The constitutive expression of the chrysanthemum gene CmERF110 in *Arabidopsis thaliana* affects lateral branching

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Abstract

Background: Producers of cut flower chrysanthemum are obliged to manually remove lateral buds, a procedure which consumes one third of the total production cost. The formation of lateral buds in 'SEI NO ISSEI' is suppressed when the plants are exposed to high temperatures, but the molecular basis of this phenomenon is not well understood. Here, the transcriptome of buds formed by decapitated chrysanthemum plants grown under a high temperature regime was characterized with a view to revealing which genes known to be involved in a pathway determining shoot branching were induced/repressed by the treatment. **Results:** The transcriptomic data was acquired using RNA-Seq technology, based on the Illumina HiSeq™ 2000 platform. Four libraries were generated from pooled lateral buds of decapitated 'SEI NO ISSEI'. To predict the potential functions of unigenes in the 'SEI NO ISSEI' buds, after assembly, we performed seven functional database annotations. 132,396 unigenes were assembled, of which 79,116 unigenes were annotated in the seven functional databases. The percentage of unigenes annotated in the NR, NT, Swiss-Prot, KEGG, COG, Interpro, and GO databases were 54.59%, 42.96%, 39.05%, 41.84%, 20.72%, 37.25%, and 13.01%, respectively. Multiple differentially expressed transcription factors and auxin-related genes were identified in buds of decapitated 'SEI NO ISSEI' under 28°C/23°C or 38°C/33°C. Constitutively expressing CmERF110 in wild type *Arabidopsis thaliana* produced a bushy plant. A quantitative analysis of gene expression changes in the CmERF110 transgenic *A. thaliana* plants showed that the presence of the transgene altered the abundance of transcript produced by TIR1, ARF2, ARF16, IAA3 and IAA9 (encoding auxin signaling proteins) and PIN1, AUX1, LAX1, LAX2 and ABCB1 (auxin transport). **Conclusions:** This study reported a highly complete transcriptome from the buds of decapitated 'SEI NO ISSEI'. Differential abundant transcripts during high temperature treatments were identified and validated by qPCR, many of these differentially abundant transcripts as key players in bud outgrowth. These include known members of the AP2/ERF, MYB, WRKY, bHLH families and auxin-related genes. CmERF110 regulated shoot branching when its encoding gene was heterologously expressed in *A. Thaliana*. The regulation acted through the auxin-related genes.

Background

The ability of the plant shoot to form branches has a major effect on the plant's architecture, a trait of major importance in the context of crop domestication and improvement [1, 2]. Axillary meristems are composed of a group of cells which have retained their meristematic potential [3]. Following their initiation, these structures develop into axillary buds, which either remain dormant or develop into a new branches, depending a number of both internal and/or external cues [4]. Three forms of bud dormancy have been recognized: paradormancy occurs when growth ceases because of physiological factors external to the bud; endodormancy when growth is regulated by internal physiological factors; and ecodormancy when external environmental factors are most important [5]. The major environmental cues regulating the induction and release of bud dormancy are temperature and light, although the extent of the influence and the importance of crosstalk between temperature- and light-regulated signaling pathways appear to be somewhat species-dependent [6]. The relevant physiological factors include

phytochrome, sugar and phytohormones, which are basically associated with direct phenotypic changes when plants perceive environmental signals [7]. The phytohormones associated with bud growth and development include abscisic acid, ethylene, gibberellic acid, cytokinin, strigolactone and auxin.

The involvement of auxin in the regulation of axillary branching has been known for many decades [8]. The auxin indole-3-acetic acid (IAA) affects, among many other developmental phenomena, both shoot apical dominance and lateral root development [9-11]. Its major site of production is in young expanding leaves [12], from where it is transported within the polar auxin transport stream [13, 14]. To date, three types of auxin transporter have been reported, namely auxin influx carriers such as AUXIN RESISTANT 1/LIKE AUX1s (AUX1/LAXs), PIN-FORMED (PIN) carriers and the B subfamily of ATP-binding cassette proteins (ABCBs) which act as auxin efflux carriers [15, 16]. Auxin signaling is controlled by members of three protein families: TIR1/AFB auxin receptors, Aux/IAA transcriptional repressors and B3 type auxin response factors (ARFs) [10, 17, 18]. Auxin stabilizes the interaction between TIR1/AFBs and Aux/IAAs [19], promoting the degradation of Aux/IAA [20][28-31]. Many *Aux/IAAs* can be rapidly induced by auxin in an SCF^{TIR1/AFB}-dependent manner [21][32].

Chrysanthemum is a valuable ornamental species [22]. Producers of cut flowers are required to manually remove lateral buds, a costly and energy-consuming procedure. In the cultivar 'SEI NO ISSEI', the formation of lateral buds has been shown here to be suppressed when the plants are exposed to a high temperature regime. In order to reveal the molecular basis of this suppression, the transcriptome of this cultivar was characterized, which resulted in the recognition that the gene encoding a specific ethylene response factor was up-regulated in response to a high temperature exposure. By expressing this gene heterologously in *Arabidopsis thaliana*, it was possible to determine that its product regulates shoot branching, acting through the auxin-related genes. The conclusion was that it may be possible, through the manipulation of this gene within chrysanthemum, to achieve a branchless chrysanthemum plant, which would be of substantial value to breeders of chrysanthemums directed at the market for cut flowers.

Results

The effect of high temperature on bud outgrowth in 'SEI NO ISSEI'

Decapitated 'SEI NO ISSEI' plants exposed to the 28°C/23°C treatment presented an acrotonic pattern of bud out-growth along their stems, whereas those exposed to 38°C/33°C produced very few buds (Fig. 1a). After a four day exposure to two temperature regimes (28°C/23°C or 38°C/33°C), there was no difference in the bud burst rate. However, when decapitated chrysanthemum was exposed to 28°C/23°C or 38°C/33°C on the seventh day, the bud burst rate was significantly different in the fifth and sixth nodes (the first node n-1 refers to the first bud of decapitated chrysanthemum from top to bottom). Buds formed by plants exposed for ten days to 28°C/23°C exhibited a significantly higher bud burst rate than those of plants exposed to 38°C/33°C (Fig. 1b). In the former plants, bud growth was acrotonic, as also shown by the pattern of leaf development, whereas bud growth under the higher temperature regime was inhibited

and no acrotonic gradient was observed. The length of a bud of different position was two to twelve fold greater in plants exposed for ten days to the lower temperature regime than in those exposed to the higher temperature regime (Fig. 1c).

Transcriptome sequencing and read assembly

In order to study the differentially expressed genes of buds of decapitated 'SEI NO ISSEI' between 38°C/33°C and 28°C/23°C, four cDNA libraries were generated using the RNA-Seq platform, i.e., h-24 h (38/33°C for 24 h), h-96 h (38/33°C for 96 h), n-24 h (28/23°C for 24 h) and n-96 h (28/23°C for 96 h). After filtering the raw reads, we obtained 44.13 Mb, 44.52 Mb, 44.63 Mb and 45.46 Mb clean reads from four cDNA libraries, containing 6.62 Gb, 6.68 Gb, 6.69 Gb and 6.82 Gb clean bases, respectively. The clean reads ratios, the Q20 percentages, the Q30 percentages of the four samples were more than 83%, 98% and 94%, respectively (Table 1). Using Tgicl [26] to cluster the transcripts, we obtained 132,396 unigenes with the total length of 140,318,943 bp, the mean length of 1,059 bp and the GC percentage of 39.48% (Table 2).

Unigene functional annotation

To predict the potential functions of unigenes in the 'SEI NO ISSEI' buds, after assembly, we performed seven functional database annotations (NR, NT, Swiss-Prot, KEGG, COG, Interpro and GO). 132,396 unigenes were assembled, of which 79,116 unigenes were annotated in the seven functional databases. The number of unigenes annotated in the NR, NT, Swiss-Prot, KEGG, COG, Interpro, and GO databases were 72,274, 56,878, 51,697, 55,399, 27,433, 49,316 and 17,244, respectively, meanwhile, the percentages of annotations were 54.59%, 42.96%, 39.05%, 41.84%, 20.72%, 37.25%, and 13.01%, respectively (Table 3). Among them, the NR database had the largest number of unigenes, so we counted the species distribution according to the NR annotation results. The most common species was *Vitis vinifera*, which accounted for 16.71% of species distribution, followed by *Solanum tuberosum* (8.54%), *Theobroma cacao* (6.95%) and *Erythranthe guttata* (6.00%). The proportion of other species was 61.81% (Figure S1).

To clarify the potential functions of unigenes in the buds of 'SEI NO ISSEI', we calculated its functional classification based on the COG annotation results. The COG annotation divided unigenes into 25 categories, the most of which was "general functional prediction only" (7601, 16.89%), followed by "replication, recombination and repair" (4309, 9.57%) and "transcription" (4187, 9.30%). However, the least two categories were "nuclear structure" and "extracellular structures", accounting for 0.019% and 0.024% of unigenes, respectively (Figure S2).

To further understand the potential functions, GO term enrichment analysis was performed. A total of 85,084 unigenes were divided into 53 functional groups, which mainly included biological process (23), cellular component (16) and molecular function (14). In biological process, the main functional groups were “metabolic process” (8904) and “cellular process” (8337). In cellular component, the main functional groups were “cell” (6393) and “cell part” (6393). Among the molecular function, “catalytic activity” (9984) and “binding” (8266) were most (Figure S3). The results suggested that these main categories might play an important role in buds.

In addition, we calculated its functional classification based on the results of the KEGG database. A total of 58,140 unigenes were clustered, including cellular processes, environmental information processing, genetic information processing, human diseases, metabolism and organismal systems. The three main pathways were the “global and overview maps” in metabolism (12,893, 22.18%), the “translation” in genetic information processing (5,562, 9.57%), and the “carbohydrate metabolism” (5,163, 8.88%) (Figure S4).

Identification of transcription factors (TFs) involved in buds under contrasting temperature regimes

Multiple differentially expressed TFs were identified in buds of decapitated ‘SEI NO ISSEI’ under 28°C/23°C or 38°C/33°C. Most transcription factors belonged to the *AP2/ERF*, *MYB*, *WRKY* and *bHLH* families. We analyzed 8 *AP2/ERFs*, 6 *MYBs*, 3 *WRKYs* and 1 *bHLH* (Fig. 2 and Table S2). In the *AP2/ERFs* family, the expression levels of CL5614.Contig4_All (*RAP2-7*), CL2842.Contig2_All (*ERF12*), Unigene38388_All (*ERF13*), Unigene38278_All (*ERF53*) and Unigene28455_All (*ERF110*) were significantly higher in h-96h than n-96h, however, the expression levels of Unigene34736_All (*WIN1*), CL5120.Contig2_All (*ERF017*) and Unigene69087_All (*RAP2-7*) were significantly inhibited at 96 h after high temperature treatment (Fig. 2 and Table S2). In the *MYBs* family, Unigene 5596_All (*MYB46*), Unigene9775_All (*MYB4*), CL10164.Contig2_All (*MYB6*), CL262.Contig9_All (*MYB*) and CL1495.Contig1_All (*MYB44*) were induced more strongly in h-96h than in n-96h. CL6283.Contig2_All (*MYB6*) was more suppressed in 96 h after high temperature treatments (Fig. 2 and Table S2). In the *WRKYs* family, CL3506.Contig5_All (*WRKY33*) and CL2694.Contig5_All (*WRKY40*) were all more up-regulated during 38°C/33°C and down-regulated during 28°C/23°C, however, CL3506.Contig11_All (*WRKY33*) was inhibited in h-96h (Fig. 2 and Table S2). In the *bHLH* family, bHLH-related gene (CL3647.Contig6_All) was more up-regulated in 38°C/33°C than in 28°C/23°C (Fig. 2 and Table S2). DEGs suggested that these TFs played important roles in the buds of the decapitated ‘SEI NO ISSEI’ under contrasting temperature conditions.

Differentially transcribed of auxin-related genes in buds

Auxin plays a key role in bud growth and development, we analyzed auxin-related differential genes from RNA-seq data. There were 32 differential transcriptions associated with auxin, 21 genes in the signalling pathway and 11 genes in the transport pathway (Fig. 3 and Table S3). Among 21 transcripts associated with auxin signalling process, the abundance of *TIR1*, *ARF2*, *ARF4*, *ARF5*, *ARF8*, *ARF16*, *ARF18*, *ARF19*, *IAA3* and *IAA9* transcript were low in buds outgrowth (n-24h and n-96h) compared to other time points (h-24h and h-96h), while *PIN1*, *PIN2*, *AUX1*, *LAX1*, *LAX2* and *ABCB1* (associated with auxin transport process) were significantly up-regulated in buds outgrowth compared to inhibited buds.

Verification of RNA-seq data by qRT-PCR

In the library of bud outgrowth, fourteen differentially expressed genes were selected for qRT-PCR to test the reliability of RNA-seq data. The qRT-PCR assays largely validated the RNA-Seq based identification of differential transcription (Fig. 4 and 5). We selected four transcription factors, including Unigene28455_All (*ERF110*), Unigene9775_All (*MYB4*), CL2694.Contig5_All (*WRY40*) and CL3647.Contig6_All (*bHLH36*) and ten auxin-related genes (*TIR1*, *ARF2*, *ARF16*, *IAA3* and *IAA9*), which were all related to shoot branching and potential candidate genes for regulating chrysanthemum branching.

The phenotype of *A. thaliana* plants heterologously expressing *CmERF110*

When *CmERF110* was constitutively expressed in *A. thaliana*, the number of branches formed by 45 day old plants varied between wild type (WT) plants, WT plants harboring the transgene p35S::*CmERF110* (WT/*ERF110*), *brc1* mutant plants and *brc1* mutant plants harboring p35S::*CmERF110* (*brc1*/*ERF110*): the latter genotype produced the highest number of branches, followed by WT/*ERF110* and *brc1* (Fig. 6a and 6b). Axillary buds were rarely formed in the first three rosette leaf axils of WT plants, whereas nearly all rosette leaves carried buds or branches in the axils of WT/*ERF110* (active buds), *brc1* (inactive buds) and *brc1*/*ERF110* (active buds) plants. A greater number of high order branches was produced by WT/*ERF110*, *brc1* and *brc1*/*ERF110* plants than by WT plants (Fig. 6g-j). WT/*ERF110*, *brc1* and *brc1*/*ERF110* plants formed a similar number of primary cauline leaf branches (CI) as did WT ones (Fig. 6k). WT/*ERF110* and *brc1*/*ERF110* plants produced significantly more secondary cauline leaf branches (CII) than did, respectively, WT and *brc1* plants (Fig. 6l). The *brc1*/*ERF110* plants formed more rosette branches (RI and RII) than did WT plants. The RI and RII phenotype of *brc1* plants was weaker than that of WT/*ERF110* (Fig. 6m and 6n). The conclusion was that the product of *BRC1* influenced the plants' RI and RII performance, while the constitutive expression of *CmERF110* mainly affected CII as well as RI and RII.

The constitutive expression of *CmERF110* in *A. thaliana* reprogrammed the transcription of auxin-related genes

The effect of constitutively expressing *CmERF110* on the transcription of the auxin-related genes was tested in 21 day old *A. thaliana* seedlings. The abundance of *PIN1*, *AUX1*, *LAX1*, *LAX2* and *ABCB1* transcript was from 1.30 to 3.14 fold higher in the transgenic plants than in WT plants, while *TIR1*, *ARF2*, *ARF16*, *IAA3* and *IAA9* were down-regulated by between 1.23 and 1.33 fold (Fig. 7). The suggestions that in the transgenic plants, the *CmERF110* product participated in the shoot branching, acting through the auxin-related genes.

Discussion

The capacity to form branches is an important determinant of crop productivity. Shoot branching includes the formation of axillary buds in the axil of leaves and subsequent outgrowth of the buds [29]. The presence of an apical shoot meristem inhibits lateral bud outgrowth, and removal of this meristem initiates lateral bud outgrowth [30]. The outgrowth of lateral buds in the chrysanthemum cultivar 'SEI NO ISSEI' was found to be significantly compromised when the plants were exposed to a high temperature regime. In *Rosa*, temperature has also been shown to influence the budburst gradient along the stem [31] [43], which laid the foundation for us to study the burst of chrysanthemum buds under the contrast temperature treatments. In chrysanthemum, does the effect of temperature on the burst of buds may act locally through the modulation of the individual bud's capacity to burst or, instead, through the modulation of long-distance mechanisms?

To solve this problem, we performed RNA-Seq. The molecular basis of the high temperature-induced inhibition of bud outgrowth was explored here by comparing the transcriptomes of plants grown under a non-inhibitory and an inhibitory temperature regime, an experiment which revealed that a number of auxin-related genes were differentially transcribed: while *TIR1* (*CL6519.Contig2_All*), *ARF2* (*Unigene4746_All*), *ARF16* (*CL5081.Contig1_All*), *IAA3* (*CL9965.Contig1_All*) and *IAA9* (*Unigene1646_All*) were up-regulated by high temperature, *PIN1* (*Unigene23859_All*), *AUX1* (*Unigene23517_All*), *LAX1* (*Unigene23518_All*), *LAX2* (*CL142.Contig2_All*) and *ABCB1* (*CL2492.Contig2_All*) were all down-regulated (Fig. 3 and Table S3). Physiological experiments conducted many years ago established auxin as a key regulator of axillary shoot branching [8]. *TIR1* is an F-box protein, which acts as an auxin receptor [17]. It has been shown in tomato that a deficiency of *ARF2a* transcript results in the promotion of axillary shoot formation [32] and *ARF16* has been established as auxin-inducible [33][45]. Several proteins are known to regulate auxin transport: these include the auxin efflux carrier PIN and the auxin influx facilitators AUX1 and LAX [34-36][49-51]. *ABCB1* is expressed in shoot and root apices [37], where it functions primarily to introduce auxin synthesized in the meristem into long-distance polar auxin transport streams [38-40][53-55]. Our experimental results were consistent with the previous studies, indicating that under high temperature treatments the inhibition of bud outgrowth in chrysanthemum may be regulated by auxin-related genes.

A large number of transcription factors act in plants to regulate their growth and development [41]. Here, the ERF family became the particular focus of the transcriptomic analysis, because these factors are prominent in the response to abiotic stress [42, 43], as well as being involved in numerous signal transduction pathways [44]. The sequence of five of the unigenes classed as being differentially transcribed as a result of the high temperature treatment identified them as likely members of the *ERF* family: both were more abundantly transcribed under h-96h than under n-96h (Fig. 2 and Table S2), consistent with their products exerting control over bud outgrowth under high temperature conditions. The poplar gene *EBB1* is known to encode an AP2/ERF transcription factor; plants over-expressing it exhibit early budbreak and those under-expressing it suffer from delayed budbreak [45]. The chrysanthemum ERF transcription factor *CmERF053* has been shown to regulate shoot branching [46], while the product of the *Larix kaempferi* AP2/ERF gene *LkAP2L2* exerts a pleiotropic effect on both branching and seed development [47]. The *A. thaliana* gene *EBE* encodes an AP2/ERF transcription factor, which has a notable effect on shoot architecture [48]. Finally, the peach transcription factor PpERF3b promotes precocious side-branching [49]. These previous studies suggested that ERFs played essential roles in the control of plant bud development, so a more in-depth investigation regarding this important group of temperature-related genes is required. *CmERF110* was up-regulated by the high temperature regime inhibiting bud outgrowth in 'SEI NO ISSEI', however, its constitutive expression in *A. thaliana* induced a bush-like phenotype. Based on the above results, we supposed that the function of *CmERF110* in both *A. thaliana* and chrysanthemum, may regulate different downstream genes or form protein complex with different protein interactions to regulate the shoot branching through affecting the auxin-related genes, but the mechanism needs further experimental verification. Such as, auxin has been shown to enhance root-growth inhibition under aluminum (Al) stress in *Arabidopsis*. However, in maize (*Zea mays*), auxin may play a negative role in the Al-induced inhibition of root growth [50]. Previously, TSRF1, an ethylene response factor (ERF) protein from tomato, binds to GCC box in the promoters of pathogenesis-related genes positively regulates pathogen resistance in tomato and tobacco [51, 52], but negatively regulates osmotic response in tobacco [52]. However, overexpression of *TSRF1* enhances the osmotic and drought tolerance of rice by modulating the increase in stress responsive gene expression [53]. Our study also showed that the function of *CmERF110* in chrysanthemum and *Arabidopsis* was opposite, and the previous studies further demonstrated that our findings were reliable.

In *A. thaliana*, the *BRC1* product suppresses bud formation, since in the loss-of-function *brc1* mutant, axillary meristem formation is promoted, the buds develop more rapidly and the plant branches more profusely [54, 55]. When a transgene comprising *CmERF110* driven by the CaMV 35S promoter was introduced into the *brc1* mutant, the resulting plants produced more branches than when the same transgene was introduced into a WT background, although the latter transgenic formed more branches than did either *brc1* or WT plants (Fig. 6). *brc1* mutants has a significantly higher number of rosette branches (RI and RII) than WT plants, and *brc1* plants has a similar number of primary and secondary cauline leaf branches (CI and CII) as the wild type [54]. Consistent with previous studies, the loss of *BRC1* influenced the number of rosette branches formed, while the *CmERF110* transgene affected both the number of secondary cauline leaf branches and rosette branches.

A qRT-PCR analysis identified a number of genes which were transcriptionally reprogrammed as a result of constitutively expressing *CmERF110* in *A. thaliana*. The abundance of *PIN1*, *AUX1*, *LAX1*, *LAX2* and *ABCB1* transcript was higher in the transgenic plants than in the WT ones, so the transgenic lines exhibited a multi-branched phenotype (Fig. 6). The high expression level of auxin transport protein in lateral buds is positively correlated with shoot branching. For example, the expression of *RhPIN1* is upregulated during the process that sucrose promotes lateral buds [56]. In *Arabidopsis*, the expression level of *PIN1* is significantly upregulated [57]. In decapitated peas, the growth of lateral buds are promoted and rapidly upregulate the expression of *PsAUX1* and *PsPIN1* [58]. In addition, the auxin signalling genes are also involved in the development of shoot branching. Increasing the R/FR in *Arabidopsis* can promote branching and inhibit the expression of *IAA3* [59]. The lateral bud outgrowth of leafy spurge is accompanied by down-regulation of *ARF2* expression [60]. In tomato, *ARF2a* acts downstream of *IAA3* and *IAA9* during the development of axillary shoots [32], *SlARF2a* inhibits lateral bud growth, and the down-regulated strains of *IAA3* and *IAA9* show an increased branching phenotype [61, 62]. Similar expression patterns of *IAA3*, *IAA9* and *ARF2a* suggest that they may function closely in axillary buds [32]. In our study, a further effect of constitutively expressing *CmERF110* in *A. thaliana* was to lower the abundance of *TIR1* transcript, and reduced that of *IAA3* and *IAA9*, with a knock-on effect on the transcription of *ARF2* and *ARF16* (Fig. 7). These transcriptional changes were consistent with the previous studies, thereby driving branch induction to completion.

Conclusions

In summary, this study reported a highly complete transcriptome from the buds of decapitated 'SEI NO ISSEI'. Differentially expressed genes during high temperature treatments were identified and validated by qPCR, many of these differentially abundant transcripts as key players in bud outgrowth, including the *AP2/ERF*, *MYB*, *WRKY*, *bHLH* families and auxin-related genes. To our knowledge, the present study has identified that *CmERF110* regulated shoot branching when its encoding gene was heterologously expressed in *A. Thaliana*. The regulation acted through the auxin-related genes. The gene therefore represents a potential candidate for manipulating the shoot branching behavior of chrysanthemum. What remains to be established is how *CmERF110* regulates bud outgrowth, how it interacts with other gene products influencing bud growth, and whether shoot branching in chrysanthemum can be manipulated by over-expressing *CmERF110*.

Methods

Plant materials and growing conditions

Chrysanthemum morifolium cultivar 'SEI NO ISSEI' cuttings were obtained from the Chrysanthemum Germplasm Resource Preserving Centre (Nanjing Agricultural University, Nanjing, China). Uniformly-sized cuttings were rooted in a 2:1 mixture of peat and perlite, grown in a greenhouse under natural light, then transferred into a 1:3 mixture of vermiculite and garden soil. The plants were exposed to an 16 h photoperiod ($160 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density), a relative humidity of 70% and a day/night

temperature of 28°C/23°C until they had formed around 15 leaves. *A. thaliana* (ecotype Col-0) plants were raised at 22°C, either on a solidified medium containing half strength Murashige and Skoog [23] salts, or in a vermiculite/peat moss/perlite (9:3:1) mixture. The plants were given a 16 h photoperiod of 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light.

High temperature treatment

Decapitated 'SEI NO ISSEI' plants retaining about 15 internodes were subjected to two contrasting temperature regimes, both associated with a 16 h photoperiod provided by white light. The four treatments were 38°C (day) / 33°C (night) for either 24 h (h-24h) or 96 h (h-96h) and 28°C/23°C for either 24 h (n-24h) or 96 h (n-96h). At least three replicates, each comprising 15 plants, were raised for each treatment. Harvested buds were snap-frozen in liquid nitrogen and stored at -80°C until processed for RNA extraction.

cDNA library construction and RNA-Seq analysis

RNA was extracted using a Total RNA Isolation System (Takara Bio Inc., Otsu, Japan) following the manufacturer's protocol [24]. After extract total RNA and treated with DNase I, mRNA was isolated using oligo (dT) and fragmented in mixed fragmentation buffer. Using the mRNA fragments as templates, the first strand of cDNA was synthesized, then the second strand was synthesized by using DNA polymerase I, RNase H, dNTPs and buffer. Purified double strands cDNA was resolved with EB buffer for end reparation and single nucleotide A (adenine) was added to each 3' end. After that, the A-tailed fragments were connected with adapters. The suitable fragments were selected for the PCR amplification. The constructed library was tested with the Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System, then the quality-tested samples were sequenced using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA) [22].

Raw reads were edited by removing low-quality, adaptor-polluted and high content of unknown base(N) reads [25], after which we got clean reads. Trinity software [26] was then used to assemble the transcriptome and the resulting unigenes were assigned a putative function based on homologs present in the NR (NCBI non-redundant protein), NT (NCBI non-redundant nucleotide), Swiss-Prot, KEGG (Kyoto Encyclopedia of Genes and Genomes) and COG (Cluster of Orthologous Groups) databases. Conflicting assignments were resolved by applying priority in the order NR, Swiss-Prot, KEGG and COG. Differentially expression genes (DEGs) were identified by imposing the criteria P value <0.05 , false discovery rate (FDR) ≤ 0.0001 and $|\log_2 \text{ratio}| \geq 1.0$. All sequencing data have been deposited in the NCBI sequence read archive (www.ncbi.nlm.nih.gov/sra).

Quantitative real-time PCR (qRT-PCR) assay

Total RNA was extracted from the buds of chrysanthemum plants subjected to each of the various temperature treatments and from 21 day old *A. thaliana* plants carrying the transgene *p35S::CmERF110*. Each treatment was represented by three biological replicates. A 1 µg aliquot of each RNA was subjected to an RNase-free DNase I treatment, then converted to ss cDNA (after) using PrimeScript® Reverse Transcriptase (Takara). The subsequent 20 µL qRT-PCRs (three technical replicates per biological replicate) each contained 10 µL SYBR Green PCR master mix (Takara), 10 ng cDNA and 0.2 µM of each primer; the reactions were given an initial denaturation (95°C/2 min) followed by 40 cycles of 95°C/15 s, 55°C/15 s and 72°C for 20 s. Details of the various gene-specific primer pairs given in Supplementary Table 1 were synthesized by Shanghai Generay Biotech Co., Ltd. Experiments involving chrysanthemum were based on *EF1a* (Genbank accession number KF305681) as the reference [27], while those involving *A. thaliana* used *AtActin2* (*At3g18780*). Relative transcript abundances were calculated using the $2^{-\Delta\Delta CT}$ method [28].

Abbreviations

TFs: Transcription factors; IAA: Auxin indole-3-acetic acid; AUX1/LAXs: Auxin resistant 1/like aux1s; ABCBs: B subfamily of ATP-binding cassette proteins; cDNA: Complementary deoxyribonucleic acid; Cl: Primary cauline-leaf branches; Cll: Secondary cauline-leaf branches; Rl: Primary rosette-leaf branches; Rll: Secondary rosette-leaf branches; FDR: False discovery rate; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genome pathway; NCBI: National Center for Biotechnology Information; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; RNA-seq: Ribonucleic acid sequencing; SRA: Sequence Read Archive; TIR1: Transport inhibitor response 1; ARF2: Auxin response factor 2; ARF16: Auxin response factor 16; ERF110: Ethylene-responsive transcription factor 110; IAA3: Auxin-responsive protein IAA3; IAA9: Auxin-responsive protein IAA9; PIN1: Pin-formed 1; AUX1: Auxin resistant 1; LAX1: Like aux 1; LAX2: Like aux 2; ABCB1: ATP-binding cassette protein 1

Declarations

Acknowledgements

Not applicable.

Authors' contributions

WF and JJ conceived and designed the project. WF, FC and JJ provided the materials. JJ, AS and XX conducted the experiments. KZ, YH, YT, QM, ZZ and WL analyzed the data. XX and JJ wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Raw data has been uploaded to the National Centre for Biotechnology Information Sequence Read Archive (SRA) under the accession BioProject number: PRJNA578175. Annotation results and full-list of differentially expressed transcripts are available in Additional file 6: Tables S2 and Additional file 7: Table S3.

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 Summary of sequencing reads after filtering in lateral buds of ‘SEI NO ISSEI’

Sample	Total raw reads (Mb)	Total clean reads (Mb)	Total clean bases (Gb)	Q20 percentage (%)	Q30 percentage (%)	Clean reads ratio (%)
h-24h	53.11	44.13	6.62	98.43	94.97	83.10
h-96h	52.94	44.52	6.68	98.46	95.03	84.10
n-24h	52.56	44.63	6.69	98.41	94.97	84.92
n-96h	51.40	45.46	6.82	98.56	95.92	88.44

Table 2 Quality metrics of unigenes in lateral buds of ‘SEI NO ISSEI’

Sample	Total number	Total length	Mean length	GC percentage (%)
h-24h	84,759	72,240,579	852	39.70
h-96h	87,615	75,257,081	858	39.61
n-24h	80,704	67,772,052	839	39.76
n-96h	72,974	61,738,236	846	40.15
All-Unigene	132,396	140,318,943	1,059	39.48

Table 3 Summary of functional annotation result of unigenes in lateral buds of ‘SEI NO ISSEI’

Database	Annotated number	Annotated percentage (%)
Total	132,396	100%
NR	72,274	54.59%
NT	56,878	42.96%
Swiss-Prot	51,697	39.05%
KEGG	55,399	41.84%
COG	27,433	20.72%
Interpro	49,316	37.25%
GO	17,224	13.01%
Overall	79,116	59.76%

Figures

Figure 1

Bud development in decapitated plants of 'SEI NO ISSEI' chrysanthemum as affected by temperature. a The appearance of the plants over the first ten days following exposure to a temperature regime of either 38°C/33°C or 28°C/23°C (bar: 3.5 cm) b Bud burst rate (%) c Length of bud. Bars represent SE *, **: performances differ significantly ($P < 0.05$, < 0.01) between the treatments. n-1 to n-15 refers to the first bud to the last bud of decapitated chrysanthemum from top to bottom.

Figure 2

Heatmap of 18 differentially expression transcription factors (TFs) from four libraries. The bar represents the expression (FPKM) of each gene in n-24h, h-24h, n-96h and h-96h as indicated by blue/white/red rectangles. Blue means low expression and red means high expression. The detail information for each list can be found in Table S2.

Figure 3

Heatmap of 18 differentially expression transcription factors (TFs) from four libraries. The bar represents the expression (FPKM) of each gene in n-24h, h-24h, n-96h and h-96h as indicated by blue/white/red rectangles. Blue means low expression and red means high expression. The detail information for each list can be found in Table S2.

Figure 4

The transcriptional response after 24 h of high temperature treatment of a selection of four transcription factors and ten auxin-related genes. Quantification of differentially transcribed derived from qRT-PCR assays. FPKM is their abundance in the sequencing data of the transcriptome libraries.

Figure 5

The transcriptional response after 96 h of high temperature treatment of a selection of four transcription factors and ten auxin-related genes. Quantification of differentially transcribed derived from qRT-PCR assays. FPKM is their abundance in the sequencing data of the transcriptome libraries.

Figure 6

The phenotypic consequence of constitutively expressing CmERF110 in *A. thaliana* in the wild type or absence of BRC1. a, b The appearance of 45 day old plants a Col-0: WT, 35S::ERF110-1, -2, -3: three constitutive expressors of CmERF110 in a WT background b *brc1*: the *brc1* mutant, *brc1/35S::ERF110*-1, -2, -3: three constitutive expressors of CmERF110 in a *brc1* background. Bars: 3.5 cm c-f Schematic representation of branching in the contrasting genotypes g-j Quantification of branching and bud formation (n=7). Each column represents the order of branch, and each row presents the position of the axils. P: primary branch, S: secondary branch. Grey boxes indicate the absence of a bud, yellow ones the presence of a dormant bud, green ones the presence of primary rosette branches (RI), red ones of secondary rosette branches (RII), blue ones of primary cauline branches (CI), purple ones of secondary cauline branches (CII). The white boxes indicate the absence of a leaf. k-n The number of (k) CIs, (l) CIIIs, (m) RIs, (n) RIIs. o The total number of branches. Error bars represent the SE (n=20).

Figure 7

The abundance of transcript generated from auxin-related genes involved in the regulation of shoot branching in 21 day old WT *A. thaliana* seedlings and those constitutively expressing CmERF110. Values given in the form mean $\pm$ SE (n=3).

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