellular localizations among the CKII family indicated that all of the members, except for MiCKIIβ-like-1 from wild banana, were the same among wild banana, ‘Tianbaojiao’ and the A genome. Furthermore, all of the CKIIβ subunit members, except for

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Table 1. The nucleotide sequences characteristics of CKII family members between the wild banana and ‘Tianbaojiao’.

| CKII family in wild banana | Accession NO. | CKII family in ‘Tianbaojiao’ | Accession NO. |
|---------------------------|--------------|-----------------------------|---------------|
| MiCKIIα-1                | 1002 MF598885| MaCKIIα-1                   | 1002 MF598873 |
| MiCKIIβ-4-1              | 843 MF598886| MaCKIIβ-4-1                 | 843 MF598874  |
| MiCKIIβ-like-1           | 681 MF598887| MaCKIIβ-like-1              | 681 MF598875  |
| MiCKIIβ-4-2              | 861 MF598888| MaCKIIβ-4-2                 | 858 MF598876  |
| MiCKIIβ-4-3              | 840 MF598889| MaCKIIβ-4-3                 | 843 MF598877  |
| MiCKIIβ-3-like           | 843 MF598890| MaCKIIβ-3-like              | 873 MF598878  |
| MiCKIIβ-4-4              | 843 MF598891| MaCKIIβ-4-4                 | 843 MF598879  |
| MiCKIIα-2                | 1251 MG451828| MaCKIIα-2                   | 1251 MF598880 |
| MiCKIIα-3                | 1002 MF598892| MaCKIIα-3                   | 1002 MF598881 |
| MiCKIIα-4                | 1227 MF598893| MaCKIIα-4                   | 1215 MG451818 |
| MiCKIIβ-like-2a          | 855 MF598894| MaCKIIβ-like-2              | 639 MF598882  |
| MiCKIIβ-like-2b          | 639 MF598895| MaCKIIβ-like-2              | 1176 MF598883 |
| MiCKIIα-5                | 1176 MF598896| MaCKIIα-5                   | 1176 MF598883 |
| MiCKIIβ-like-3           | 843 MF598897| MaCKIIβ-like-3              | 843 MF598884  |

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CKIIβ-like-1 from 'Tianbaojiao' and the A genome, were localized to the nucleus. However, the subcellular localizations of the CKII α subunit members varied, including cytoplasmic, mitochondrial, and extracellular (including cell wall). In particularly, MaCKIIα-4, Ma06_p36630.1 and MiCKIIα-4 were predicted to localize to the mitochondrial with 100%, 100%, and 95.7% probabilities, respectively, which indicated that CKIIα-4 likely functioned in the mitochondria.

The clustering analysis, combined with the subcellular localizations of the CKII family members from the wild banana, 'Tianbaojiao' and the A genome are shown in Fig 2, and both the CKII α and the CKII β members were clearly assigned to two branches. The CKII β members were assigned to one branch (Fig 2A), which were localized to the nucleus except for CKIIβ-like-1 from 'Tianbaojiao' and the A genome. The CKII α members were assigned to another branch, and being further clustered which were consistent with subcellular localizations site, i.e. cytoplasmic (Fig 2B), extracellular, including cell wall (Fig 2C) and mitochondrial (Fig 2D).

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3.5 Analyses CKII family members gene structures among the wild banana, ‘Tianbaojiao’ and A genome

The gene structures of the CKII family members among wild banana, ‘Tianbaojiao’ and the A genome were analyzed (Fig 3). The CKII α members contained 10 exons and 9 introns, while all the CKII β members contained 5 exons and 4 introns, except for MiCKIIβ-like-2b from the wild banana and MaCKIIβ-like-2 from ‘Tianbaojiao’, which contained 4 exons and 3 introns. The differences in these two members may have resulted from an alternative splicing event during evolution that resulted in an exon deletion. The structures define the functions, therefore, the CKII α and β subunit members likely functioned differently.

### Table 2. The sequences comparisons of the CKII family members among the wild banana, ‘Tianbaojiao’ and the A genome.

| Gene name |一致性 (%) | Gene name |一致性 (%) | Gene ID |一致性 (%) |
|-----------|------------|-----------|------------|--------|------------|
| MiCKIIα-1 | 91.82 | 79.42 | MiCKIIα-1 | 93.71 | 79.88 | Ma06_p18320.2 | 92.61 | 79.54 |
| MiCKIIα-3 | MaCKIIα-3 | | Ma10_p11700.1 | | |
| MiCKIIα-5 | MaCKIIα-5 | | Ma05_p01580.1 | | |
| MiCKIIα-2 | 84.68 | MaCKIIα-2 | 85.37 | Ma09_p09220.1 | 86.01 |
| MiCKIIα-4 | MaCKIIα-4 | | Ma06_p36630.1 | | |
| MiCKIIβ-4-1 | 96.96 | 91.55 | MiCKIIβ-4-1 | 96.28 | 91.43 | Ma04_p36590.1 | 94.78 | 90.48 |
| MiCKIIβ-4-3 | MaCKIIβ-4-3 | | Ma02_p12220.2 | | |
| MiCKIIβ-4-4 | MaCKIIβ-4-4 | | Ma04_p32940.1 | | |
| MiCKIIβ-4-2 | 81.71 | 59.55 | MiCKIIβ-4-2 | 82.11 | 59.67 | Ma04_p18400.1 | 81.13 | |
| MiCKIIβ-like-3 | 73.57 | MaCKIIβ-like-3 | | Ma05_p16000.1 | 80.30 |
| MiCKIIβ-like-2b | MaCKIIβ-like-2b | | Ma08_p15420.1 | | |
| MiCKIIβ-like-2a | MaCKIIβ-like-2a | | Ma05_p32940.1 | | |
| MiCKIIβ-like-1 | 68.56 | MaCKIIβ-like-1 | 66.90 | Ma02_p23160.1 | 67.12 |
| MiCKIIβ-like-like | MaCKIIβ-like-like | | Ma06_p13370.1 | | |

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### Table 3. Predicted and analysis of the subcellular localization of the CKII family members among the wild banana, ‘Tianbaojiao’ and A genome.

| Subunit | Genome A | The wild banana | ‘Tianbaojiao’ |
|---------|-----------|-----------------|---------------|
| | Gene ID | subcellular localization | Gene name | subcellular localization | Gene name | subcellular localization |
| α | Ma06_p18320.1 | cytoplasmic | MiCKIIα-1 | cytoplasmic | MaCKIIα-1 | cytoplasmic |
| β | Ma04_p36590.1 | nuclear | MiCKIIβ-4-1 | nuclear | MaCKIIβ-4-1 | nuclear |
| β | Ma02_p23160.1 | cytoplasmic | MiCKIIβ-like-1 | nuclear | MaCKIIβ-like-1 | cytoplasmic |
| β | Ma04_p18400.1 | nuclear | MiCKIIβ-4-2 | nuclear | MaCKIIβ-4-2 | nuclear |
| β | Ma02_p12220.1 | nuclear | MiCKIIβ-4-3 | nuclear | MaCKIIβ-4-3 | nuclear |
| β | Ma06_p13370.1 | nuclear | MiCKIIβ-like-3 | nuclear | MaCKIIβ-like-3 | nuclear |
| β | Ma04_p32940.1 | nuclear | MiCKIIβ-4-4 | nuclear | MaCKIIβ-4-4 | nuclear |
| α | Ma09_p09220.1 | mitochondrial | MiCKIIα-2 | mitochondrial | MaCKIIα-2 | mitochondrial |
| α | Ma10_p11700.1 | cytoplasmic | MiCKIIα-3 | cytoplasmic | MaCKIIα-3 | cytoplasmic |
| α | Ma06_p36630.1 | mitochondrial | MiCKIIα-4 | mitochondrial | MaCKIIα-4 | mitochondrial |
| β | Ma08_p15420.1 | nuclear | MiCKIIβ-like-2a | nuclear | MaCKIIβ-like-2a | nuclear |
| β | Ma05_p15880.1 | extracellular, including cell wall | MiCKIIβ-5 | extracellular, including cell wall | MaCKIIβ-5 | extracellular, including cell wall |
| β | Ma05_p16000.1 | nuclear | MiCKIIβ-like-3 | nuclear | MaCKIIβ-like-3 | nuclear |

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3.6 Predicted occurrence of CKII phosphorylation sites among the wild banana, 'Tianbaojiao' and A genome

CKII phosphorylation sites were predicted among the wild banana, 'Tianbaojiao' and A genome (S3 Table). The results showed that the numbers of phosphorylation sites in the CKII family members among the wild banana, 'Tianbaojiao' and A genome varied from 1 to 12, and the numbers of phosphorylation sites in the CKIIβ members (except for the proteins from the 2 alternatively spliced transcripts MiCKIIβ-like-2b and MaCKIIβ-like-2) were generally greater than those in the CKIIα members. The former were between 5 and 12, and the latter were between 1 and 2, which was in accordance with the differences in the functions of CKIIα and CKIIβ. The CKIIα members acted mainly as catalysts of phosphorylation, while the CKIIβ members were highly conserved and acted mainly in the regulation of enzymatic activities.

The sites and numbers of amino acid residues of CKII phosphorylation in CKIIα-1, CKIIβ-4-4, CKIIα-2, CKIIα-3 and CKIIα-4 members were the same, while the others showed some differences among the wild banana, 'Tianbaojiao' and A genome. For example, the amino acid residue serine 104 in Ma05_p01580.1 and serine 148 in Ma04_p36590.1 were both specific to the A genome, while the amino acid residue serine 10 in MiCKIIβ-4-3 was specific to wild banana.
3.7 Analysis of the conserved motifs of CKII family among the wild banana, ‘Tianbaojiao’ and A genome

The conserved motifs of CKII family members were analyzed among the wild banana, ‘Tianbaojiao’ and A genome (Fig 4). All 5 CKII α members had the same numbers of the 10 conserved motifs in the wild banana, ‘Tianbaojiao’ and A genome. However, CKII α-2 had the specific motif 3, CKII α-4 had the specific motif 10 and CKII α-5 had the specific motif 4 (Fig 4A). The CKII β members of CKII β-4-1, CKII β-4-2, CKII β-4-3, CKII β-4-4 and CKII β-like-3 in both A genome and the wild banana, CKII β-like-2 in A genome and CKII β-like-2a in
wild banana, had the same numbers of the 10 conserved motifs. However, CKII β-like-2b and CKII β-like-2 both had 8 conserved motifs, lacking motifs 3 and 6, and CKII β-like-1 and CKII β-3-like both had 8 conserved motifs, lacking motifs 4 and 8 (Fig 4B). Thus, the motifs among CKII α or CKII β family members were highly conserved.

3.8 Expression levels of the CKII family members in wild banana at different temperatures and in different tissues by qPCR

The expression levels of the CKII family members in wild banana were detected by qPCR in leaf tissue at different temperatures (growth temperature of 28˚C as the control, and the low temperatures of 13˚C for critical growth, 4˚C for chilling and 0˚C for freezing) (Fig 5) and in...
different tissues (leaves, pseudo-stems and roots) (Fig 6). The expression levels of CKIIβ-like-2a, CKIIα-5 and CKIIβ-4-2 were the highest at 4˚C, especially those of CKIIβ-like-2a and CKIIα-5, which were significantly higher than those of the control, and the expression levels of CKIIβ-like-2a, CKIIα-5 and CKIIβ-4-2 in leaves were also higher than those in pseudo-stems and roots. The expression level of CKIIα-3 was lowest at 4˚C, which was highest in leaf. While the expression level of CKIIα-2 in root was higher than that in leaf (the only member). The expression levels of CKIIα-1, CKIIβ-4-1 and CKIIβ-4-4 were all high, but not significant, at 13˚C. The expression levels of these 3 members in leaves were significantly higher than in the other two tissues, even though they showed similar expression patterns. The expression levels

Fig 5. Gene expression of CKII family members at different temperatures in the wild banana. Transcripts abundance was quantified using qRT-PCR. SPSS software was used to perform all statistical analyses of data, and all data are expressed as the means ± SDs of three independent replicates. Duncan’s multiple range test was used for the significant differences. *: significant difference (at p-value <0.05) identified by comparing with 28˚C, **: very significant difference (at p-value <0.01) identified by comparing with 28˚C. The CKII gene family members of CKIIα-1, CKIIβ-4-1, CKIIβ-like-1, CKIIβ-4-2, CKIIβ-3-like, CKIIβ-4-4, CKIIα-2, CKIIα-3, CKIIα-4, CKIIβ-like-2a, CKIIα-5, CKIIβ-like-3, were abbreviated CKII-1, CKII-2, CKII-3, CKII-4, CKII-5, CKII-6, CKII-7, CKII-9, CKII-10, CKII-13, CKII-14a, CKII-15 and CKII-16.

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Fig 6. Gene expression of CKII family members in different tissues in the wild banana. Transcripts abundance was quantified using qRT-PCR. SPSS software was used to perform all statistical analyses of data, and all data are expressed as the means ± SDs of three independent replicates. Duncan’s multiple range test was used for the significant differences. *: significant difference (at p-value <0.05) identified by comparing with 28˚C, **: very significant difference (at p-value <0.01) identified by comparing with 28˚C. G, roots; J, pseudo-stems; Y, leaves. The CKII gene family members of CKIIα-1, CKIIβ-4-1, CKIIβ-like-1, CKIIβ-4-2, CKIIβ-3-like, CKIIβ-4-4, CKIIα-2, CKIIα-3, CKIIα-4, CKIIβ-like-2a, CKIIα-5, CKIIβ-like-3, were abbreviated CKII-1, CKII-2, CKII-3, CKII-4, CKII-5, CKII-6, CKII-7, CKII-9, CKII-10, CKII-13, CKII-14a, CKII-15 and CKII-16.

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of the 5 members, CKIIβ-like-1, CKIIβ-4-3, CKIIβ-3-like, CKIIα-4 and CKIIβ-like-3, changed remarkably at 2 or 3 of the low temperature points, and the expression patterns of CKIIβ-like-1, CKIIβ-4-3, CKIIα-4 and CKIIβ-like-3 were similar at 28˚C, 13˚C and 4˚C. Interestingly, the expression level of CKIIα-4 at 0˚C was 117 times higher than at 4˚C, and it had a significantly higher expression level in pseudo-stem than that in leaves. It was also the only member that expressed more highly in pseudo-stem than in leaves and roots. CKIIα-2 and CKIIα-4, with root and pseudo-stem specific expression, both belonged to CKII α subunit members and had a sequence similarity of 84.68%, and were expressed relatively higher at 0˚C. This suggested that wild banana could activate not only the CKII members in leaves but also the CKII members in roots and pseudo-stems for cold acclimation in response to the 0˚C cold stress. In addition, alternatively spliced transcript CKIIβ-like-2b was expressed at very low levels or could not be detected at different temperatures and in different tissues (data not shown), which was quite different from the expression patterns of the normal transcript CKIIβ-like-2a. The wild banana of Musa itinerans from Sanming City and A genome from 'DH-Pahang' (Musa acuminiata, AA group) of the Malaysian wild banana had the normal transcript CKIIβ-like-2a, but the cultivated ‘Tianbaojiao’ (AAA group) had only the lowly expressed alternatively spliced transcript CKIIβ-like-2. It might be a key gene related to cold stress. To further validate the expression levels of alternatively spliced transcript CKIIβ-like-2 in response to different temperatures in cultivated banana. For above mentioned reasons that the 'Tianbaojiao' cannot grow well at 4˚C low temperature stress, the expression profile of alternatively spliced transcript CKIIβ-like-2 was performed at 28˚C (control) and 13˚C in ‘Tianbaojiao’ (S6 Fig). The expression level of CKIIβ-like-2 at 13˚C was lower than those of the control. The growth of 'Tianbaojiao' is retarded or stopped at 13˚C, which is thought to be the critical temperature of growth and relatively low temperature for most banana cultivars in China, while CKIIβ-like-2 was down-regulated expression at 13˚C in 'Tianbaojiao'. So the alternatively spliced transcript CKIIβ-like-2 respond negatively to 13˚C low temperature in 'Tianbaojiao', which is opposite trend for the normal transcript CKIIβ-like-2a at 13˚C low temperature in wild banana.

Discussion

4.1 The function of CKII family members might be different in Musa plants

CKII is considered a tetrameric complex consisting of two catalytic α subunits and two regulatory β subunits [14, 29–30]. The gene structures, phosphorylation sites, and conserved motifs of CKII α and β members were specific in three Musa plants. In addition, most of the CKII β subunit members localized in the nucleus, but the CKII α subunit members varied in Musa plants. However, it is opposite in Arabidopsis. All of the CKII α members, except for the members of Alphacp (localized in chloroplast), localized to the nucleus, but the CKII β members localized to various sites in Arabidopsis [14]. CKII subunits localized frequently to the nucleus and cytosol, but they have also been found in other organelles, such as mitochondria, the endoplasmic reticulum and the external and internal surfaces of the plasma membrane [14, 31–32]. In plants, CKII has been found to localize to the cytosol and the nucleus [16], as well as to the chloroplast in mustard and Arabidopsis [8, 14]. In this study, the members of CKII family localized mostly in the nucleus, followed by the cytoplasmic and mitochondrial, and then extracellular sites. The maize CKII α subunit was the first catalytic subunit identified in plants [33], and contains 10 exons separated by 9 introns. Similarly, the CKII α members contained 10 exons and 9 introns in three Musa plants. The characteristic of CKII α subunits are highly conserved among different species were reported [10]. In present study, all the CKII β members contained 5 exons and 4 introns except for the alternatively spliced transcript. All the plant CKII β proteins present the major conserved features described for CKII β subunits from
other organisms [34]. This high-degree conservation indicates that the CKII functions may be conserved between the different species. In addition, the conserved motifs analysis of CKII family members in three Musa plants showed the motifs among CKII α or CKII β family members were highly conserved. These above results may further illustrate protein kinase CKII is a ubiquitous and highly conserved Ser/Thr kinase. The numbers of CKII phosphorylation sites in the CKII β members (except for the 2 alternatively spliced transcripts) were generally greater than those in the CKII α members in three Musa plants, and it will be worth investigating relevance of CKII subunit functions.

Expression levels of CKII family members at different temperatures and in different tissues were also different in wild banana. In particularly, the wild banana of Musa itinerans contained both the normal CKIIβ-like-2α transcript and the alternatively spliced CKIIβ-like-2β transcript. Genome A had CKIIβ-like-2a, but the cultivated ‘Tianbaojiao’ had only alternatively spliced transcript CKIIβ-like-2. The two transcripts of CKIIβ-like-2 had distinct expression levels in response to low temperature. The three Musa plants differ in their responses to environmental stress [20, 35–39], and CKII is involved in various plant developmental processes and in responses to biotic and abiotic stresses [2–3, 40]. Plant breeders require access to genetic diversity to satisfy the demands for more and higher quality foods that can be produced in a variable or changing climate, and the crop’s wild relatives represent a practical gene pool from which to expand the genetic diversity in crop plants [41]. Ortiza and Swennenb (2014) [42] proposed changing from crossbreeding to the biotechnology-facilitated improvement of banana and plantain. CKIIβ-like-2a may be a target gene for cold resistant in cultivated banana breeding by biotechnology technology.

4.2 The alternative splicing events of CKII may result from the complex origin and evolution of Musa lineage

The origin of Musa plant is quite complex. Musa acuminata (A genome) and Musa balbisiana (B genome) were the ancestors of Musa lineage, and the banana cultivars mainly involve both and are sometimes diploid but generally triploid. The ‘Tianbaojiao’ (Musa spp., Cavendish, AAA group) which is the famous traditional cultivar in China, originated from the wild banana Musa acuminate (AA group). The wild banana from Sanming City is Musa itinerans, which is one wild banana different from the Musa acuminata and Musa balbisiana. In additions, the Musa lineage experienced long-term evolution. Lescot et al. have provided the evidence of a whole-genome duplications (WGDs) event in the Musa lineage for the first time [43]. D’Hont et al. have detected three rounds of WGDs (denoted as α, β and γ) in the Musa lineage; α and β events was dated at a similar period around 65 Myr (million years) ago, and γ event occurred around 100 Myr ago [23]. After WGD, most (65.4%) of the genes included in the Musa α/β ancestral blocks are singletons and only 10% are retained in four copies, in agreement with the loss of most gene-duplicated copies [44]. Genes are more prone to be co-retained or co-lost after WGD [45]. WGDs have played a major role in angiosperm genome evolution [46]. Alternative splicing, which is common in plants, is a rapidly evolving process after gene duplication [47–48]. Alternative splicing could affect gene regulation, gene function and cause functional divergence between duplicates [47, 49]. Moreover, alternative splicing plays a crucial role in defense response of plants [50]. Abiotic stresses are known to cause changes in alternative splicing patterns in plants [51–52]. Plants alter splicing patterns in response to temperature stress [53–56]. After gene and genome duplication, alternative splicing patterns have diverged considerably in an organ- or stress-specific manner during the evolutionary history of Arabidopsis lineage [49]. These above reports may explain the phenomenon of alternative splicing events occurred in Musa plants. In our study, the alternative splicing events resulted in an exon
deletion in both MiCKIIβ-like-2b from the wild banana and MaCKIIβ-like-2 from ‘Tianbaojiao’. Both the wild bananas of and genome A had the normal CKIIβ-like-2 transcript, CKIIβ-like-2a, which was highly expressed under cold stress in the wild banana. However, the cultivar ‘Tianbaojiao’ did not contain CKIIβ-like-2a, and had only the alternatively spliced transcript CKIIβ-like-2, which was expressed at very low levels or could not be detected in response to cold stress in wild banana and respond negatively to 13°C low temperature in ‘Tianbaojiao’. Furthermore, from single gene duplication to WGD, gene duplication has occurred throughout eukaryotic evolution and contributed greatly to the duplicated genes [49]. The majority of duplicated genes are retained gain new functions and/or expression patterns (neofunctionalization) [49]. In maize leaf, 13% of homology gene pairs have undergone regulatory neofunctionalization [57]. WGD has formed novel functions genes and altered expression patterns [58]. This may be the factors of the different expression patterns in response to cold stress of the two CKIIβ-like-2 transcripts in wild banana. The character of poorly conserved between duplicated genes by WGD was reported [47]. The two CKIIβ-like-2 transcripts are also not conserved in three Musa plants. Besides, Feng et al. suggested the WGD, segmental duplication and complex transcriptional regulation contributed to the gene expansion and mRNA diversity of the MaSODs by the genome-wide identification of SOD gene family in ‘Tianbaojiao’ [25]. The alternative splicing events were occurred with high frequency in previous study related Musa plant [59–61]. Therefore, the likely factors of alternative splicing events occurred in present study are the complex origin and long-term evolution of Musa lineage.

Conclusions

In this study, based on the banana genome database, the cloning, identification and characterization of the CKII family members in Musa spp. cv. Tianbaojiao (AAA group) and the wild banana (Musa itinerans) were reported. 13 cDNA sequences of the CKII family members were obtained from the ‘Tianbaojiao’ and the wild banana, respectively. The bioinformatics and qPCR analyses of CKII family members suggested that the function of CKII family members might be different in Musa plants. Furthermore, CKIIβ-like-2a might be a gene related to cold resistant. In addition, the CKIIβ-like-2 gDNA sequences in wild banana and ‘Tianbaojiao’ were obtained, and the analysis of sequences and gene structures showed the MiCKIIβ-like-2b in wild banana and MaCKIIβ-like-2 in ‘Tianbaojiao’ might be the exon deletion alternative splicing transcripts. The alternative splicing events of CKIIβ-like-2 may result from the complex origin and evolution of Musa lineage.

Supporting information

S1 Fig. The leaves phenotypes of the wild banana and ‘Tianbaojiao’ in January 2016 showing the different response to the cold stress of these two Musa plants. A-B, ‘Tianbaojiao’ in the field; C, the wild banana in the field.

(TIF)

S2 Fig. The leaves phenotypes of the wild banana and ‘Tianbaojiao’ at 4˚C stress. A, ‘Tianbaojiao’ at 4˚C, 3 h; B, ‘Tianbaojiao’ at 4˚C, 5 h; C, ‘Tianbaojiao’ at 4˚C, 7 h; D-E, 4˚C stressed ‘Tianbaojiao’ at 28˚C to recover; F, the wild banana at 4˚C, 3 h; G, the wild banana at 4˚C, 5 h; H, the wild banana at 4˚C, 7 h.

(TIF)

S3 Fig. The information of qPCR efficiency calibration curves for each primer pairs of CKII family genes and CAC. The amplification efficiency for each primer pairs of the CKII family genes and CAC was determined in a qPCR assay using a five-fold dilution series from a
pooled cDNA template. A, CKIIα-1; B, CKIIβ-4-1; C, CKIIβ-like-1; D, CKIIβ-4-2; E, CKIIβ-4-3; F, CKIIβ-3-like; G, CKIIβ-4-4; H, CKIIα-2; I, CKIIα-3; J, CKIIα-4; K, CKIIβ-like-2a; L, CKIIβ-like-2b; M, CKIIα-5; N, CKIIβ-like-3; O, CAC.

(TIF)

S4 Fig. Phylogenetic analysis of the CKII family members in banana genome A and B. The phylogenetic tree was constructed using MEGA5 by the neighbor-joining (NJ) method and 1000 bootstrap replicates. The tree was divided into two phylogenetic subgroups. The CKII β members of the two Musa plants were assigned to one branch, and the CKII α members combined with unclassified subunit CKII members were assigned to another branch.

(TIF)

S5 Fig. Gene structures of CKIIβ-like-2a, CKIIβ-like-2b in wild banana and CKIIβ-like-2 in 'Tianbaojiao'. Structural analyses of CKIIβ-like-2a, CKIIβ-like-2b in wild banana and CKIIβ-like-2 in 'Tianbaojiao' were performed using GSDS showing the CKIIβ-like-2b in wild banana and CKIIβ-like-2 in 'Tianbaojiao' might be the exon deletion alternative splicing transcript. The exons and introns are represented by colored boxes and black lines, respectively. A, the gene structure of MiCKIIβ-like-2a; B, the gene structure of MiCKIIβ-like-2b; C, the gene structure of MaCKIIβ-like-2. The wild banana and 'Tianbaojiao' were abbreviated as 'SM' and 'TB'. CKIIβ-like-2 was abbreviated as '14'.

(TIF)

S6 Fig. Gene expression of alternatively spliced transcript CKIIβ-like-2 from 'Tianbaojiao' at 28˚C and 13˚C. Transcripts abundance was quantified using qRT-PCR. The expression levels from three independent biological replicates were analyzed.

(TIF)

S1 Table. The primers used for gene cloning in this study. 'Tianbaojiao' and the wild banana were abbreviated as 'TB' and 'SM'. The CKII gene family members of CKIIα-1, CKIIβ-4-1, CKIIβ-like-1, CKIIβ-4-2, CKIIβ-4-3, CKIIβ-like-2, CKIIα-3, CKIIα-4, CKIIβ-like-2a, CKIIβ-like-2b, CKIIα-5, CKIIβ-like-3, were abbreviated CKII-1, CKII-2, CKII-3, CKII-4, CKII-5, CKII-6, CKII-7, CKII-9, CKII-10, CKII-13, CKII-14a, CKII-14b, CKII-15 and CKII-16.

(DOC)

S2 Table. The primers used for qPCR assay in this study. The CKII gene family members of CKIIα-1, CKIIβ-4-1, CKIIβ-like-1, CKIIβ-4-2, CKIIβ-4-3, CKIIβ-like-2, CKIIα-3, CKIIα-4, CKIIβ-like-2a, CKIIβ-like-2b, CKIIα-5, CKIIβ-like-3, were abbreviated CKII-1, CKII-2, CKII-3, CKII-4, CKII-5, CKII-6, CKII-7, CKII-9, CKII-10, CKII-13, CKII-14a, CKII-14b, CKII-15 and CKII-16.

(DOC)

S3 Table. Predict of the CKII phosphorylation sites among the wild banana, 'Tianbaojiao' and genome A. 'Ser' and 'Thr' were abbreviated as 'S' and 'T'.

(DOC)

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References
1. Armengot L, Marquès-Bueno MM, Soria-Garcia A, Müller M, Munné-Bosch S, Martínez MC. Functional interplay between protein kinase CK2 and salicylic acid sustains PIN transcriptional expression and root development. Plant Journal. 2014; 78: 411–423. https://doi.org/10.1111/tpj.12481 PMID: 24547808
2. Mulekar JJ, Huq E. Expanding roles of protein kinase CK2 in regulating plant growth and development. Journal Experimental Botany. 2014; 65: 2883–2893.
3. Riera M, Vélez-Bermudez IC, Legnaioli T, Pagès M. “Specific features of plant CK2,” in Protein Kinase CK2, ed. L. Pinna (Wiley-Blackwell). 2013; 267–279.
4. Mulekar JJ, Bu Q, Chen F, Huq E. Casein kinase II α subunits affect multiple developmental and stress-responsive pathways in Arabidopsis. Plant Journal. 2012; 69: 343–354. https://doi.org/10.1111/j.1365-310X.2011.04794.x PMID: 21950772
5. Kang HG, Klessig DF. Salicylic acid-inducible Arabidopsis CK2-like activity phosphorylates TGA2. Plant Molecular Biology. 2005; 57: 541–557. https://doi.org/10.1007/s11103-005-0409-1 PMID: 15821979
6. Meggio F, Pinna LA. One-thousand-and-one substrates of protein kinase CK2?. FASEB Journal. 2003; 17: 349–368. https://doi.org/10.1096/fj.02-0473rev PMID: 12631575
7. Kato K, Kidou S, Miura H, Sawada S. Molecular cloning of the wheat CK2 alpha gene and detection of its linkage with Vrn-A1 on chromosome 5A. Theoretical and Applied Genetics. 2002; 104: 1071–1077. https://doi.org/10.1007/s00122-001-0805-0 PMID: 12582614
8. Ogrezwalla K, Piotrowski M, Reinothe S, Link G. The plastid transcription kinase from mustard (Sinapis alba L.). European Journal of Biochemistry. 2002; 294: 3329–3337. PMID: 12064075
9. Hidalgo P, Garreton V, Berrios CG, Ojeda H, Jordana X, Holuigue L. A nuclear casein kinase 2 activity is involved in early events of transcriptional activation induced by salicylic acid in tobacco. Plant Physiology. 2001; 125: 396–405. PMID: 11154347
10. Riera M, Peracchia G, Pagès M. Distinctive features of plant protein kinase CK2. Molecular and Cellular Biochemistry. 2001; 227: 119–127. PMID: 11827162
11. Espunya MC, Combettes B, Dot J, Chaubet-Gigot N, Martinez MC. Cell-cycle modulation of CK2 activity in tobacco BY-2 cells. Plant Journal. 1999; 19: 655–666. PMID: 10571851
12. Lee Y, Lloyd AM, Roux SJ. Antisense expression of the CK2 alpha-subunit gene in Arabidopsis. Effects on light-regulated gene expression and plant growth. Plant Physiology. 1999; 119: 989–1000. PMID: 10069836
13. Sugano S, Andronis C, Ong MS, Green RM, Tobin EM. The protein kinase CK2 is involved in regulation of circadian rhythms in Arabidopsis. Proceeding of the National Academy of Science of the United States of America. 1999; 96: 12362–12366.
14. Salinas P, Fuentes D, Vidal E, Jordana X, Echeverría M, Holuigue L. An extensive survey of CK2 alpha and beta subunits in Arabidopsis: multiple isoforms exhibit differential subcellular localization. Plant and Cell Physiology. 2008; 47: 1295–1308. https://doi.org/10.1093/pcp/pcp100 PMID: 18926165
15. Portolés S, Más P. The functional interplay between protein kinase CK2 and CCA1 transcriptional activity is essential for clock temperature compensation in Arabidopsis. Plos Genetics. 2010; 6: e1001201. https://doi.org/10.1371/journal.pgen.1001201 PMID: 21079791
25. Feng X, Lai Z, Lin Y, Lai G, Lian C. Genome-wide identification and characterization of the superoxide dismutase gene family in Musa acuminata cv. Tianbao jiao (AAA group). BMC Genomics. 2015; 16: 18544928. https://doi.org/10.1186/s12864-015-2046-7 PMID: 26486759
26. Riera M, Figueras M, López C, Goday A, Pagès M. Protein kinase CK2 modulates developmental functions of the abscisic acid responsive protein Rab17 from maize. Proceeding of the National Academy of Science of the United States of America. 2004; 101: 9879–9884.
27. Pinna LA. Protein kinase CK2: a challenge to canons. Journal of Cell Science. 2002; 115: 3873–3878.
28. Dennis MD, Browning KS. Differential phosphorylation of plant translation initiation factors by Arabidopsis thaliana CK2 holoenzymes. Journal of Biological Chemistry. 2009; 284: 20602–20614. https://doi.org/10.1074/jbc.M109.006692 PMID: 19509278
29. Liu HY, Qin JJ, Fan H, Cheng JJ, Li L, Liu Z. Genome-wide identification, phylogeny and expression analyses of SCARECROW-LIKE (SCL) genes in millet (Setaria italica). Physiology and Molecular Biology of Plants. 2017; 23: 629–640. https://doi.org/10.1016/j.pmbs.2017.04.009 PMID: 28878501
30. Davey MW, Gudimella R, Harikrishna JA, Sin LW, Khalid N, Keulemans J. A draft genome sequence for molecular genetics in polyploid, inter- and intra-specific Musa hybrids. BMC Genomics. 2013; 14: 683. https://doi.org/10.1186/1471-2164-14-683 PMID: 24094114
31. Chen FL. Cloning and cold resistance analysis of β-1,3-Glucanase gene Mugsp from the wild banana. M.S. thesis of Fujian Agriculture and Forestry University. 2016. (in chinese)
32. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochemical Journal. 2003; 369: 1–15. https://doi.org/10.1042/BJ20021469 PMID: 12396231
33. Dobrowolska G, Boldyreff B, Issinger OG. Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme. EMBO Journal. 2001; 20: 5320–5331. https://doi.org/10.1093/emboj/20.19.5320 PMID: 11574463
34. Reed JC, Bidwai AP, Glover CV. Cloning and disruption of CKB2, the gene encoding the 32-kDa regulatory beta'-subunit of Saccharomyces cerevisiae casein kinase II. Journal of Biological Chemistry. 1994; 269: 18192–18200. PMID: 8027080
35. Faust M, Montenarh M. Subcellular localization of protein kinase CK2: a key to its function. Cell and Tissue Research. 2000; 301: 329–340. PMID: 10994779
36. Rodríguez F, Allende CC, Allende JE. Protein kinase casein kinase 2 holoenzyme produced ectopically in human cells can be exported to the external side of the cellular membrane. Proceedings of the National Academy of Science of the United States of America. 2005; 102: 4716–4723.
37. Dobrowolska G, Boldyreff B, Issinger OG. Cloning and sequencing of the casein kinase 2 alpha subunit from Zea mays. Biochimica et Biophysica Acta. 1991; 1129: 139–140. PMID: 1756176
38. Reed JC, Bidwai AP, Glover CV. Cloning and disruption of CKB2, the gene encoding the 32-kDa regulatory beta'-subunit of Saccharomyces cerevisiae casein kinase II. Journal of Biological Chemistry. 1994; 269: 18192–18200. PMID: 8027080
39. Yang QS, Wu JH, Li CY, Wei YR, Sheng O, Hu CH, et al. Quantitative proteomic analysis reveals that antioxidation mechanisms contribute to cold tolerance in plantain (Musa paradisiaca L., ABB Group) seedlings. Molecular & Cellular Proteomics. 2012; 30: 1853–1869.
40. Dobrowolska G, Boldyreff B, Issinger OG. Cloning and sequencing of the casein kinase 2 alpha subunit from Zea mays. Biochimica et Biophysica Acta. 1991; 1129: 139–140. PMID: 1756176
41. Perrier X, De Langhe E, Donohue M, Lentfer C, Vrydaghs L, Bakry F, et al. Multidisciplinary perspectives of banana (Musa spp.) domestication. Proceeding of the National Academy of Science of the United States of America. 2011; 108: 11311–11316.
42. Lescol T. The genetic diversity of banana in figures. Fruitrop. 2011; 189: 58–62.
43. Azhar M, Heslop-Harrison JS. Genomes, diversity and resistance gene analogues in Musa species. Cytogenetic and Genome Research. 2008; 121: 59–66. https://doi.org/10.1159/000124383 PMID: 18544928
39. Heslop-Harrison JS, Schwarzacher T. Domestication, genomics and the future for banana. Annals and Botany. 2007; 100: 1073–1084.

40. Vilela B, Pagès M, Riera M. Emerging roles of protein kinase CK2 in abscisic acid signaling. Frontiers in Plant Science. 2015; 6: 966. https://doi.org/10.3389/fpls.2015.00966 PMID: 26579189

41. Brozynska M, Furtado A, Henry RJ. Genomics of crop wild relatives: expanding the gene pool for crop improvement. Plant Biotechnology Journal. 2016; 14: 1070–1085. https://doi.org/10.1111/pbi.12454 PMID: 26311018

42. Ortiza R, Swennebn R. From crossingbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnology Advances. 2014; 32: 158–169. https://doi.org/10.1016/j.biotechadv.2013.09.010 PMID: 24091289

43. Lescolt M, Piffanelli P, Ciampi A Y, Ruiz M, Blanc G, Leebens-Mack J, et al. Insights into the Musa genome: Syntenic relationships to rice and between Musa species. BMC Genomics. 2008; 9: 58. https://doi.org/10.1186/1471-2164-9-58 PMID: 18234080

44. Schnable J C, Springer N M, Freeling M. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. Proceeding of the National Academy of Science of the United States of America. 2011; 108: 4069–4074.

45. Veitia R A, Bottani S, Birchler J A. Cellular reactions to gene dosage imbalance: genomic, transcriptomic and proteomic effects. Trends in Genetics. 2008; 24: 390–397. https://doi.org/10.1016/j.tig.2008.05.005 PMID: 18585818

46. Van de Peeyer F, Fawcett J A, Proost S, Sterck L, Vandepoele K. The flowering world: a tale of duplications. Trends in Plant Science. 2009; 14: 680–688. https://doi.org/10.1016/j.tplants.2009.09.001 PMID: 19818673

47. Rapid evolutionary divergence in alternative splicing patterns following whole genome duplication in the Arabidopsis lineage. PGY Zhang. M.S. thesis of British Columbia university. 2008;

48. Wang BB, Brendel V. Genome wide comparative analysis of alternative splicing in plants. Proceeding of the National Academy of Science of the United States of America. 2006; 103: 7175–7180.

49. Zhang PG, Huang SZ, Pin AL, Adams KL. Extensive divergence in alternative splicing patterns after gene and genome duplication during the evolutionary history of Arabidopsis. Molecular Biology and Evolution. 2010; 27: 1686–1697. https://doi.org/10.1093/molbev/msq054 PMID: 20185454

50. Chamala S, Jackson S, Schmutz J, Barbazuk B. Evolution of alternative splicing patterns after whole-genome duplication. International Plant and Animal Genome Conference Xxi. 2014;

51. Iida K, Seki M, Sakurai T, Satou M, Akiyama K, Toyoda T, et al. Genome-wide analysis of alternative pre-mRNA splicing in Arabidopsis thaliana based on full-length cDNA sequences. Nucleic Acids Research. 2004; 32: 5096–5103. https://doi.org/10.1093/nar/gkh845 PMID: 15452276

52. Palusa SG, Ali GS, Reddy AS. Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins: regulation by hormones and stresses. Plant Journal for Cell & Molecular Biology. 2007; 49: 1091–1107.

53. Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, et al. Genome-wide mapping of alternative splicing in Arabidopsis thaliana. Genome Research. 2010; 20: 45–58. https://doi.org/10.1101/gr.093302.109 PMID: 19858364

54. James AB, Syed NH, Bordage S, Marshall J, Nimmo GA, Jenkins GI, et al. Alternative splicing mediates responses of the Arabidopsis circadian clock to temperature changes. Plant Cell. 2012; 24: 961–981. https://doi.org/10.1105/tpc.111.093948 PMID: 22408072

55. Chang CY, Lin WD, Tu SL. Genome-wide analysis of heat-sensitive alternative splicing in phycocyanin genes. Plant Physiology. 2014; 165: 826. https://doi.org/10.1104/pp.133.230540 PMID: 24777346

56. Capovilla G, Kajrowski R. Mapping of alternative pre-mRNA splicing in soybean. Current opinion in plant biology. 2015; 27: 97–103. https://doi.org/10.1016/j.tplants.2015.06.016 PMID: 26190743

57. Hughes TE, Langdale JA, Kelly S. The impact of widespread regulatory neofunctionalization on homeolog gene evolution following whole-genome duplication in maize. Genome Research. 2014; 24: 1348–1355. https://doi.org/10.1101/gr.172684.114 PMID: 24788921

58. Richardson, Newton D. Genome duplication and alternative splicing: gateways to functional diversity. PhD thesis of zu Köln University. 2010;

59. Feng X. Genome-wide identification and function analysis of the superoxide dismutase gene family in Musa spp. PhD thesis of Fujian Agriculture and Forestry University. 2016; (in Chinese)

60. Liu W. Micropropagation and cloning and quantitative expression of resistant genes of the wild bananas in Fujian Province. M.S. thesis of Fujian Agriculture and Forestry University. 2013; (in Chinese)

61. Qi Q. Effects of Piriformospora indica on the Growth, Disease Resistance and Snakin Gene Expression in Banana. M.S. thesis of Fujian Agriculture and Forestry University. 2017; (in Chinese)