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

CURRENT STATUS: POSTED

Xiaojuan Xing
Nanjing Agricultural University

Jiafu Jiang
Nanjing Agricultural University

Kunkun Zhao
Nanjing Agricultural University

Yaoyao Huang
Nanjing Agricultural University

Yun Tang
Nanjing Agricultural University

Qi Ma
Nanjing Agricultural University

Zixin Zhang
Nanjing Agricultural University

Weixin Liu
Nanjing Agricultural University

Aiping Song
Nanjing Agricultural University

Fadi Chen
Nanjing Agricultural University

Weimin Fang  fangwm@njau.edu.cn
Nanjing Agricultural University

Corresponding Author
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\textsuperscript{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 (WRYY40) 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
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 \textit{brc1} or WT plants (Fig. 6). \textit{brc1} mutants has a significantly higher number of rosette branches (RI and RII) than WT plants, and \textit{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 \textit{BRC1} influenced the number of rosette branches formed, while the \textit{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 \textit{CmERF110} in \textit{A. thaliana}. The abundance of \textit{PIN1}, \textit{AUX1}, \textit{LAX1}, \textit{LAX2} and \textit{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 \textit{RhPIN1} is upregulated during the process that sucrose promotes lateral buds [56]. In \textit{Arabidopsis}, the expression level of \textit{PIN1} is significantly upregulated [57]. In decapitated peas, the growth of lateral buds are promoted and rapidly upregulate the expression of \textit{PsAUX1} and \textit{PsPIN1} [58]. In addition, the auxin signalling genes are also involved in the development of shoot branching.

Increasing the R/FR in \textit{Arabidopsis} can promote branching and inhibit the expression of \textit{IAA3} [59]. The lateral bud outgrowth of leafy spurge is accompanied by down-regulation of \textit{ARF2} expression [60]. In tomato, \textit{ARF2a} acts downstream of \textit{IAA3} and \textit{IAA9} during the development of axillary shoots [32], \textit{SlARF2a} inhibits lateral bud growth, and the down-regulated strains of \textit{IAA3} and \textit{IAA9} show an increased branching phenotype [61, 62]. Similar expression patterns of \textit{IAA3}, \textit{IAA9} and \textit{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 genens. 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 μmol m\(^{-2}\) 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 μmol m\(^{-2}\) 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) $\leq 0.0001$ and $|\log_2\text{ratio}| \geq 1.0$. All sequencing data have been deposed 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 EF1α (Genbank accession number KF305681) as the reference [27], while those involving A. thaliana used AtActin2 (At3g18780). Relative transcript abundances were calculated using the 2^(-ΔΔCT) 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; CI: Primary cauline-leaf branches; CII: Secondary cauline-leaf branches; RI: Primary rosette-leaf branches; RII: 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.

**Funding**

This work is supported by the National Natural Science Foundation of China (31372092), the National Natural Science Foundation of China (31870691), the earmarked fund for Jiangsu Agricultural Industry Technology System (JATS[2018]278), and a project funded by the Program Development of Jiangsu Higher Education Institutions.

**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.

Author details

1Key Laboratory of Crop Genetics and Germplasm Enhancement, Key laboratory of Landscaping, Ministry of Agriculture and Rural Affairs, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

References

1. Doebley J, Stec A, Hubbard L. The evolution of apical dominance in maize. Nature. 1997; 386:485-488.
2. Teichmann T, Muhr M. Shaping plant architecture. Frontiers in Plant Science. 2015; 6:233.
3. Schmitz G, Theres K. Shoot and inflorescence branching. Current Opinion in Plant Biology. 2005; 8(5):506-511.
4. Rameau C, Bertheloot J, Leduc N, Andrieu B, Foucher F, Sakr S. Multiple pathways regulate shoot branching. Frontiers in Plant Science. 2015; 5:741.
5. Lang GA, Early JD, Martin GC, Darnell RL. Endo-, para-ecodormancy: physiological terminology and classification for dormancy
research. Hortscience. 1987; 22(3):371-377.

6. Doğramacı M, Horvath DP, Anderson JV. Meta-analysis identifies potential molecular markers for endodormancy in crown buds of leafy spurge; a herbaceous perennial. Advances in Plant Dormancy. 2015; 197-219.

7. Chao WS, Dogramaci M, Horvath DP, Anderson JV, Foley ME. Phytohormone balance and stress-related cellular responses are involved in the transition from bud to shoot growth in leafy spurge. BMC plant biology. 2016; 16:47.

8. Thimann KV, Skoog F. On the inhibition of bud development and other functions of growth substance in Vicia faba. Proc R Soc Lond B Biol Sci. 1934; 114:317-339.

9. Wisniewska J, Xu J, Seifertova D, Brewer PB, Ruzicka K, Blilou I, et al. Polar PIN localization directs auxin flow in plants. Science. 2006; 312(5775):883.

10. Mravec J, Skupa P, Bailly A, Hoyerova K, Krecek P, Bielach A, et al. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature. 2009; 459(7250):1136-1140.

11. Vanneste S, Friml J. Auxin: a trigger for change in plant development. Cell. 2009; 136(6): 1005-1016.

12. Ljung K, Bhalerao RP, Sandberg G. Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. The Plant Journal. 2001; 28(4):465-474.

13. Snow R. The young leaf as the inhibiting organ. New Phytologist. 2010; 28(5):345-358.

14. Thimann KV, Skoog F. Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Proc Natl Acad Sci U S A. 1933; 19(7):714-716.
15. Shen CJ, Bai YH, Wang SK, Zhang SN, Wu YR, Chen M, et al. Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in Sorghum bicolor under phytohormone and abiotic stress. The FEBS journal. 2010; 277(14):2954-2969.

16. Cho M, Cho HT. The function of ABCB transporters in auxin transport. Plant Signal Behav. 2013; 8:e22990.

17. Dharmasiri N, Dharmasiri S, Estelle M. The F-box protein TIR1 is an auxin receptor. Nature. 2005; 435(7041):441-445.

18. Salehin M, Bagchi R, Estelle M. SCF$^{TIR1/AFB}$-based auxin perception: mechanism and role in plant growth and development. Plant Cell. 2015; 27(1):9-19.

19. Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, et al. Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature. 2007; 446(7136):640-645.

20. Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M. Auxin regulates SCF$^{TIR1}$-dependent degradation of AUX/IAA proteins. Nature. 2001; 414:271–276.

21. Park JY, Kim HJ, Kim J. Mutation in domain II of IAA1 confers diverse auxin-related phenotypes and represses auxin-activated expression of Aux/IAA genes in steroid regulator-inducible system. The Plant Journal. 2002; 32:669-683.

22. Ren LP, Sun J, Chen SM, Gao JJ, Dong B, Liu YN, et al. A transcriptomic analysis of Chrysanthemum nankingense provides insights into the basis of low temperature tolerance. BMC Genomics. 2014; 15:844.

23. Murashige TS, Skoog FA. A revised medium for rapid growth and bioassays with tobaco tissue cultures. Physiologia Plantarum. 1962; 15(3):473-497.

24. Song A, Gao T, Li P, Chen S, Guan Z, Wu D, et al. Transcriptome-wide
identification and expression profiling of the DOF transcription factor gene family in *Chrysanthemum morifolium*. Frontiers in Plant Science. 2016; 7:199.

25. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology. 2011, 29(7):644-652.

26. Pertea G, Huang X, Liang F, Antonescu V, Sultana R, Karamycheva S, et al. TIGR gene indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics. 2003; 19(5):651-652.

27. Wang H, Chen S, Jiang J, Zhang F, Chen F. Reference gene selection for cross-species and cross-ploidy level comparisons in *Chrysanthemum* spp. Scientific Reports. 2015; 5:8094.

28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^ΔΔCT method. Methods. 2001; 25(4):402-408.

29. McSteen P, Leyser O. Shoot branching. Annual Review of Plant Biology. 2005; 56(56):353-374.

30. Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, et al. *DWARF10*, an *RMS1/MAX4/DAD1* ortholog, controls lateral bud outgrowth in rice. The Plant Journal. 2007; 51:1019-1029.

31. Djennane S, Oyant LHS, Kawamura K, Lalanne D, Laffaire M, Thouroude T, et al. Impacts of light and temperature on shoot branching gradient and expression of strigolactone synthesis and signalling genes in rose. Plant, Cell and Environment. 2014; 37:742-757.

32. Xu T, Liu X, Wang R, Dong X, Guan X, Wang Y, et al. *SIARF2a* plays a negative role in mediating axillary shoot formation. Scientific Reports. 2016; 6:33728.

33. Wang JW, Wang LJ, Mao YB, Cai WJ, Xue HW, Chen XY. Control of root cap
formation by MicroRNA-targeted auxin response factors in Arabidopsis. The Plant cell. 2005; 17(8):2204 -2216.

34. Okadala K, Ueda J, Komaki MK, Bell CJ, Shimuraa Y. Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. The Plant cell. 1991; 3:677-684.

35. Marchant A, Kargul J, May ST, Muller P, Delbarre A, Perrot-Rechenmann C, et al. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. The EMBO Journal. 1999; 18:2066-2073.

36. Murphy AS, Hoogner KR, Peer WA, Taiz L. Identification, purification, and molecular cloning of N-1-naphthylphthalmic acid-binding plasma membrane-associated aminopeptidases from Arabidopsis. Plant Physiology. 2002; 128(3):935-950.

37. Sidler M, Hassa P, Hasan S, Ringli C, Dudler R. Involvement of an ABC transporter in a developmental pathway regulating hypocotyl cell elongation in the light. The Plant cell. 1998; 10:1623-1636.

38. Noh B, Murphy AS, Spalding EP. Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. The Plant cell. 2001; 13:2441-2454.

39. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, et al. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. The Plant Journal. 2005; 44(2):179-194.

40. Yang H, Murphy AS. Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in Schizosaccharomyces pombe. The Plant Journal. 2009; 59(1):179-191.

41. Udvardi MK, Kakar K, Wandrey M, Montanari O, Murray J, Andriankaja A, et al.
Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiology. 2007; 144(2):538-549.

42. Licausi F, Ohmetakagi M, Perata P. APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytologist. 2013; 199(3):639-649.

43. Sears MT, Zhang H, Rushton PJ, Wu M, Han S, Spano AJ, et al. NtERF32: a non-NIC2 locus AP2/ERF transcription factor required in jasmonate-inducible nicotine biosynthesis in tobacco. Plant Molecular Biology. 2014; 84(1-2):49-66.

44. Zhang G, Chen M, Chen X, Xu Z, Guan S, Li LC, et al. Phylogeny, gene structures, and expression patterns of the ERF gene family in soybean (Glycine max L.). Journal of Experimental Botany. 2008; 59(15):4095.

45. Yordanov YS, Ma C, Strauss SH, Busov VB. EARLY BUD-BREAK 1 (EBB1) is a regulator of release from seasonal dormancy in poplar trees. Proc Natl Acad Sci U S A. 2014; 111(27): 10001-10006.

46. Nie J, Wen C, Xi L, Lv S, Zhao Q, Kou Y, et al. The AP2/ERF transcription factor CmERF053 of chrysanthemum positively regulates shoot branching, lateral root, and drought tolerance. Plant Cell Reports. 2018; 37(7):1049-1060.

47. Li A, Yu X, Cao BB, Peng LX, Gao Y, Feng T, et al. LkAP2L2, an AP2/ERF transcription factor gene of Larix kaempferi, with pleiotropic roles in plant branch and seed development. Russian Journal of Genetics. 2017; 53(12):1335-1342.

48. Mehrnia M, Balazadeh S, Zanor MI, Mueller-Roeber B. EBE, an AP2ERF transcription factor highly expressed in proliferating cells, affects shoot architecture in Arabidopsis. Plant Physiology. 2013; 162:842-857.

49. Sherif S, El-Sharkawy I, Paliyath G, Jayasankar S. PpERF3b, a transcriptional
repressor from peach, contributes to disease susceptibility and side branching in EAR-dependent and -independent fashions. Plant Cell Reports. 2013; 32(7):1111-1124.

50. Zhang M, Lu X, Li C, Zhang B, Zhang C, Zhang X-s, et al. Auxin efflux carrier ZmPGP1 mediates root growth inhibition under aluminum stress. Plant Physiology. 2018; 177(2):819-832.

51. Zhang H, Zhang D, Chen J, Yang Y, Huang Z, Huang D, et al. Tomato stress-responsive factor TSRF1 interacts with ethylene responsive element GCC box and regulates pathogen resistance to Ralstonia solanacearum. Plant Molecular Biology. 2004; 55:825-834.

52. Zhang H, Li W, Chen J, Yang Y, Zhang Z, Zhang H, et al. Transcriptional activator TSRF1 reversely regulates pathogen resistance and osmotic stress tolerance in tobacco. Plant Molecular Biology. 2007; 63:63-71.

53. Quan R, Hu S, Zhang Z, Zhang H, Zhang Z, Huang R. Overexpression of an ERF transcription factor TSRF1 improves rice drought tolerance. Plant biotechnology journal. 2010; 8(4):476-488.

54. Aguilar-Martinez JA, Poza-Carrion Cs, Cubas P. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. The Plant cell. 2007; 19(2):458-472.

55. Finlayson SA. Arabidopsis Teosinte Branched1-like 1 regulates axillary bud outgrowth and is homologous to monocot Teosinte Branched1. Plant & Cell Physiology. 2007; 48(5):667-677.

56. Barbier F, Peron T, Lecerf M, Perez-Garcia MD, Barriere Q, Rolcik J, et al. Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in Rosa hybrida. Journal of Experimental Botany. 2015; 66(9):2569-
57. Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O. The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. Current Biology. 2006; 16(6):553-563.

58. Kalousek P, Buchtová D, Balla J, Reinöhl V, Procházka S. Cytokinins and polartransport of auxin in axillary pea buds. Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis. 2010; 58:79-88.

59. Holalu SV, Finlayson SA. The ratio of red light to far red light alters Arabidopsis axillary bud growth and abscisic acid signalling before stem auxin changes. Journal of Experimental Botany. 2017; 68(5):943-952.

60. Dogramaci M, Foley ME, Horvath DP, Hernandez AG, Khetani RS, Fields CJ, et al. Glyphosate's impact on vegetative growth in leafy spurge identifies molecular processes and hormone cross-talk associated with increased branching. BMC Genomics. 2015; 16:395.

61. Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, et al. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. The Plant cell. 2005; 17(10):2676-2692.

62. Chaabouni S, Jones B, Delalande C, Wang H, Li Z, Mila I, et al. Sl-IAA3, a tomato Aux/IAA at the crossroads of auxin and ethylene signalling involved in differential growth. Journal of Experimental Botany. 2009; 60(4):1349-1362.

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. α = 0.05. 0d to 10d 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 libra
Figure 3

Heatmap of 18 differentially expression transcription factors (TFs) from four libraries.
Figure 4

The transcriptional response after 24 h of high temperature treatment of a select
Figure 5

The transcriptional response after 96 h of high temperature treatment of a select
Figure 6

The phenotypic consequence of constitutively expressing CmERF110 in A. thaliana.

Figure 7

The abundance of transcript generated from auxin-related genes involved in the...
Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Supplemental Figure S3.png
Supplemental Figure S4.png
Supplemental Figure S2.png
Supplemental Table S2.docx
Supplemental Table S3.docx
Supplemental Figure S1.png
Supplemental Table S1.docx