Genome wide analysis of kinesin gene family in *Citrullus lanatus* reveals an essential role in early fruit development

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Abstract

Background

Kinesin (KIN) as a motor protein is a versatile nano-machine and involved in diverse essential processes in plant growth and development. However, the kinesin gene family has not been identified in watermelon, a valued and nutritious fruit, and yet their functions has not been characterized. Especially, their involvement in early fruit development, which directly determines the size, shape, yield and quality of the watermelon fruit, remains unclear.

Results

In this study, we performed a whole-genome search and comprehensive analysis in *C. lanatus*. In total, 48 kinesins were identified and categorized into 10 kinesin subfamilies groups based on phylogenetic analysis. Their uneven distribution on 11 chromosomes was revealed by chromosomal distribution analysis. Conserved motif analysis showed that the ATP-binding motif of kinesins was conserved within all subfamilies, but not the microtubule-binding motif. 10 segmental duplication pairs genes were detected by the syntenic and phylogenetic approaches, which explains the expansion of the kinesin gene family in *C. lanatus* genome. Moreover, 5 *CIKINs* genes are specifically and abundantly expressed in early fruit developmental stages according to comprehensive expression profile analysis, indicating their critical regulatory roles during early fruit development. Our current data also demonstrated that the majority of kinesin genes were responsive to plant hormones, implying their involvement in the signaling pathways of plant hormones.

Conclusions

This study is the first comprehensive analysis of the kinesin gene family in watermelon, which establishes a foundation for further functional investigation of *C. lanatus* kinesin genes and provides novel insights into their biological functions. In addition, these results will provide a useful resource for further selecting an artificial regulator of fruit development in *C. lanatus*.

Background

Kinesins, widely distributed in all eukaryotic organisms [1], are a group of microtubule-based motor proteins that move along microtubule (MT) protofilaments and power by hydrolyzing ATP to drive various essential biological processes[2]. All kinesin proteins share a conserved motor domain of approximately 350 amino acids. The “motor head” domain consists of an ATPase catalytic site and MT-binding sites, which possesses catalytic ATPase and MT-binding abilities[3]. The kinesin family is classified as N-type, the middle and C- type kinesins, respectively with the motor head domain at or near to the N-terminus, in the middle, and close to the C-terminus of the molecule. The “motor head” domain is followed by the stalk region and finally the “small globular tail” at the opposite end of the kinesin molecule. The “motor head” domain is responsible for protein movement powered by the hydrolysis of ATP [4–6]. And the “stalk/tail”

domain is important for the interaction with subunits of the holoenzyme or with the cargo molecules [4, 7, 8]. The short “neck” region between the “head” and “stalk/tail” is essential for functions such as the direction of motility or regulation of activity [9]. The motor domain is well conserved in each kinesin subfamily, whereas the stalk/tail region outside the motor domain is highly divergent even in the same subfamily, which reflects the diverse biological functions even within the same subfamily.

Based on phylogenetic analysis using the conserved motor domain sequences, the kinesins are divided into fourteen families, designated as kinesin-1 to kinesin-14. Kinesins that do not belong to any of these subfamilies are considered orphans, but most kinesins identified can easily be assigned to a specific family [10]. Most members of kinesin families have an N-terminal motor domain named as N-type kinesins whereas few families have an internal motor domain or a C-terminal motor domain. The directionality of kinesins varies between families, and is sometimes correlated with the position of the motor domain. In general, kinesins with the N-terminal motor domain travel to the plus ends of MTs whereas the C-terminal motors move toward the minus ends of MTs ([11–14].

Expression profiles analysis revealed that plant kinesin genes were demonstrated to play important roles in fruit development. In apple (*Malus domestica* Borkh.) cultivar Fuji, the kinesin gene *KIN2* was strongly expressed in early stage of fruit development [15]. Further investigation showed that *KIN2* gene was also expressed primarily in two other apple genotypes “Gala” and “Golden Delicious” [16]. This demonstrated that the kinesin gene *KIN2* carries out regulatory role in early fruit development in apple. In cucumber, the kinesin genes *CsKF1-7* were highly expressed and involved in rapid cell division or expansion phase during early fruit development [17]. Furthermore, the *CsKF1* and *CsKF3* were dramatically active in the fruit elongation stages, implicating their essential roles in the fruit length regulation in cucumber [18]. Intriguingly, in tomato (*Solanum lycopersicum*), the kinesin gene *SpPAKRP* was predominantly expressed in the placenta tissue in the 4-DPA fruit, indicating that *SpPAKRP* is likely involved in controlling early fruit development by regulating placenta development ([19, 20]. The watermelon fruit as well as tomato is classified as a berry fruit and the edible parts of the fruit develop from placenta [21]. In addition, the size, yield and quality of the cucurbits fruit depend on the regulation of the placenta during early fruit development [22–24]. So, identification and the functional analysis of kinesin is the ideal entry point for exploring molecular mechanism of kinesin genes in regulating watermelon early fruit development. Moreover, other kinesin genes have been verified to possess different biological functions, including root, stem and leaf various vegetative tissues development genes (e.g., *DBS1*, *BC12/GDD1*, *AtKINESIN-4A/FRA1*) [25–28]; plus anther, male gametophyte, embryo, endosperm and seed development genes (e.g., *SRS3* and *NtKRP*) [29–31]. These works revealed the critical roles of plant kinesins in many essential processes in plant development, including not only plant vegetative growth but also plant reproductive process. However, very few kinesins have been functionally identified during early fruit development. Therefore, it is well worthy to extensively investigate their roles in economic crops, like watermelon (*Citrullus lanatus*). Up to now, little is known about kinesin family genes in watermelon.

Watermelon (*Citrullus lanatus*) is the fifth consumed fresh fruit in the world. It is considered as “the king of summer fruits” and a highly nutritional valued fruit. The early fruit development directly determines the

size, shape, yield and quality of the watermelon fruit. Considering the fact that the kinesin gene can control early fruit development via regulating placenta development in tomato ([19, 20], therefore, genome-wide study of the kinesin genes in watermelon is the ideal pointcut for exploring their real roles in the critical developmental processes, especially in watermelon early fruit development. Thus, in this study, a total of 48 Kinesin genes were identified in the *Citrullus lanatus* genome. The phylogenetic relationships, gene structure, chromosomal locations, and conserved motifs of the encoded proteins were analyzed. The tissue-specific expression patterns of all *CIKINs* genes in watermelon were further studied, and *CIKINs* expression under hormone-treated condition was also investigated. Particularly, five *CIKINs* genes showed specific and abundant expression in early fruit development stage. Our work provides useful information regarding the molecular mechanism of kinesin genes regulating early fruit development and a new insight into the yield and quality control mechanism in watermelon.

Results

Genome-wide identification of kinesin genes in *Citrullus lanatus*

A total of 63 candidate genes were identified from the watermelon genome (Cucurbit Genomics Database, <http://www.icugi.org/>). Based on amino acids sequence analysis, 15 candidate genes didn't contain conserved kinesin motor domain and then excluded from further analysis. In addition, these remaining 48 Kinesin genes can also be identified by hidden Markov models (HMMs) analysis search of function conserved Pfam domains, which was consistent with the above sequence similarity blasting. In conclusion, a total of 48 kinesin genes with complete and functional structures are presented in the watermelon genome, designated as *CIKINs* hereafter.

To explore basic properties of each kinesin, the lengths of genome DNA and protein sequences, the numbers of the introns and exons, the isoelectric point and the theoretical molecular weight were predicated, respectively (Table 1). The kinesin genes in *Citrullus lanatus* genome had coding sequence lengths of 927-8670 base nucleotides, encoding proteins length ranged from 308-2889 amino acids with predicted molecular weight in the range of 25.0-330.3 KDa. The theoretical isoelectric point calculation indicated that the kinesin protein isoelectric points (pI) were distributed in the range of 5.10-9.65 (Table 1).

To characterize the distribution of kinesin genes in the watermelon genome, the physical locations of kinesin genes on the watermelon chromosomes were further determined. 48 kinesin genes were mapped to the 11 chromosomes (Fig. 1), exhibiting an uneven distribution in the watermelon chromosomes. Chr10 contains the maximum 8 kinesin genes, while only two genes locate on Chr7. The other chromosomes, including Chr1, Chr2, Chr3, Chr4, Chr5, Chr6, Chr8, Chr9, Chr11, contain 3-6 kinesin genes, respectively.

Phylogenetic analysis of kinesin family

To estimate the phylogenetic relationships of watermelon kinesins to other known kinesins in different plants, multiple sequence alignment of watermelon kinesins motor domain sequences to the sequences from one dicotyledonous plant, *A. thaliana*, and one monocotyledonous crop, *O. sativa*, was conducted using the software MUSCLE and then the phylogenetic tree of these kinesins was generated with MEGA 6.06 using neighbor joining method (Fig.2). The phylogenetic analysis suggested that kinesin proteins from three different species can be categorized into 10 families: KIN1, KIN4, KIN5, KIN7, KIN8, KIN10, KIN12, KIN13, KIN14 (Fig.S1 and Fig.S2). Among them, the KIN14 family is the largest family consisting of 52 kinesins. The KIN7 family is the second largest family, which has 36 kinesin members. KIN11 is the smallest family with only 3 kinesins. Kinesin numbers from watermelon followed the same distribution tendency as the other two species. 13 watermelon kinesins belong to the KIN14 subfamily which is the largest family of all. 7 kinesins are grouped into KIN7, the second largest subfamily. Only 1 kinesin is in KIN11 family, representing the smallest subfamily. The phylogenetic relationship does not show any recognizable distinction between dicot and monocot species analyzed, indicating functional conservation of kinesin in plant kingdom.

Gene structure and conserved motif distribution analysis of watermelon kinesin family genes

The gene structure and intron/exon arrangements of the *CIKINs* genes were determined by the comparison of the cDNA sequence of each *CIKIN* with its genomic DNA sequence. The analysis results revealed that the intron number of all *CIKINs* genes ranged from 4 to 34. *CIKIN14F* only has 4 introns while there are 34 introns presented in *CIKIN12C* (Fig 3).

Further, to explore sequence features and functional motifs of each *CIKIN* protein, a multiple sequence alignment of watermelon kinesin protein sequences was performed and analyzed using MEME online software. Seven typical conserved motifs for kinesin family proteins, named as motifs 1-7, have been identified (Fig 4). Motif 1, highly conserved peptide sequence (FAYGQTGSGKT) inside the ATP-binding site and motif 6, a conserved microtubule-binding site (SSRSH), were found in all watermelon kinesins. Motif 4, another conserved microtubule-binding site 'VDLAGSE', could be detected in most *CIKINs* with the exception of *CIKIN7C*, *CIKIN10D*, *CIKIN14A* and *CIKIN14C* [32]. The motif 2, the microtubule-binding site 'HIPYR' existed in most *CIKINs* with the exclusion of *CIKIN1D*, *CIKIN7C*, *CIKIN10D*, *CIKIN12A*, *CIKIN14M* and *CIKIN14G*. Motif 3 is a conserved motif of K/RxIxNxxxVIN at the beginning of the neck region. Motif 5 is the highly conserved neck motif consisting of a hydrophobic repeat pattern of ϕ -xx(x)- ϕ -xxx- ϕ -xx- ϕ -G. Motif 3 and Motif 5 were found in the majority of *CIKINs* [33]. The results demonstrated that *CIKINs* proteins contained the typical conserved feature motifs of kinesin family.

Duplication and syntenic analysis of kinesin gene families

Tandem and segmental duplications play important roles in the expansion and function of a gene family [34, 35]. To further explore the possible evolutionary relationships of kinesin gene families, duplication events, segmental and tandem duplication gene pairs of the kinesin family were investigated in *C. lanatus* and *A. thaliana*. The results showed there are no tandem genome duplication events occurred for kinesin genes. However, 15 pairs of segmental duplication events were identified, where each pair of

genes were situated at separate chromosome in watermelon genome, such as *CIKIN1B/ CIKIN1D*, *CIKIN7F/CIKIN7J*, *CIKIN14L/ CIKIN14A*, *CIKIN13B/ CIKIN13C* (Fig.5). Overall, the synteny analyses suggested that the kinesin family in watermelon expanded only through segmental duplication.

Expression profiles of kinesin genes in different tissues in watermelon

The expression pattern is important for assessing the potential roles of *CIKINs* genes in the processes of plant growth and development. Therefore, we examined the expression patterns of 48 *CIKINs* genes in five different tissues including the root, stem, leaf, pistil and stamen by real-time reverse transcription PCR (RT-qpcr) (Fig.6). The RT-qpcr results indicated that the kinesin genes in watermelon exhibited a narrow expression pattern and could only be detected in one or two tissue tested. Eight watermelon kinesin genes (*Cla013748*, *Cla001716*, *Cla003337*, *Cla011614*, *Cla000622*, *Cla008965*, *Cla016598* and *Cla010272*) were highly expressed in the root, stem and leaf vegetative organs, which indicates their potential roles in vegetative organs development. In addition, 20 kinesin genes were preferentially expressed in the pistil, which implies the possible participation of these genes in the pistil development. 9 kinesin genes were specifically expressed in the stamens, indicating their potential involvement in stamen development. Overall, 38 kinesin genes were abundantly expressed in the reproductive organs, suggesting that they play critical roles in the growth and development of reproductive tissues.

Key kinesin genes identification involved in early fruit development

Fruit development of watermelon, as a cucurbit species, follows the canonical developmental progression of four stages: ovary development; fruit set; expansive fruit growth; and maturation and ripening [21, 22, 36]. Among the four stages, the first three stages of development (ovary development, fruit set, expansive fruit growth) are defined as the early fruit development stage. The early fruit development stage completes in about 10 days after pollination (DAP) and directly determines the size, shape and quality of fruit [37]. In addition, previous transcriptome and RT-qPCR analysis results demonstrated that kinesin family genes participated in the regulation of early fruit development in *Malus domestica* and *Cucumis sativus* [15, 17]. Therefore, to identify potential roles of kinesin family genes in the process of watermelon early fruit development, RT-qPCR was performed using cDNA prepared from the fruits at -1, 0, 1, 2, 3, 5, 7, 9, 10, 12, 34 days after pollination (DAP). Hierarchical clustering and heatmap analysis were executed and gave a visual analysis of kinesin gene expression. From the overview of the kinesin expression profiles, the transcripts of all *CIKINs* genes could be detected in the fruit at different development stages, with different transcription levels at specific stage of fruit development (Fig. 7). Among them, twenty-one *CIKINs* showed high transcription levels in the fruits at 34 days DAP, which is at maturation and ripening development phase. A total of twenty-seven *CIKINs* exhibited different expression levels in the fruits at -1, 0, 1, 2, 3, 5, 7, 9, 10, 12 days DAP, which is at the early fruit development stage. Further detailed analysis revealed that fourteen kinesins (*Cla022645*, *Cla013925*, *Cla010272*, *Cla009301*, *Cla019890*, *Cla007599*, *Cla014608*, *Cla014076*, *Cla000622*, *Cla018908*, *Cla014106*, *Cla015441*, *Cla022444* and *Cla008965*) showed specifically or abundantly expressed in the early developing fruits. Moreover, the major edible sections of watermelon fruit develop

and differentiate from the pistil tissue. In consequence, comparative analysis of expression levels between the early fruit and the pistil tissue demonstrated that five kinesin *CIKINs* genes (*Cla022645*, *Cla013925*, *Cla019890*, *Cla007599* and *Cla018908*) showed relatively specifically or highest expression level simultaneously both in the early developmental fruit and pistil. All these data indicated that the different kinesin members displayed diverse expression patterns and may have stage-specific roles in the process of watermelon fruit development. Especially, above five kinesin genes may function in the process of watermelon early fruit development.

Potential roles of kinesin family genes in response to hormone treatments

Plant hormones are a group of small signal molecules which have been approved to play essential roles in different processes of plant growth and development. The expression levels of a larger number of genes are known to regulated by different plant hormones. Previous studies have discovered the contribution of hormones such as ethylene (ETH) in sex determination and development of sex-specific floral organs in the Cucurbitaceae [38, 39]. In addition, study has indicated that kinesin-4 gene *OsGDD11* is involved in the signaling pathways of plant hormone [27]. More importantly, in cucurbits crops, pollination was believed to be the key process to release hormonal enzymes, most specifically auxin, which in turns to stimulate fruit enlargement [23]. To understand the possible relationship of kinesin genes in watermelon and major hormones, the relative transcriptional levels of each kinesin gene after ABA and ETH hormones treatments were investigated and compared with untreated controls. The relative expression levels of each *CIKIN* gene under two kinds of hormones treatments were investigated by RT-qPCR and the heat map were created based on the relative expression levels (Fig 8). The results revealed that at least 1/3 of kinesin genes were responsive to ABA or ETH treatments. Moreover, 23 *CIKINs* genes were regulated by both ABA and ETH, but showing very different expression pattern under different hormone treatment. Following ABA treatment, the expression levels of two *CIKINs* (*CIKIN11A* and *CIKIN7G*) were sharply down-regulated (<2 fold). Whereas the expression levels of 21 *CIKINs* increased significantly (>2 fold) after ABA treatment. However, unlike ABA treatment, most of kinesin genes (32 *CIKINs*) exhibited significant up-regulation in response to ETH stimuli. In general, these detailed expression level analyses implied that *CIKINs* genes could participate in the regulation of the plant hormones pathway.

Discussion

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] comprises the major cucurbits and is the fifth consumed fruit in the world, known as “the king of summer fruits”. It is also one of the most important economic crops grown worldwide. Fruit development traits are the very important agronomic traits in watermelon breeding and production. The early fruit development in watermelon directly affects the subsequent agronomic traits, including the fruit size, shape and quality. Kinesins are important microtubule-based motor proteins with conserved motor domains among all eukaryotic organisms. They play critical roles in the unidirectional transport of vesicles and organelles, cytokinesis, signal transduction, morphogenesis, cell division and cell growth in the plant development [40–43]. Furthermore,

previous researches have revealed that the kinesin family genes also participated in plant reproductive development [29–31, 44]. Especially, kinesin family genes have been proved to be essential for the regulation of early fruit development in *Malus domestica* and *Cucumis sativus* [15, 17]. Therefore, these works declared the urgency of extensively investigation of kinesin family genes in plants, especially in economic crops, with the expectation to the improvement of crop yield. However, the identification and analysis of detailed expression characteristics and functions of kinesin family genes in watermelon, especially in the fruit reproductive tissues, still remain elusive. With the completion of the *C. lanatus* genome sequence, the *CIKINs* genes can be systematically identified and analyzed [45]. In the present study, we have identified 48 kinesin family genes *CIKINs* in the watermelon genome and comprehensively analyzed these genes for their phylogenetic relationships, chromosomal locations, gene structures, conserved motif distributions, and duplication and syntenic analysis. In addition, we performed the extensive analysis of *CIKINs* expression patterns in different tissues, at different early fruit developmental stages and in response to hormones treatments. Especially, expression patterns analysis during fruit developmental process elaborated the overall characteristics and the specific dynamics of watermelon kinesin family genes in watermelon development. Conclusively, our work provided reliable and useful information of distribution and functions of kinesin genes in watermelon development. In addition, this work also provides clear clues to further investigation of their detailed roles in watermelon reproductive development and response to hormones influence.

Characteristics of kinesin family genes in watermelon

As described above in results section, some typical conserved motifs for kinesin family proteins exists in most watermelon kinesins. Kinesin motor domain is comprised of a Walk A ATP binding motif “FAYGQTGSGKT” and a microtubule binding domain [46–48]. Microtubule binding domain commonly contains three microtubule binding motifs (SSRSH, xdlagse and HxPYR)([49]. Highly conserved peptide sequence “FAYGQTGSGKT” in the ATP-binding motif could be found in all of watermelon kinesins through the alignments of their amino acid sequences, which is responsible for hydrolyze ATP to produce a direct force to travel unidirectionally along microtubule protofilaments and power multiple critical cellular process. The typical microtubule binding site “SSRSH” could be detected in all of kinesins, but the other two microtubule binding motifs “xdlagse and HxPYR” could be found in most of kinesins with few exceptions. In particular, only one microtubule binding motif “SSRSH” could be detected in two kinesins *CIKIN7C* and *CIKIN10D*, but the other two of three microtubule binding motifs couldn't be found. The results suggested that the microtubule binding site “SSRSH” is the most conserved microtubule binding site. However, whether *CIKIN7C* and *CIKIN10D* proteins possess the microtubule binding abilities with only one microtubule binding site needs further verification.

Phylogenetic analysis based on kinesin protein sequences categorized the kinesin genes from watermelon, *Arabidopsis* and rice into ten families. Interestingly, the number distribution tendency of all these genes from three species was almost the same in the ten groups and did not show distinct monocot or dicot distribution characteristics. The analysis of the consistent tendency showed that the kinesin-14 and kinesin-7 respectively were the first and the second largest group, including at least

kinesin-14 or kinesin-7 proteins in the three plants. The kinesin-14 family was one well-conserved family and played important roles in chromosome segregation at mitosis and organelle transport [50]. The number of kinesin-14 family members is the maximum both in animals and plants [50, 51]. The kinesin-11 subfamily contained the minimum amount of kinesin protein, which had only one kinesin-11 protein in each of the three species. Kinesin-11 family members function in signal transduction or divergent catalytic core and are rarely found. The results of phylogenetic analysis implied that kinesin family genes were spatially and functionally conserved in some essential developmental processes in different plant taxa.

Essential roles of kinesin family genes in early fruit development

Previous microarray and expression profiling analysis have revealed that some kinesin genes were necessary for early fruit development in apple and cucumber [52, 53], [18, 54]. However, the exact roles of kinesin genes in the process of early fruit development are still unknown in most economic species, including watermelon. Therefore, the expression profiles of kinesin family genes in early fruit development were comprehensively analyzed by RT-qPCR in this study. RT-qPCR results demonstrated that the transcripts of most kinesin genes could be detected in early developmental fruits at different development stages. A striking feature was that the expression levels of most kinesin genes were higher in fruits at early development stages and then decrease sharply at fruit maturation and ripening. Studies of early fruit development in cucurbits showed that the early fruit growth is primarily due to cell number increments, or in the other words, primarily driven by cell division [55]. Moreover, the period of rapid cell division was accompanied by increased peak expression of microtubule related kinesin genes [18]. Microtubules facilitate alignment of chromosomes at the spindle equator in mitosis [56]. Therefore, the high expression level of kinesin genes in the early fruit development stages may regulate chromosome organization during mitosis via regulation of cytoskeleton and microtubule dynamics and finally caused the change of the cell amounts or sizes [56].

Which developmental process does the watermelon kinesin genes regulate to ultimately control the early fruit development? This question is intriguing and needs to be characterized in future. The watermelon fruit is classified as a berry fruit as well as tomato fruit because the thick pericarp encloses many seeds. The edible parts of two kinds of fruits are either mainly composed of placentas or develop and differentiate from the placenta tissues [21]. The comprehensive tissue-specific transcriptome analysis revealed that the kinesin gene *SpPAKRP1* showed peak expression in the placenta tissue during the early stage of fruit development in *Solanum pimpinellifolium*, a wild cultivated tomato [19, 20]. This suggested that the tomato kinesin gene *SpPAKRP1* could be involved in the early fruit development by regulating the placenta tissue development. This implies that the watermelon kinesin genes *CIKINs*, specifically or abundantly expressed in the early fruit development stage, could also control the early fruit development via regulating the placenta tissue development. This provided an ideal entry point to study the molecular mechanism of early fruit development through analyzing the role in the placenta development. Nevertheless, the exact roles of these kinesin genes need to be further studied and confirmed.

Potential roles of kinesin family genes in response to hormones treatments

Early studies demonstrated that plant hormones have been implicated to facilitate early fruit development in cucurbits, although there is debate as to which hormones are most critical [23, 57]. Mitotic kinesins play important roles in chromosome organization during mitosis in developing cucurbits cucumber fruits [17, 56]. These studies implied that there is a connection between plant hormones and kinesins. The connection has been first verified in rice, in which kinesin protein gene *BC12/GDD1* mediated cell elongation by regulating the hormone GA biosynthesis pathway [27]. In order to further explore the relationships between plant hormones and kinesins in watermelon, in our present work, the relative expression levels of kinesin genes after hormones treatments were investigated. The results showed that the transcription levels of most kinesin genes changed after hormones treatments, indicating their critical roles in response to different hormones. Although some genes could respond to the same hormones, some other members of kinesin family genes showed their roles differentially in response to certain hormones. Interestingly, *CIKIN7E* and *CIKIN7G* were down-regulated after ABA treatment, which is quite different from other kinesin genes, implying their unique roles in response to ABA hormone treatment. Taken together, the data provided useful clues for the further investigations of molecular mechanism of kinesins in response to plant hormones during the plant development process.

Conclusions

In conclusion, a total of 48 *CIKINs* genes were identified in *C. lanatus* at the whole-genome level. These genes were divided into 10 subfamilies. The chromosomal locations, exon/intron structures, conserved motif distributions, and syntenic analysis of kinesin family members in *C. lanatus* were determined. Comprehensive analysis and expression profiling of *CIKINs* genes were performed to determine the potential functions in early fruit development and in response to hormones stimuli. Furthermore, detailed expression analysis revealed the tissue-specific and highly expression pattern of *CIKINs* genes. Finally, 5 *CIKINs* genes, including *CIKIN7D*, *CIKIN7K*, *CIKIN10B*, *CIKIN12D* and *CIKIN14M*, demonstrated relatively specifically and highest expression level simultaneously in the early fruit developmental, indicating their important roles in the early fruit developmental. In general, these results will provide a useful resource for further selecting an artificial regulator for fruit development in *C. lanatus*.

Methods

Identification of kinesin gene family in *Citrullus lanatus*

All BLAST searches were conducted in the watermelon genome database (Cucurbit Genomics Database, <http://www.icugi.org/>) by using three motor domain sequences from the KHC (N-terminus motor in human), KIF2 (internal motor in mouse), and KCBP (C-terminus motor in *Arabidopsis*) as queries. 63 candidate genes generated by using an E-value cut-off of 1, which contained kinesins and some unrelated proteins. In addition, Hidden Markov Model (HMM) profiles of the motor domain (PF00225)

was downloaded from the Pfam database (<http://pfam.xfam.org/>). Then HMMER 3.0 software was used to search for kinesins. Motor domain analysis in SMART (<http://smart.embl-heidelberg.de/>) and INTERPROSCAN (<http://www.ebi.ac.uk/interpro/>) were performed and the proteins without conserved motor domain were deleted. Finally, the candidate kinesin genes in watermelon were further analyzed with the online tools ExPASy (<http://www.expasy.org/tools/>) to predict the isoelectric point (PI) and molecular weight (MW).

Chromosome localization analysis of watermelon kinesin genes

The chromosome locations information of all *CKINs* genes were downloaded from watermelon genomics database. The information, including localizations and length of the chromosomes, were visualized by MapChart online software.

Phylogenetic analysis of kinesin genes

Kinesin amino acid sequences from *C. lanatus* with *A. thaliana* and *O. sativa* were aligned using the software Muscle with the default multiple alignment parameters. The phylogenetic trees were constructed via MEGA 6.06 using the neighbor joining method. The bootstrap replicates test value was set as 1000.

Gene structures and conserved motifs analysis of kinesin proteins

The *CKINs* gene structures were visualized by using the program GSDS2.0 (Gene Structure Display Server, <http://gsds.cbi.pku.edu.cn/>). The conserved motifs in *C. lanatus* kinesin proteins were identified by using the program MEME (Multiple Em for Motif Elicitation, <http://meme-suite.org/tools/meme>). The maximum number of motifs was set to 7 and the others were default.

Syntenic analysis of watermelon *CKINs* genes

The homolog pairs between *C. lanatus* and *A. thaliana* were identified using the BLASTp program. GFF files serves as input documents for MCScanX to analyze the synteny relationship [58]. The analysis result was visualized using the software CIRCOS (<http://circos.ca/>).

Plant materials and hormones treatments

Under normal conditions, various tissues including root, stem, leaf, pistil, stamen and fruits at different days after pollination of *C. lanatus* were collected for RNA extraction. The watermelon plants were grown under natural light with temperatures of 28-35°C/16-20°C (day/night) in a greenhouse in spring. For hormones treatments, four-week-old seedlings after sowing were used phytohormones treatments. The leaves of the seedlings were sprayed with 100µM Absciscic acid (ABA) [59] and 10mM Ethephon (ETH) [60] and collected after 12h treatments. The control seedlings were sprayed with the same solutions except for corresponding hormones. The taken samples with three biological replicates were immediately frozen in liquid nitrogen and stored at -80°C before RNA extraction.

RNA extraction and qRT-PCR

The total RNA of viroous tissues were extracted using the Quick RNA isolation kit (Huayueyang Biotechnologies Co. Ltd, Beijing, China) according to the manufacturer's instructions. The first-strand cDNA was synthesized with 1µg total RNA using SuperScript III transcriptase (Invitrogen).

Quantitative reverse transcription PCR (qRT-PCR) was conducted on an ABI StepOnePlus machine using SYBR Premix Ex TaqTM (TaKaRa). Three independent biological repeats were performed for each *CIKIN* gene. Specific primers for all *CIKINs* genes were designed using Primer3Plus online software (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>) and listed in Supplementary Table S1. The relative expression levels of *CIKINs* genes were normalized against that of the watermelon *ACTIN* gene (gene ID: *Cla007792*) transcript.

Abbreviations

Cl: *Citrullus lanatus*; KIN:kinesin; MT:microtubule; qRT-PCR:Quantitative reverse transcription PCR; PAKRP:phragmoplast-associated kinesin-related protein.

Declarations

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Availability of data and materials

All data generated or analyzed during this study were included in this published article and the additional files.

Authors contributions

TS and YL were responsible for the data analysis and experimental design. TS and YL wrote the manuscript. TS, JJ, XG, WT, LQ, CX, LM, LZ, LM and YL were responsible for the programs and the manuscript revision. All authors have commented, read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Kinesin gene family members identified in *Citrullus lanatus*

Gene name	Gene ID	Length (Kb)	Amino Acids (aa)	No. of introns	No. of exons	Isoelectric point (pI)	Molecular Weight (KDa)
<i>CIKIN1A</i>	<i>Cla000622</i>	5.01	442	13	14	5.1	49.1
<i>CIKIN1B</i>	<i>Cla011525</i>	3.86	377	6	7	7.07	40.8
<i>CIKIN1C</i>	<i>Cla013748</i>	12.11	1002	19	20	7.18	112.4
<i>CIKIN1D</i>	<i>Cla021298</i>	10.28	870	17	18	6.31	96.6
<i>CIKIN4A</i>	<i>Cla023054</i>	10.7	1221	24	25	7.49	137
<i>CIKIN5A</i>	<i>Cla008272</i>	6.43	1051	21	22	5.65	118
<i>CIKIN5B</i>	<i>Cla017676</i>	6.86	1001	22	23	5.53	113
<i>CIKIN5C</i>	<i>Cla018236</i>	7.46	1051	22	23	5.8	118.4
<i>CIKIN7A</i>	<i>Cla001883</i>	11.89	941	12	13	8.55	106.4
<i>CIKIN7B</i>	<i>Cla002884</i>	21.5	1205	29	30	5.38	137.7
<i>CIKIN7C</i>	<i>Cla011614</i>	18.23	367	8	9	9.47	41.2
<i>CIKIN7D</i>	<i>Cla013925</i>	13.09	1146	23	24	6.72	128
<i>CIKIN7E</i>	<i>Cla014608</i>	10.29	696	11	12	5.38	78
<i>CIKIN7F</i>	<i>Cla015885</i>	7.66	962	12	13	*	*
<i>CIKIN7G</i>	<i>Cla015893</i>	10.6	1078	23	24	5.45	119.7
<i>CIKIN7H</i>	<i>Cla016598</i>	10.71	1083	23	24	6.14	120.1
<i>CIKIN7I</i>	<i>Cla018481</i>	5.12	960	13	14	6.92	109.3
<i>CIKIN7J</i>	<i>Cla022444</i>	7.38	946	11	12	6.04	107.1
<i>CIKIN7K</i>	<i>Cla022645</i>	6.29	830	16	17	5.83	94.2
<i>CIKIN8A</i>	<i>Cla014076</i>	7.74	716	14	15	7.2	80.3
<i>CIKIN8B</i>	<i>Cla019001</i>	4.75	757	5	6	9.65	84.4
<i>CIKIN10A</i>	<i>Cla003633</i>	6.83	700	15	16	7.95	76.5
<i>CIKIN10B</i>	<i>Cla007599</i>	4.66	837	5	6	5.8	93.1
<i>CIKIN10C</i>	<i>Cla008965</i>	12.67	892	16	17	6.01	102.1
<i>CIKIN10D</i>	<i>Cla011526</i>	5.18	419	9	10	6.41	46.4
<i>CIKIN10E</i>	<i>Cla017895</i>	4.61	616	11	12	8.61	67.6
<i>CIKIN11A</i>	<i>Cla022414</i>	10.13	835	18	19	5.94	93.6

<i>CIKIN12A</i>	<i>Cla004496</i>	6.94	1142	14	15	5.59	128.8
<i>CIKIN12B</i>	<i>Cla009212</i>	15.33	2202	29	30	5.21	250.1
<i>CIKIN12C</i>	<i>Cla010272</i>	16.07	2889	34	35	5.13	330.3
<i>CIKIN12D</i>	<i>Cla018908</i>	9.93	1439	21	22	5.12	162.7
<i>CIKIN12E</i>	<i>Cla021862</i>	9.26	1305	15	16	5.28	145.4
<i>CIKIN13A</i>	<i>Cla009301</i>	7.05	811	11	12	6.04	89.6
<i>CIKIN13B</i>	<i>Cla010631</i>	5.63	724	11	12	6.35	81.8
<i>CIKIN13C</i>	<i>Cla015441</i>	6.11	679	11	12	6.05	76.1
<i>CIKIN14A</i>	<i>Cla001716</i>	5	752	16	17	5.59	84.9
<i>CIKIN14B</i>	<i>Cla003337</i>	9.72	1148	16	17	8.67	127.4
<i>CIKIN14C</i>	<i>Cla005012</i>	17.45	858	14	15	9.11	95.5
<i>CIKIN14D</i>	<i>Cla005176</i>	7.51	1064	18	19	5.49	117.7
<i>CIKIN14E</i>	<i>Cla005413</i>	6.26	1117	16	17	6.42	124.9
<i>CIKIN14F</i>	<i>Cla008964</i>	3.68	308	4	5	8.77	35
<i>CIKIN14G</i>	<i>Cla011288</i>	4.16	632	9	10	9.33	71.3
<i>CIKIN14H</i>	<i>Cla011444</i>	10.84	1068	19	20	6.29	119
<i>CIKIN14I</i>	<i>Cla014106</i>	19.12	1276	22	23	5.99	141.2
<i>CIKIN14J</i>	<i>Cla017386</i>	6.97	1017	18	19	8.43	112.1
<i>CIKIN14K</i>	<i>Cla017457</i>	7.52	1336	19	20	5.85	150.4
<i>CIKIN14L</i>	<i>Cla019422</i>	5.99	773	15	16	7.51	87.1
<i>CIKIN14M</i>	<i>Cla019890</i>	5.03	810	10	11	9.49	89.8

* show that there are continuous “Ns” in the CIKINs protein sequences, making impossible to calculate their PI and MW.

Figures

Figure 1

Distribution of *Citrullus lanatus* kinesin genes on 11 chromosomes. Chromosome numbers were marked as Chr1- Chr11 at the top of each chromosome. The sizes of chromosome were labeled on the left of the figure. Forty-eight kinesin genes of watermelon were mapped to different chromosomes using Map Chart.

Figure 2

Phylogenetic relations of the kinesins from *C. lanatus*, *A. thaliana* and *O. sativa*. The tree was calculated with MEGA6.06 software using neighbor-joining method.

Figure 3

Genomic structures of kinesin genes in *C. lanatus*.

Figure 4

Distributions of conserved motifs in watermelon kinesin proteins. The phylogenetic tree of CIKINs is on the left panel. The motifs of corresponding proteins are shown on the right panel using different colors on behalf of specific motifs with the Multiple Em for Motif Elicitation (MEME).

Figure 5

Syntenic analysis between watermelon and Arabidopsis kinesin genes. Chromosomes of watermelon and Arabidopsis are shown in different colors (red and yellow) and in partial circles. The approximate distribution of each kinesin gene is presented by short black line on the circle. Colored curves indicate the syntenic relations between watermelon and Arabidopsis kinesin genes. The prefixes 'WM' and 'AT' respectively indicate watermelon *Citrullus lanatus* and Arabidopsis *thaliana*.

Figure 6

Expression profiles analysis of kinesin family in different tissues in watermelon. The data were showed as means value+ SD. All experiments were performed with three independent replicates. Log2-transformed data were used for the cluster analysis (n=3). The inset shows the colour legend used in the cluster representation (Log2 ratios). The red dots indicate the higher expression level, whereas the blue

dots indicate the lower expression level. CI α CTIN gene was used for normalization of quantitative RT-PCR results.

Figure 7

Expression profiles analysis of CIKINs genes during the early fruit development in watermelon. (A) The morphological characteristics of the watermelon fruits at different days after pollination. -1,0,1,4,5,8,10 respectively displays the days after pollination. (B) Expression patterns of CIKINs genes during the fruit

development. Log2-transformed data were used for the cluster analysis (n=3). The inset shows the colour legend used in the cluster representation (Log2 ratios). A red box indicates the higher expression level, whereas the purple box indicates the lower expression level. C_IACTIN gene was used for normalization of quantitative RT-PCR results. (C) The dynamic changes of expression levels analysis from the 5 C_IKINs genes specifically or abundantly expressed in the early fruit development. The standard deviations of three biological replicates are represented by the error bars.

Figure 8

Expression levels analysis of kinesin family in watermelon under ABA and ETH hormones treatments. (A) Expression profiles of C_IKINs genes under ABA stress treatment visualized as a heat map. (B) Expression levels of C_IKINs genes under ETH stress treatment. The relative transcript levels were Log2 transformed and visualized as a heatmap via Mev6.0, using red to indicate increased expression level and green to indicate decreased expression level (displayed at the bottom).