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The high concentrations of abscisic, jasmonic, and salicylic acids produced under long days do not accelerate flowering in *Chenopodium ficifolium* 459

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ABSTRACT

The survival and adaptation of angiosperms depends on the proper timing of flowering. The weedy species *Chenopodium ficifolium* serves as a useful diploid model for comparing the transition to flowering with the important tetraploid crop *Chenopodium quinoa* due to the close phylogenetic relationship. The detailed transcriptomic and hormonomic study of the floral induction was performed in the short-day accession *C. ficifolium* 459. The plants grew more rapidly under long days but flowered later than under short days. The high levels of abscisic, jasmonic, and salicylic acids at long days were accompanied by the elevated expression of the genes responding to oxidative stress. The increased concentrations of stress-related phytohormones neither inhibited the plant growth nor accelerated flowering in *C. ficifolium* 459 at long photoperiods. Enhanced content of cytokinins and the stimulation of cytokinin and gibberellic acid signaling pathways under short days may indicate the possible participation of these phytohormones in floral initiation. The accumulation of auxin metabolites suggests the presence of a dynamic regulatory network in *C. ficifolium* 459.

1. Introduction

The transition from vegetative growth to flowering is an essential commitment in plant life. It relies on the integration of both endogenous (age, phytohormone content), and external stimuli (photoperiod or day length, temperature, vernalization) [38,42,61,86] Izawa, [38]. The proper timing of reproduction is a fundamental prerequisite for adapting to the rapidly changing environment. The climate warming in the Holocene triggered the migration of plant species to higher latitudes, which could accelerate under recent climate changes [39,73]. Whereas the plants encountered suitable temperatures further from the equator, they had to adapt to variable day lengths, the prominent environmental cue informing plants about the proper time to flower. The capability to flower at the beginning of summer when the days become very long is also necessary for the successful cultivation of crops at higher latitudes [24]. At the same time, short-day plants start flowering at the beginning of autumn.

Chenopodium quinoa, initially grown in the Andean region of South America, is highly tolerant to drought and high salinity [40], making it a promising crop for future cultivation worldwide. Most *C. quinoa* accessions flower earlier at short days (SD), but some varieties, mainly originating from southern Chile, accelerate flowering under long days (LD) [10]. The adaptation of *C. quinoa* to LD flowering was associated with the sequence variation in the paralogs of the *FLOWERING LOCUS T* (*FT*) gene [67], the essential regulator of flowering in angiosperms [18]. *Chenopodium quinoa* is an allotetraploid species containing *FT* genes in 12 loci [83]; the expression of some *FT* homeologs is biased toward one of the two subgenomes [67]. These features complicate the clarification of the roles of the particular *FT* paralogs in floral induction in *C. quinoa*.

The diploid species *Chenopodium ficifolium* is closely related to the ancestor of the subgenome B of *C. quinoa* [81,91]. It may start to flower as early as 20 days after sowing (DAS), making it possible to obtain developmentally synchronized populations of seedlings and follow the pace of floral induction accurately. Like *C. quinoa*, two accessions with contrasting photoperiod responses were described in *C. ficifolium* [67, 82].

Chenopodium ficifolium has a very short juvenile phase and possesses only about a half of the loci than the C. *quinoa* genome because it is

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diploid. At the same time, it shares similarities in photoperiod response with its close relative *C. quinoa*. All these features suggest *C. ficifolium* could be an excellent comparative model for the studies of flowering in the more complex crop *C. quinoa*. *Chenopodium ficifolium* has been recently utilized to analyze agronomically relevant traits, including flowering time or plant height, associated with the *FLOWERING LOCUS T LIKE* (*FTL*) genes [85].

This study investigates the effect of photoperiod length on plant development. Day length informs plants about suitable time for flowering. It also impacts photosynthesis and growth rate. A complex methodical approach is necessary to capture multifaceted effects of short or long photoperiods on plant growth and development. We performed a detailed transcriptomic and hormonomic analysis of the floral induction in *C. ficifolium* under LD and SD. We focused on the accession 459 [82], which flowers earlier under SD similarly to the majority of *C. quinoa* cultivars. We aimed to analyze the changes in phytohormone concentrations accompanying the transition to the reproductive phase and the transcription patterns of phytohormone-related genes. We searched for the homologs of the flowering-related genes in *A. thaliana*, checking which transcript profiles were congruent with their participation in the floral induction in *C. ficifolium* 459.

We discovered highly elevated levels of abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) produced at long days. The high content of the stress-related hormones might have been induced by the oxidative stress occurring during the prolonged photosynthesis under LD. The high concentrations of stress-related phytohormones did not accelerate flowering in *C. ficifolium* 459.

2. Material and methods

2.1. Plant material and experimental conditions

We used the accession *C. ficifolium* 459, originally collected in Central Asia [82], as our experimental plant model. The Eurasian distribution area of *C. ficifolium* was plotted using the R package *rangemap* [16] based on presence records (preserved specimens and human observations) obtained from the available databases at the website GBIF.org [(13 August 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.e29s2v]. Fig. 1a shows the distribution area of *C. ficifolium* in Eurasia and the site where the accession 459 was collected. We also used *C. quinoa*, QQ74 [41] to compare growth and development. The plants were cultivated in the Institute of Experimental Botany greenhouse and propagated by self-pollination. Seeds were surface-sterilized and germinated as described by Štorchová et al. [82]. Average-sized individuals with opened cotyledons and uniform growth were selected for the experimental analysis. Plants planted in 96-well flat-bottom ELISA plates, single seedling per well, soaked in half-strength Hoagland solution, were maintained under 22 °C, 70% humidity, and cool-white fluorescent light (130 μmol m^{-2} s^{-1}) or dark in growth chamber E-36L2. Two photoperiodic regimes were applied: SD (6 h light and 18 h dark, and LD (18 h light and 6 h dark) for the floral induction analysis.

Growth analyses started using vegetative seedlings ten days after sowing. Analyses were made 5 times in the interval of 4–5 days (until flowering). Usually six plants from each treatment (LD or SD) were used.

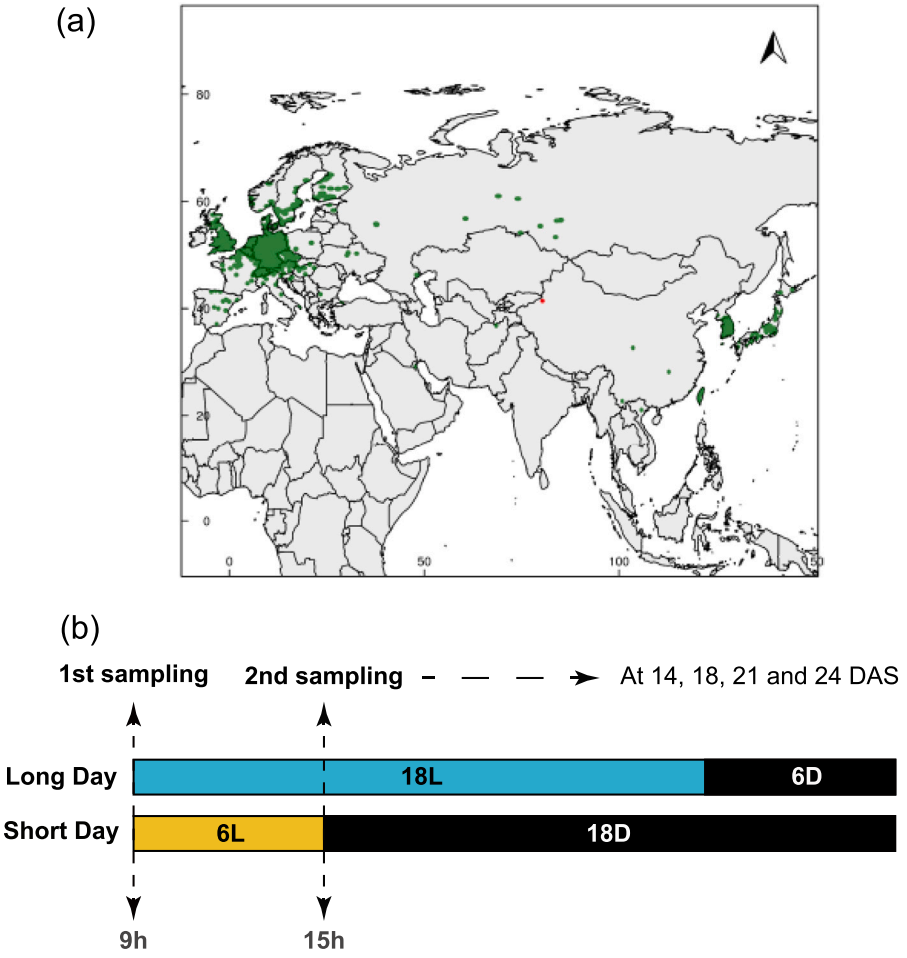

Fig. 1. (a) Distribution area of *Chenopodium ficifolium* in Euroasia. Green dots shows *C.ficifolium* presences. The site, where the accession 459 was found is marked by a red dot. (b) Experimental design: plant material was collected twice a day (9 h and 15 h) at 14, 18, 21 and 24 days after sowing (DAS) under two photoperiodic regimes; long day, (LD), and short day (SD).

The images of the whole seedlings, isolated cotyledons and leaves placed into the Petri dishes were examined under Navitar Machine Vision (Navitar Inc., Rochester, NY, USA). The length of shoot apex and flowering rate were assessed under a stereomicroscope Zeiss Stemi 305. The rate of flowering was stated as the number of plants with terminal flower bud (in % from the whole set of tested plants). The images were recorded using a DFK 33UX250 camera (The Imaging Source, Bremen, Germany) and processed using NIS-Elements 5,0 (Laboratory Imaging, Prague, Czech Republic).

2.2. RNA sampling and extraction

The plantlets were collected twice a day (in the morning at 9.00 and the afternoon at 15.00) at 14, 18, 21 and 24 days after sowing (DAS) under SD and LD (Fig. 1b). Aerial parts of the seedlings (14 and 18 DAS) or upper leaves and stems with apices of young plants (21 and 24 DAS) from each photoperiodic regime were collected and flash-frozen in liquid nitrogen. Three biological replicates, each consisting of three to four seedlings from LD conditions and eight to ten seedlings from SD conditions, were sampled at each time point. Total RNA was extracted using a Plant RNeasy Mini kit (Qiagen, Valencia, CA, USA). DNase I treatment was performed according to the manufacturer's protocol (DNA-free, Ambion, Austin, TX, USA) to eliminate DNA contaminations. If necessary, the DNase I treatment was done twice to eliminate any traces of genomic DNA. RNA concentration and quality were checked on 0.9% agarose gel and using the NanoDrop (Thermo Fisher Scientific, Vantaa, Finland).

2.3. RT qPCR

One μg of RNA and oligo dT primers (500 ng) were heated for 5 min at 65 °C, chilled on ice and mixed with Transcriptor buffer (Roche), 0.5 μl of Protector RNase Inhibitor (Roche, Diagnostics, Mannheim, Germany), 2 μl of 10 mM dNTPs and 10 units of Transcriptor Reverse Transcriptase (Roche). The first strand of cDNA was produced at 55 °C for 30 min. Two technical replicates of cDNA were produced with each RNA specimen. qPCR was performed using the LightCycler 480 SYBR Green I Master (Roche) in a final volume of 10 μl with 500 nM of each of the primers. The LightCycler LC 480 (Roche) was programmed as follows: 10 min of initial denaturation at 95 °C, then 40 cycles for 10 s at 95 °C, 10 s at 60°, followed by 15 s at 72 °C. PCR efficiencies were estimated on the basis of serial dilutions of cDNAs and used to calculate relative expression using the formula E_R^{CpR}/E_T^{CpT}, where E_T/E_R represents the PCR efficiency and CpT /CpR represents the cycle number at the threshold (crossing point). The *ACTIN11* (*ACT11*) gene was used as the reference. The primers for the reference and target genes (Table S1) were the same as used by Štorchová et al. [82].

2.4. Sampling for phytohormone measurements

The plant material for the estimation of phytohormone concentrations was sampled simultaneously as the samples for RNA extraction to ensure the correspondence of the transcriptomic data with phytohormone estimations. Aerial parts of the seedlings were collected at 14 and 18 DAS, upper leaves and stems with apices of young plants were taken at 21 and 24 DAS. The samples were flash-frozen in liquid nitrogen. Three biological replicates (about 20 mg of fresh weight) were taken at each sampling point. The samples were stored at −80 °C until performing the phytohormone analyses.

2.5. Extraction, purification, and quantification of endogenous phytohormones

The endogenous phytohormones were determined according to Přerostová et al. [71]. Briefly, about 20 mg of fresh weight (FW) (5–7 plants) per sample were homogenized with 1.5 mm zirconium beads using a FastPrep-24 instrument (MP Biomedicals, CA, USA) with 50 μl extraction buffer 50% acetonitrile/ water (1/1, v/v) and 10 μl mixture of internal standards (10^{-7} M), vigorously mixed and left at 4 °C for 30 min. After centrifugation at 30,000*g* for 20 min, the supernatant was applied to SPE Oasis HLB 10 mg 96-well plate (Waters, Milford, MA, USA) with a 96-well sample collection plate underneath. The pellet was re-extracted with an additional 50 μl of extraction buffer, centrifuged, and applied to the SPE 96-well plate. An aliquot of 5 μl of the combined extract was injected into the LCMS system. Phytohormones were separated on a Kinetex EVO C18 column (2.6 μm, 150 × 2.1 mm, Phenomenex, Torrance, CA, USA). Mobile phases consisted of A) 5 mM ammonium acetate and 2 μM medronic acid in water, and B) 95/5 acetonitrile/water (v/v). We applied the following gradient program: 5% B in 0 min, 7% B in 0.1–5 min, 10–35% in 5.1–12 min, 100% B at 13–14 min, and 5% B at 14.1 min. Hormone analysis was done on LCMS system consisting of UHPLC 1290 Infinity II (Agilent, Santa Clara, CA, USA) coupled to 6495 Triple Quadrupole mass spectrometer (Agilent). MS analysis was done in MRM mode, using isotope dilution method. Data acquisition and processing were done with the Mass Hunter software B.08 (Agilent).

2.6. Transcriptome assembly and evaluation

Total RNA extracted from the seedlings collected at eight time points under SD and LD, and from the leaves, flowers, and roots of adult plants as described above (51 RNA samples) were sent to Macrogen (Seoul, Korea), and strand-specific cDNA libraries were constructed from polyA enriched RNA. The sequencing of total RNA from all 51 samples on the Illumina NovaSeq6000 produced 753,019,719 paired-end (PE) reads (150 nt), about 14.8 million reads per sample. Following error correction (Rcorrector) [80], ribosomal RNA filtering (SortMeRNA; [45]), and quality and adapter trimming (TrimGalore, [48]) removed approximately 25% of the data, leaving 567,261,573 paired-reads (cutoff 145 bp). The raw and resulting trimmed data were deposited under the BioProject number PRJNA771226 with SRA accessions SRR16380491-SRR16380533 for the trimmed reads and accessions SRR16327138-SRR16327180 for the raw reads.

The trimmed reads were *de novo* assembled with Trinity v.2.9.0 [33] and evaluated, applying three different methods; BUSCO (Embryophytes) [78], RSEM-EVAL [52], and a completeness and contiguity evaluation as described in Krüger et al. [47]. Redundancy of the Trinity assembly was first reduced with CD-Hit (similarity cutoff 99.9%) and second with the script EvidentialGene tr2aacds.pl (MINCDS = 50). The resulting set was used for a BLASTX search against the nr database. The BLASTX results were imported into the MEGAN pipeline [37], with only plant hits retained. The evigene assembly was used for all subsequent analyses and deposited on NCBI under the accession GJOD01000000.

BLASTX-based homology searches (BLAST + 2.9.0) for the final transcriptome assembly against the NCBI nr protein database were performed. The cutoff E-value was set to $< 10^{-4,}$ and the maximum number of allowed hits was set to 10. The OmicsBox program v. 1.3.3 (BioBam Bioinformatics S.L., Valencia, Spain) was then used to annotate the "Trinity" genes based on gene ontology (GO) terms, InterProScan, and nr database annotation.

2.7. Transcript quantification and pairwise differential expression

Transcript quantification was done with the Trinity pipeline and included scripts, using the alignment-free method Salmon [68], as described in Krüger et al. [47]. The differential gene expression analysis was carried out using the Bioconductor package DESeq2 [56] with three biological replicates for each sampling time point using the standard single time point analysis. Extraction of differentially expressed genes (DEGs) was done for each sampling day, contrasting the LD with the SD sampling with 0.05 cutoff for false-discovery rate (FDR). The resulting DEGs and matrices were used for the subsequent analyses.

To set the collection of DEGs used for the Gene Ontology Term Enrichment analysis (GO analysis), an index was created based on the Fold Change values between SD and LD treated samples obtained through DESeq2 [56]. Absolute values of log2 Fold Change for each DEG between SD and LD at each sampling time point were summed up. High values of the sum denoted high pair differences in the expression between SD and LD, both positive and negative. The thresholds of 10, 15 and 20 index summed values corresponding to 6096, 3011, and 1545 DE genes, respectively, were selected to perform GO analysis. After comparing the GO analysis outputs and the gene expression graphs of selected DEGs, the set of 3011 genes was chosen as the most informative case for the GO enrichment analysis. The Fisher exact test (p-value $<$ 0.05) implemented in OmicsBox program v. 1.3.3 was utilized for this analysis.

3. Results

3.1. The growth and floral transition in C. ficifolium 459

The morphological and anatomical changes were followed at the whole plant level during floral induction in *C. ficifolium* 459 to supplement the comprehensive transcriptomic and hormonomic analyses. Plants grown under SD (6-h day length) were more tiny and spindly compared with LD (18-h day length) grown plants (Fig. 2a). *Chenopodium quinoa* plants were cultivated under the same conditions as *C. ficifolium* for the comparison of morphological development between the crop and its weedy relative. The *C. quinoa* plants were more robust, but remained vegetative even at 28 DAS, when *C. ficifolium* started to flower under both photoperiods (Fig. 2b). *Chenopodium quinoa* formed flower buds at about a week later (at 35 DAS) under SD, being vegetative under LD.

Most *C. ficifolium* plantlets cultivated under SD produced flower buds at 20 DAS, whereas less than 25% of individuals flowered at 28 DAS under LD. The onset of flowering in *C. ficifolium* correlated with the shoot apex length, which was much higher under SD (Fig. 2c). However, the plants grew more slowly at SD than at LD, as shown by the lower number and size of leaf pairs and the longer persistence of cotyledons (Fig. 2d). Long periods of darkness supported a faster elongation of hypocotyls under SD (Fig. 2e). The observed morphological changes confirm that *C. ficifolium* 459 accelerates flowering under SD, as described previously [82].

3.2. The assembly of the reference transcriptome of C. ficifolium 459

To get insight into the time course of the global gene expression of *C. ficifolium* 459 during floral induction, we analyzed the individual transcriptomes sampled at eight time points (in the morning and the afternoon) since 14–24 DAS under SD and LD. Because of the absence of a reference genome, we constructed the *de novo* reference transcriptome of C. *ficifolium* 459 with Trinity [31].

We combined 17 transcriptomic sets – one biological replicate of seedlings from seven time point (14 specimens) supplemented with RNA from leaves, flowers, and roots of adult plants. The total number 199.5 million filtered reads were assembled to 213,741 transcripts and

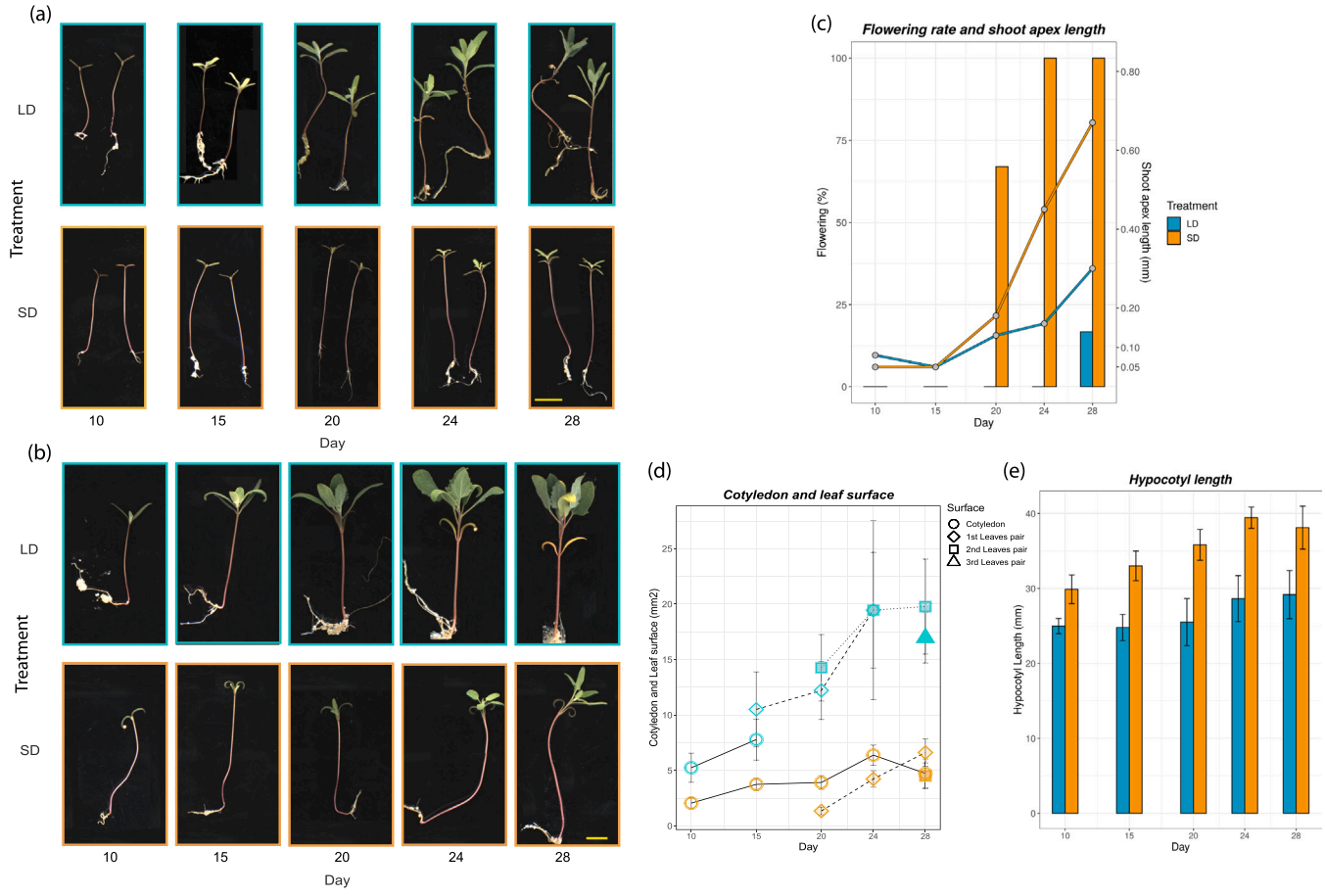

Fig. 2. The growth of *C. ficifolium* 459 and *C. quinoa* under long (LD) and short days (SD). (a) The seedlings of *C. ficifolium* at the age 10–28 days after sowing (DAS). (b)The seedlings of *C. quinoa* at the age 10–28 DAS. (c) The shoot apex length and proportion of the plants of *C. ficifolium* with visible flower buds at the age 10–28 DAS. (d) The cotyledon and leaf surface of *C. ficifolium* at the age 10–28 DAS. The third leaf pair (triangle) appeared under LD only. The cotyledons were lost before the age 20 DAS under LD, but persisted under SD. (e) The hypocotyl length in *C. ficifolium* at the age 10–28 DAS.

168,036 potential genes. The CD-Hit [51] step with the similarity threshold of 99.9% reduced the number of transcripts by 14% (to 184, 615) and the assigned genes by 16% (140,238). After the subsequent EvidentialGene [30] step, the assembly contained 55,020 transcripts and 51,146 potential genes, a reduction of 75% (transcripts) and 70% ('genes') compared to the initial Trinity assembly. The evigene assembly was checked for non-plant transcripts with BLASTX and filtered with MEGAN [37], resulting in 27,646 transcripts and 25,173 assigned genes classified as Viridiplantae. The evaluation of the evigene assembly showed completeness of 0.915, while the contiguity was 0.904. BUSCO found 92.3% complete and single-copy orthologs, 3.5% complete and duplicated orthologs, only 5.3% orthologs were missing.

3.3. The expression of FTL1, LFY, and flower-organ identity homologs increased under SD

The homolog of the key floral inducer *FTL1* was dramatically activated under SD, more in the morning than in the afternoon. The homologs of *LEAFY* (*LFY*) and flower-organ identity genes *APETALA 1* (*AP1*), *CAULIFLOWER* (*CAL*), *AGAMOUS* (*AG*) followed the same increasing trend (including higher morning levels) correlating with the onset of flowering at 20 DAS in SD grown plants. The paralog *FTL2–1* was only very slightly upregulated under SD at 24 DAS. The expression of the homolog of another angiosperm floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) [11,77] showed a stable expression during the experiment, a bit higher under LD than SD, not correlated with flowering (Fig. 3). Unlike *AP1*, the homolog of the *AP2* gene increased its expression under LD. The *FTL1, FTL2–1*, *SOC1* and *LFY* transcript levels were in general agreement with RT qPCR measurements (Fig. S1).

The transcription profiles of the *AGAMOUS-LIKE 9* (*AGL9*), *AGL11*, *UNUSUAL FLORAL ORGANS* (*UFO*), and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* 4 (*SPL4*) homologs share the increasing trends with *LFY* and other flower-organ identity genes, indicating their possible participation in flowering. In contrast, very strong but age-independent activation of *SPL7* occurred in the morning under SD. The *FLOWERING LOCUS D* (*FLD*) and *SUPRESSOR OF PHYA-105 1* (*SPA1*) homologs exhibited age-independent rhythmic expression under SD. The homolog of the *FD* gene, which interacts with *FT* to induce flower development in *A. thaliana* [17], increases its transcript levels with time under SD and a bit more under LD (Fig. S2).

3.4. The concentrations of stress-related phytohormones ABA, JA, and SA were highly elevated under LD in C. ficifolium 459

We estimated phytohormone levels in aerial parts of *C. ficifolium* seedlings taken at the same time as the RNA samples used for the construction of the transcriptomes. These measurements made it possible to correlate the phytohormone concentrations to the global gene expression profiles in the course of floral transition in *C. ficifolium*.

There were remarkable contrasts in ABA, JA, JA-Ile, and SA concentrations between SD and LD grown plants. The ABA and JA levels

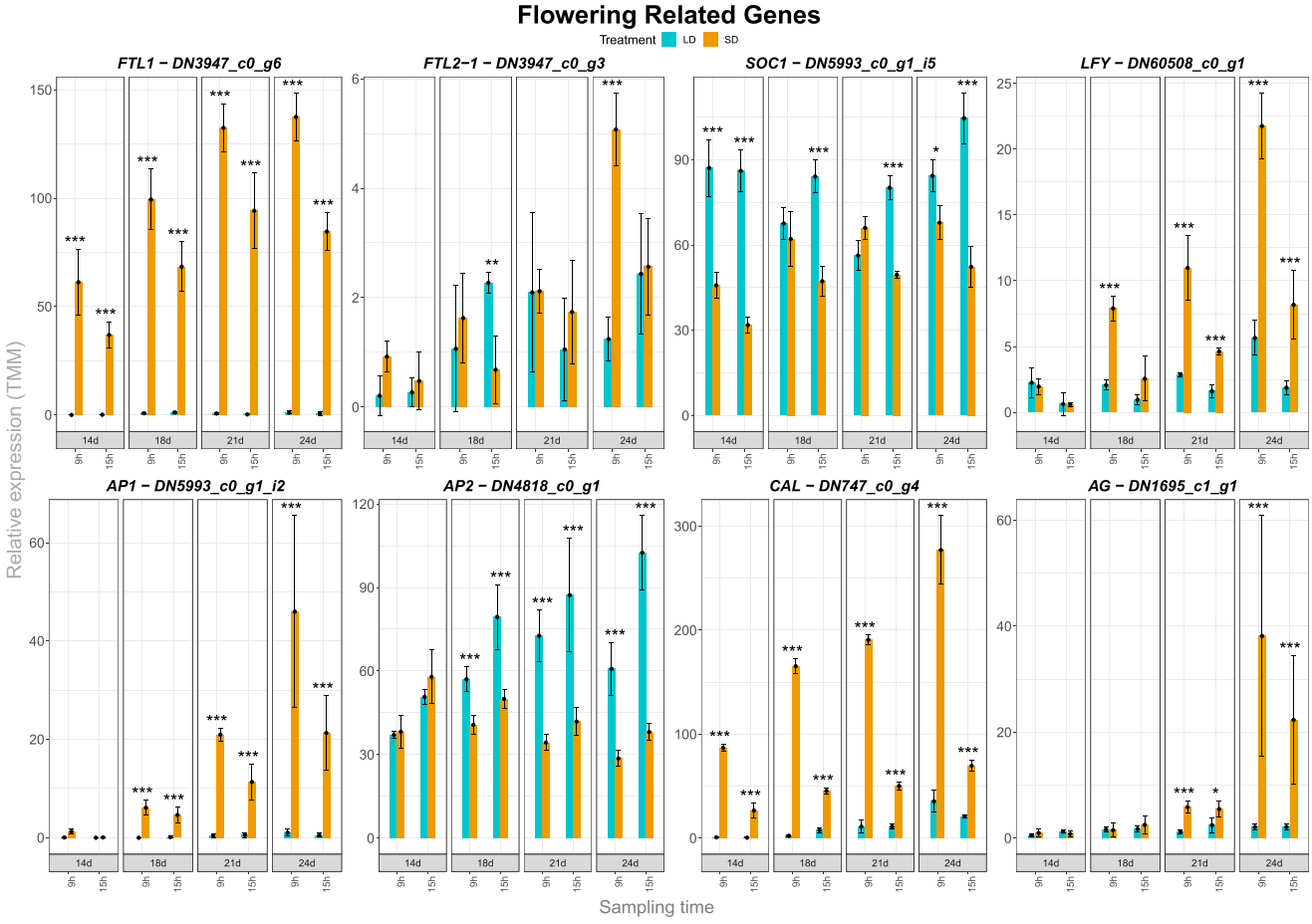

Fig. 3. The flowering-related genes expressed in *C. ficifolium* 459 at the age 14–24 DAS under long (LD) and short days (SD): blue columns correspond to LD treated samples, golden ones represent SD treated samples. Transverse lines at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * < 0.05, ** < 0.01 and ***< 0.001; Wald-test (DESeq2); three biological replicates, each consisting of 3–5 seedlings) between pairs of differentially treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning - 9.00, and afternoon −15.00; during four days corresponding to 14, 18, 21 and 24 DAS). The y-axis represents relative expression in transcript coverage (TMM).

oscillated rhythmically, being more abundant in the morning and about 10 – 50 fold higher under LD. Extreme differences between the photoperiods were found in SA concentrations, about a thousand times higher at LD than at SD (Fig. 4). The abundance of ABA metabolites (e.g., ABA-glucose ester) and JA precursor cis-12-oxo-phytodienoic acid (cisOPDA) were also highly elevated under LD. In contrast, the JA metabolite (dihydrojasmonic acid (DiH-JA) content decreased under LD (Fig. S3).

Some ABA biosynthesis-related gene homologs such as NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3 (NCED3) (Fig. 5a) or less prominently *NCED4* (Fig. S4) were upregulated under LD, suggesting their possible participation in ABA biosynthesis in *C. ficifolium*. The homolog of *SHORT VEGETATIVE PHASE* (SVP), which under stress conditions suppressed ABA catabolism in *A. thaliana* [93], was also activated under LD. In contrast, the homologs of the genes involved in ABA signaling pathways, e.g., ABA receptor *PYR1-LIKE 4* (*PYL4*) (Fig. 5a), repressor protein phosphatase 2Cs (PP2C) such as ABA-HYPERSENSITIVE GERMINATION 3 (AHG3) (Fig. S4) [6], and positive regulator of ABA response SNF1-related protein kinase 2.6 (SnRK2–6) [101] were either downregulated under LD or exhibited only small differences between both photoperiods (Fig. S4). Some transcription factors positively mediating ABA response were moderately upregulated by long photoperiods (ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2 (ABF2) and TINY gene homologs) (Fig. S4). The homolog of the transcription factors suppressing ABA response - ABA REPRESSOR 1 (ABR1) [66] (Fig. S4) or INDUCER OF CBP EXPRESSION 1 (ICE1) [54] were strongly upregulated under SD, as well as the central regulator in ABA signaling ABSCISIC ACID INSENSITIVE 3 (ABI3) (Fig. 5a). The coincident activation of both the positive and negative regulators of ABA response contributes to its fine tuning at LD.

Similar to ABA, some putative JA biosynthesis genes were highly activated under LD (*ALLENE OXIDE SYNTHASE 4* (*AOC4*) and *LIPOOXYGENASE 3* (*LOX3*) homologs). The transcription of the putative key receptor gene *CORONATINE INSENSITIVE 1* (*COI1*) was only slightly higher under LD. In contrast, the transcript level of the *JASMONATE ZIM DOMAIN PROTEIN 1* (*JAZ1*) homolog, functioning as the repressors of JA response in *A. thaliana* [32], was highly elevated under LD (Fig. 5b), which may modulate JA response, supposing the similar repressor function of *JAZ1* in *C. ficifolium*. The homolog of the transcription factor *MYC2*, the central mediator of JA and ABA response in angiosperms [44], was upregulated since 18 DAS under LD (Fig. 5b) as well as the homologs of the transcription factors *MYB108* and *bHLH14* (Fig. S4).

The putative SA biosynthesis gene *PHENYLALANINE AMMONIA LYASE* (*PAL1*) was inhibited in the afternoon under SD. The *ISOCHORISMATE SYNTHASE 1* (*ICS1*) homolog exhibited low expression, slightly increasing under SD (Fig. 5c), which may indicate lower importance of the isochorismate pathway for SA production in *C. ficifolium*. Interestingly, the *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1* (*SARD1*) gene positively regulating *ICS1* in *A. thaliana* [92] was strongly upregulated under LD, which may suggest distinct functions of these genes in *C. ficifolium* than in *A. thaliana*. The *SA-BINDING PROTEIN 2* (*SABP2*) homolog responsible for converting methylsalicylate to bioactive SA in *A. thaliana* [69] showed more than tenfold higher expression under LD. The transcription factor *TGACG MOTIF-BINDING FACTOR 4* (*TGA4*), which may be involved in SA response, was slightly downregulated under LD (Fig. 5c).

3.5. The higher concentrations of cytokinins under SD correlated with elevated expression of most CK-related genes under the short photoperiod

The bioactive cytokinin (CK) *tZ* (*trans*-zeatin) levels oscillated in diurnal rhythms under SD, showing a higher abundance in the afternoon. The metabolite *trans*-zeatin riboside (*tZR*) followed the same rhythmic pattern as *tZ*. The glycosylated metabolites *trans*-zeatin *O*-glucoside (*tZOG*) and *trans*-zeatin *N7*-glucoside (*tZ7G*) were also more abundant under SD but without differences between morning and afternoon. The other bioactive CK *cis*-zeatin (*cZ*) had about tenfold lower concentrations than *tZ*, being higher under SD (Fig. 6).

The isopentenyladenine (iP) levels were relatively invariant, reaching the same values as *tZ* (Fig. 6a). The concentrations of the iP precursor isopentenyl adenosine monophosphate (iPRMP) gradually decreased under LD, but they maintained stable values under SD. The ribosides derived from IP, *cZ*, and 2-methylthiozeatin (MeS-Z) were slightly more abundant under LD, with a clear outlier in the morning 21 DAS. The level of dihydrozeatin (DHZ) catabolite dihydrozeatin-*N7*-glucoside (DZ7G) was higher at SD at each time point (Fig. S5a).

In line with the elevated CK levels under SD, the transcription of the putative genes coding for isopentenyltransferase (IPT) enzymes, catalyzing the rate-limiting step in CK synthesis, increased under short photoperiods. *IPT2*, *IPT5*, and *IPT9* were the most expressed members of the *IPT* gene family in *C. ficifolium*. *IPT9* was strongly upregulated in the afternoon, accordingly with the rhythms of *tZ* and *cZ* concentration (Fig. S5b). The *LONELY GUY* (*LOG*) homologs, encoding CK riboside 5'-monophosphate phosphoribohydrolases necessary for the conversion of precursors to active hormones [49], were also expressed more under SD, except for *LOG5*, which was activated under LD, as well as the zeatin glucosyl transferase (*ZOG*) genes, responsible for the reversible glucosylation of zeatin (Fig. S5c). The cytokinin oxidase/dehydrogenase (CKX) genes controlling irreversible degradation of CKs were upregulated under SD, more in the morning than in the afternoon (Fig. S5b).

The activation by SD was also observed in the homologs of genes involved in CK signaling. The potential homolog of the CK receptor *HISTIDINE KINASE* (*AHK2*) was highly upregulated in the afternoon, *AHK3* in the morning. The elevated expression in the morning under SD was measured in the type-B Response Regulators - transcription factors *ARR2*, *ARR11, ARR12*. The *ARR3* gene was upregulated in the afternoon under SD (Fig. S5d). Accordingly, the homologs of CYTOKININ RESPONSE FACTOR 2 (CRF2) and CRF4 exhibited higher expression under SD, increasing in the afternoon. The *CRF10* homolog was more highly expressed under LD, both in the morning and afternoon (Fig. S5d). In conclusion, most genes putatively involved in CK metabolism and signaling were upregulated under SD, many of them showing rhythmic expression patterns.

3.6. The gibberellin and auxin concentrations varied slightly between SD and LD conditions, but the expression of some phytohormone-related genes showed dynamic oscillations

As the levels of gibberellins were close to the detection limit, only GA_1 and its precursor GA_{19} were determined. The concentration of the bioactive gibberellin GA_1 was the highest in the 14 day-old seedlings, being slightly but not significantly higher under LD than SD. Later it dropped down under both photoperiods, less under SD. The concentration of the precursor GA_{19} was higher under LD during the course of the experiment (Fig. 7a).

The transcription of the genes likely involved in bioactive GA biosynthesis varied substantially with seedling age, photoperiod, and daytime. The homolog of *GA3ox1*, responsible for GA_1 biosynthesis in *A. thaliana* [59], was transcribed more under SD. The *GA2ox6* gene, putatively engaged in the GA degradation, was highly activated in the morning under SD (Fig. 7b). The expression of putative GA biosynthesis and degradation genes is consistent with slightly elevated GA_1 levels under SD.

We also estimated the high variation among expression patterns of the genes putatively functioning in the GA signaling pathways. The GA receptor homologs *GA INSENSITIVE DWARF 1C* (*GID1C*) and *GID1B* were, in longer-term, slightly upregulated under LD, unlike the SD activation of the homolog of transcription factor *GAMYB33* mediating GA response [3] (Fig. 7b). The transcription factors *PIF*s, responsible for skotomorphogenesis hypocotyl elongation, are controlled by GAs at the posttranslational level [23]. The *PHYTOCHROME INTERACTING*

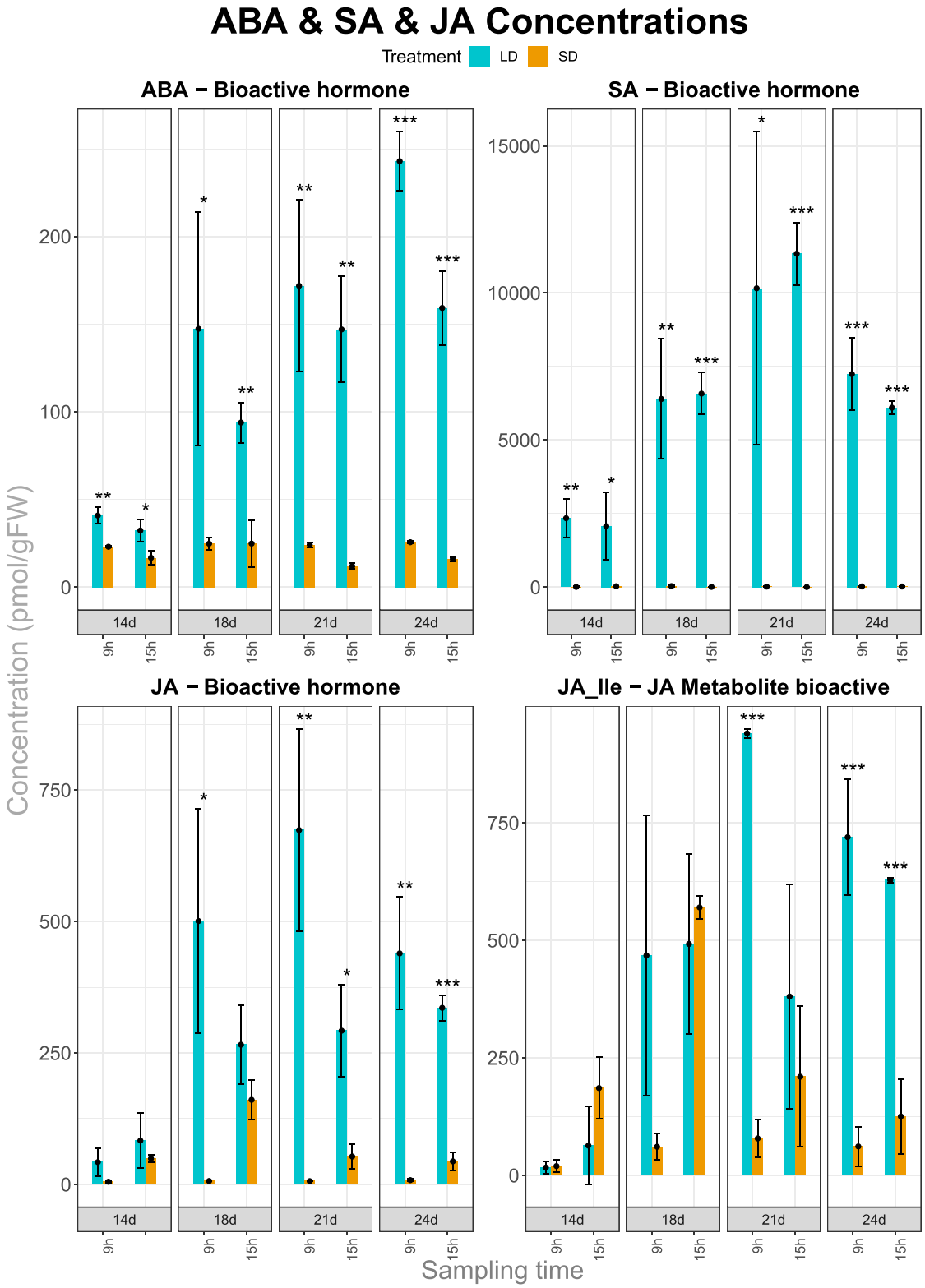

Fig. 4. The concentrations of ABA, SA, JA and JA-Ile in *C. ficifolium* 459 at the age 14–24 DAS under long (LD) and short days (SD). Blue columns correspond to LD treated samples, golden ones represent SD treated samples. Transverse lines at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * < 0.05, ** < 0.01 and ***< 0.001; t-test; three biological replicates, each consisting of 5–7 seedlings) between pairs of differentially treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning −9.00, and afternoon −15.00; during four days corresponding to 14, 18, 21 and 24 DAS. The y-axis represents phytohomone concentration measured as pmol per g of fresh weight.

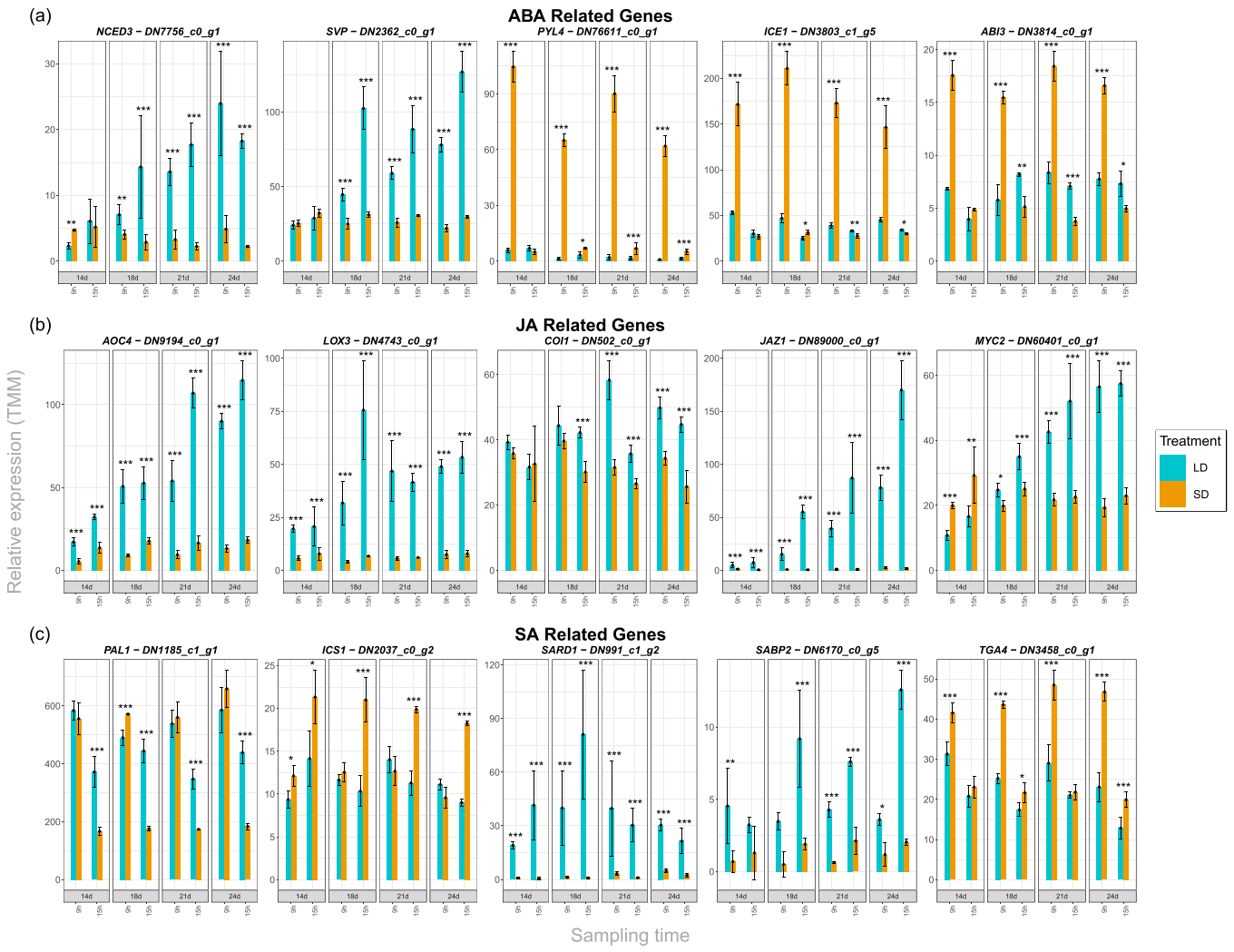

Fig. 5. The stress phytohormone-related genes expressed in *C. ficifolium* 459 at the age 14–24 DAS under long (LD) and short days (SD). (a) The ABA-related genes. (b) The SA-related genes. (c) The JA-related genes. Blue columns correspond to LD treated samples, golden ones represent SD treated samples. Transverse line at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * $<$ 0.05, ** $<$ 0.01, and *** $<$ 0.001; Wald-test (DESeq2); three biological replicates, each consisting of 3–5 seedlings) between pairs of reversely treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning −9.00, and afternoon - 15.00. The y-axis represents relative expression in transcript coverage (TMM).

FACTOR 3 (*PIF3*) homolog exhibited higher expression under SD shortly after the dark period (Fig. 7b), which suggests the presence of the transcriptional control of its expression. The homologs of GA response repressors *SPINDLY* (*SPY*) and *GIBBERELLIC ACID INSENSITIVE* (*GAI*) was detected were expressed more highly under SD shortly after the dark period (Fig. 7b), which contributes to fine modulation of GA response.

The bioactive auxins indol-3-acetic acid (IAA) and phenylacetic acid (PAA) were slightly more abundant under LD in older plantlets (21 and 24 DAS) (Fig. 7a). In contrast, the auxin metabolites IAA-glutamate (IAA-Glu) and 2-oxindole-3-acetic acid (oxIAA), were elevated under SD compared to LD (Fig. S6a).

We analyzed the expression patterns of numerous genes which may be responsible for auxin metabolism and transport. Some showed multiple differences between LD and SD conditions, e.g., *PIN-FORMED 1* (*PIN1*), *PIN3*, and *PIN7* (Fig. S6 b). We also identified the rich set of the genes putatively involved in auxin signaling *Aux/IAAs* (Fig. S6c, d) and *AUXIN RESPONSE FACTOR* (*ARFs*) (Fig. S6e) with a distinct response to the respective photoperiods. Interestingly, all potential homologs of the Small Auxin Upregulated RNA (SAUR) genes, except for SAUR71–693, were highly transcribed under SD (Fig. S6f).

3.7. The GO enrichment analysis of the genes differentially expressed between the LD and SD conditions

We assigned GO annotations to 41% of the putative genes from the final evigene transcriptome by OmicsBox v. 1.3.3. Twenty-five % of the genes received blastx hits. The rest (34%) remained without blastx hit (Fig. S7B).

We used the index calculated from the Fold Change values between the two experimental photoperiodic conditions (Fig. S7a). We identified 3011 putative genes as the representatives of the genes differentially expressed between LD and SD. The GO enrichment analysis of this data set found numerous GO categories with a significant excess of DEGs (Fig. S7c). Response to stress, particularly response to oxidative stress and defense response, oxidoreductase activity, cell wall biogenesis, or transcription regulator activity, appeared among the highly enriched GO categories.

Most of the genes participating in stress response were upregulated under LD (Fig. 8). Their homologs in *A. thaliana* or other plants are induced by biotic stress, e.g. the genes encoding defensin D2 [88], acidic endochitinase [76], antiviral ribosome-inactivating protein (RIP) [14] or Mildew Resistance Locus (MLO) protein [1]. Both abiotic and biotic

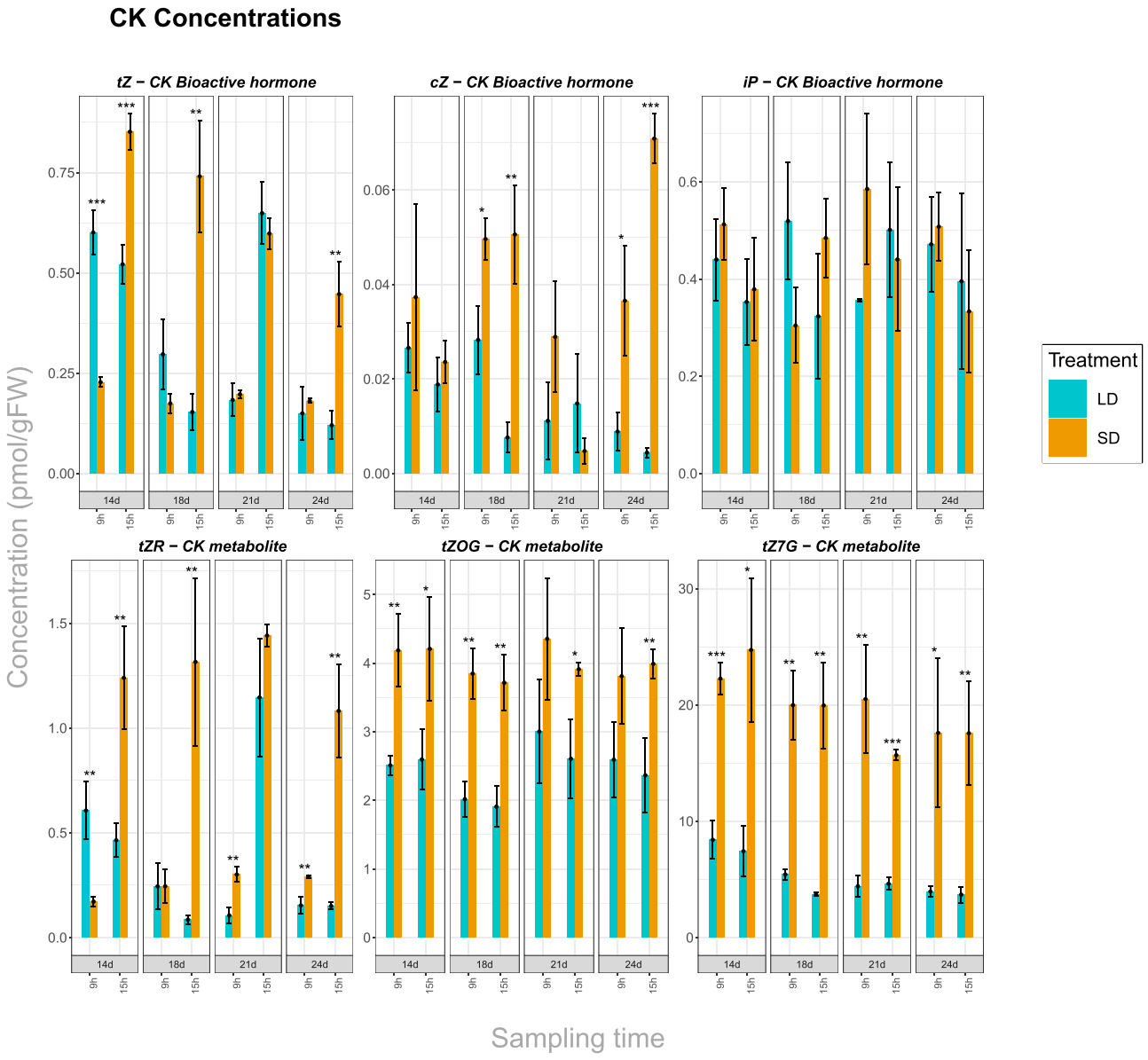

Fig. 6. The cytokinin (CK) concentrations in *C. ficifolium 459* at the age 14–24 DAS under long (LD) and short days (SD). Blue columns correspond to long LD treated samples, golden ones represent SD treated samples. Transverse lines at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * < 0.05, ** < 0.01 and *** < 0.001; t-test; three biological replicates, each consisting of 5–7 seedlings) between pairs of differentially treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning −9.00, and afternoon −15.00). The y-axis represents phytohomone concentration measured as pmol per g of fresh weight.

stresses activate the gene coding for chalcone synthase [20] or DETOXICATION protein [50]. Two highly expressed Major Latex Protein (*MLP)* homologs exhibited an opposite response to the photoperiod. Whereas *MLP28-like* was upregulated like LD, *MLP43-like* was activated by SD. An array of the genes encoding peroxidases (PERs) or L-ascorbate peroxidases (APXs) are induced by oxidative stress [65]. Many *PER* or *APX* homologs were strongly activated under LD, but some others, e.g., *PER7-like*, were also upregulated under SD.

4. Discussion

4.1. The high concentrations of ABA, JA, and SA produced under LD in C. ficifolium 459 did not inhibit growth

We estimated high ABA, JA, and SA concentrations in *C. ficifolium* seedlings grown under LD compared to the plants cultivated under SD. These phytohormones participate in plant stress responses and also control plant growth and development. ABA affects both abiotic stress responses [36,72] and developmental processes such as embryogenesis or fruit ripening, hydrotropism, or xylem formation [97]. JA is an essential mediator of biotic and abiotic stress responses [13,29,94]; it interacts with other phytohormones in the control of organogenesis, e. g., stamen maturation or stimulates trichome formation Acosta and Przybyl [2]. SA is involved in plant defense against biotrophic pathogens, it stimulates the antioxidant system and suppresses plant growth [34,100].

C. ficifolium 459 plants were cultivated under two conditions differing only in day length (18 h versus 6 h). Flowering occurred earlier under SD, as previously described [82]. The 18-h or even longer days may be encountered by *C. ficifolium* at the northern margin of its distribution area (Fig. 1a). As no other factor varied between LD and SD grown *C. ficifolium* 459 plants, the long photoperiod was responsible for the substantial increase in ABA, JA, and SA production measured under LD. The stress by altered photoperiod, accompanied by elevated levels of

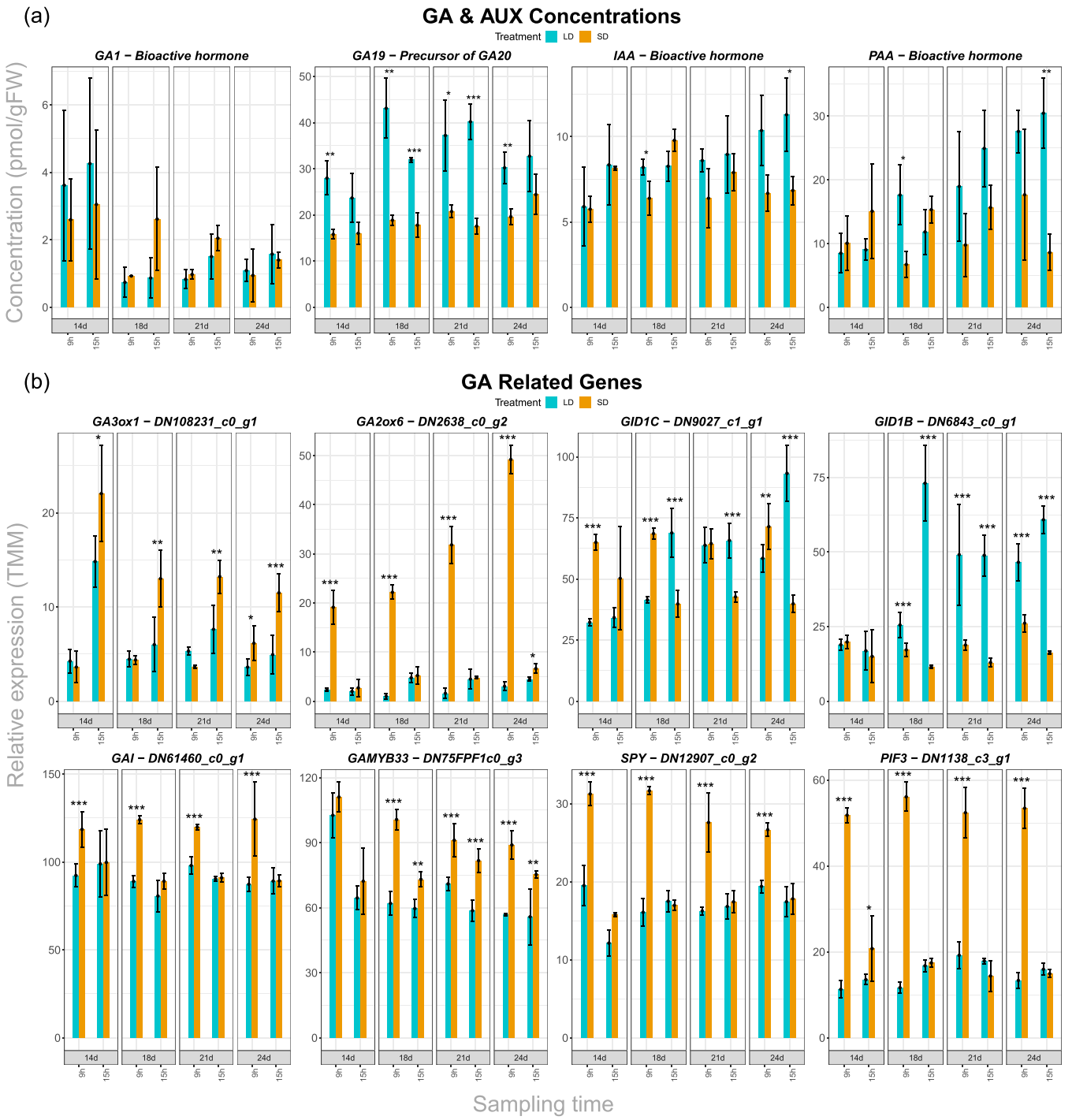

Fig. 7. The gibberellins (GA) and auxins (AUX). (a) The concentrations of GA and AUX in *C. ficifolium* 459 at the age 14–24 DAS under long (LD) and short days (SD). The y-axis represents phytohomone concentration measured as pmol per g of fresh weight. (b) The GA-related genes expressed in *C. ficifolium* at the age 14–24 DAS under LD and short days SD. The y-axis represents relative expression in transcript coverage (TMM). Blue columns correspond to LD treated samples, golden ones represent SD treated samples. Transverse lines at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * < 0.05, ** < 0.01 and *** < 0.001; t-test and Wald-test (DESeq2) corresponding to concentration and gene expression measurements respectively) between pairs of differentially treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning −9.00, and afternoon −15.00).

JA and ROS, has been described recently in *A. thaliana* [25,63]. These studies applied a single long photoperiod (32 h), which followed the SD photoperiodic regime. The increase in ABA level after the shift to longer photoperiod (17.5 h) was also described in *C. quinoa* by Bendevis et al. [8]. Our experimental conditions applied the invariant SD or LD photoperiodic regime, not changed since seedling germination, without switching between both photoperiods. We cannot, therefore, attribute the abundance of stress-related hormones produced under LD in *C. ficifolium* to the stress by altered photoperiod as described by Nitschke et al. [64], but rather to the stress caused by prolonged irradiation.

The evaluation of transcriptomes collected across ten days made it possible to investigate global gene expression profiles associated with the two contrasting photoperiods. We found many genes differentially expressed between LD and SD involved in biotic or abiotic stress

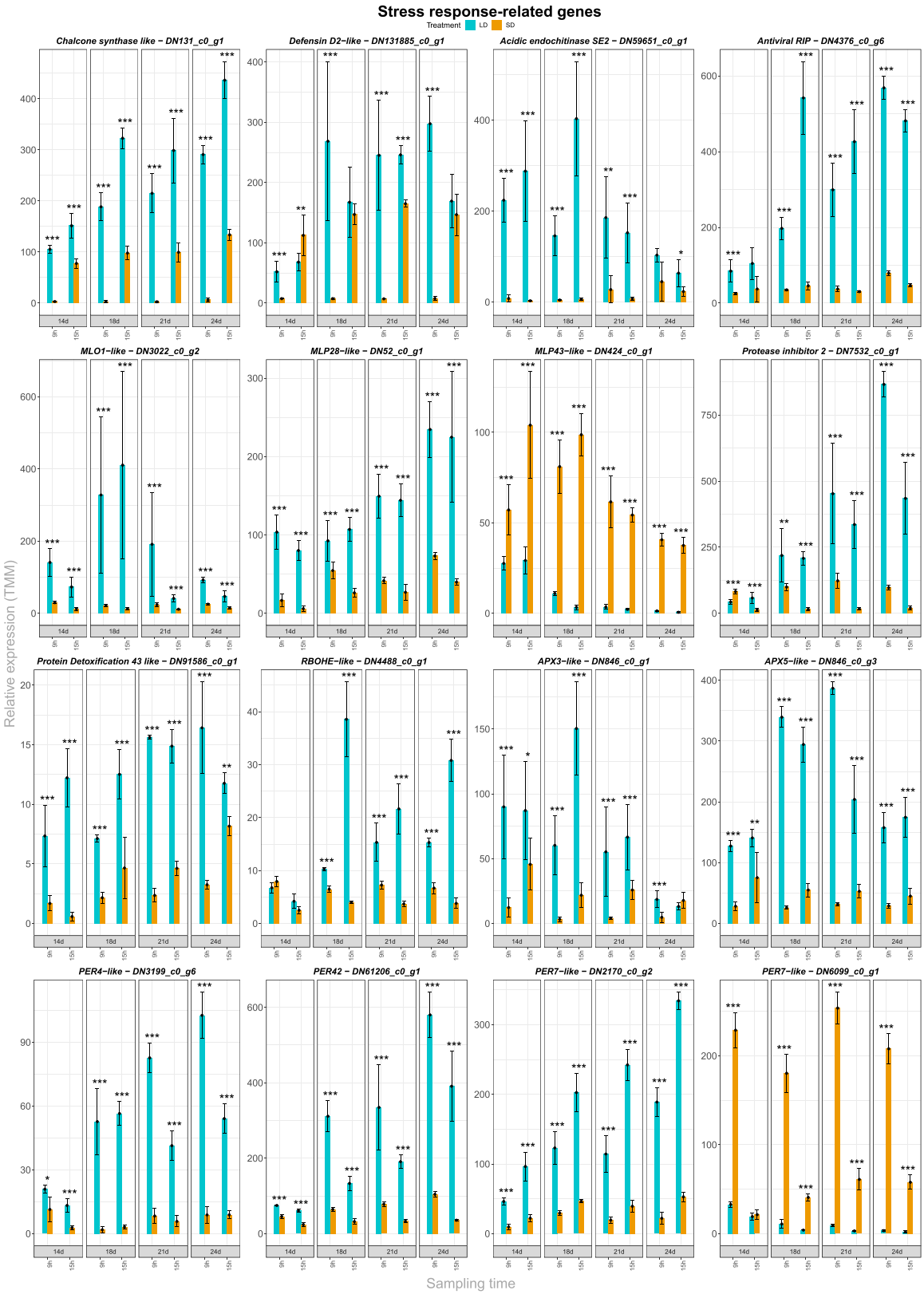

Fig. 8. The expression of important stress response related genes in *C. ficifolium* 459 at the age 14–24 DAS under long (LD) and short days (SD). Blue columns correspond to LD treated samples, golden ones represent SD treated samples. Transverse lines at each dot (median value of three biological replicates) represent standard deviation. Statistical significance (p-values * < 0.05, ** < 0.01 and ***< 0.001; Wald-test (DESeq2; three biological replicates, each consisting of 3–5 seedlings) between pairs of differentially treated samples is represented by asterisks. The x-axis represents eight sampling points (two sampling points per day: morning −9.00, and afternoon −15.00. The y-axis represents relative expression in transcript coverage (TMM).

responses, particularly in oxidative stress response. For example, genes encoding peroxidases and L-ascorbate peroxidase were abundant among DE genes, most of them highly upregulated under LD, which coincided with high ABA, JA, and SA levels. The activation of numerous stress-related genes by LD suggests the occurrence of oxidative stress, most likely provoked by photosynthesis at long photoperiods, as the main source of ROS in aboveground plant tissues are chloroplasts [5]. Enhanced ABA, JA, and SA levels may alleviate the deleterious effects of ROS generated during prolonged photosynthesis under LD by inducing antioxidant production.

Although plants under severe stress suppress the processes of growth and development [55], mild stress may be associated with growth promotion. *C. ficifolium* plants cultivated under LD grew more rapidly and produced bigger biomass. This response indicates that plants could cope efficiently with oxidative stress under LD. The high levels of stress-related phytohormones produced under LD did not inhibit the growth in *C. ficifolium*.

4.2. The high concentrations of ABA, JA, and SA produced under LD did not accelerate flowering in C. ficifolium 459

ABA, SA, and JA not only mediate stress response and influence plant growth, but they also regulate the floral transition. ABA was found to be associated with earlier flowering induced by drought stress (drought escape) in *A. thaliana* under long days [74]. However, there is also evidence about the delay of flowering caused by ABA [79]. SA was reported to activate transition to flowering in *Arabidopsis thaliana* under UV-C light stress [58]. SA has been known to promote flowering in specific species (e.g. in aquatic genus *Lemna*, [15,27]). It is also involved in stress-induced floral induction in *Pharbitis nil* [35]. JA was reported to suppress flowering in *A. thaliana* [12]. However, at low concentration, JA enhanced floral induction in *L. minor* under LD conditions [46].

We observed accelerated flowering in *C. ficifolium* 459 under SD when the ABA, JA, and SA contents were low. Thus, the simultaneous increase of endogenous concentrations of all three stress-related phytohormones under LD did not induce earlier flowering of *C. ficifolium* 459. However, it is possible that these high concentrations contributed to the delay of flowering in this accession observed under LD. The homolog of *SVP*, which functions as a negative regulator of ABA catabolism and at the same time as the repressor of flowering in *A. thaliana* [93], was highly activated by LD. It could mediate the floral inhibition, supposing that the *SVP* homolog in *C. ficifolium* possesses the same function as in *A. thaliana*. It is not easy to test this hypothesis directly, because *C. ficifolium* is recalcitrant to transformation, and no mutations are so far available. Fortunately, some populations of *C. ficifolium* behave as facultative long-day plants, flowering earlier under LD (the accession 283/9, Štorchová et al., 2019). The transcriptomic and hormonomic study on floral induction in this accession is now running in our lab with aims to elucidate the potential inhibitory effect of ABA, JA, and SA on flowering in C. *ficifolium*.

4.3. The floral induction in C. ficifolium 459 under SD was accompanied by slightly elevated CK concentrations

The possible function of gene homologs in C. *ficifolium* can in part be estimated by their sequence similarity with the *A. thaliana* genes. No phytohormone-related gene function study in *Chenopodium* has been available by now. Nevertheless, the transcriptomic data provide a useful hint when transcriptional profiles of gene homologs with putative metabolic function correlate with the estimated levels of the phytohormones, as we observed in the case of CKs.

The genes putatively involved in CK metabolism were upregulated in *C. ficifolium* under SD, in parallel with higher concentrations of *t*Z and *c*Z. The transcripts of *IPT5*, *IPT9*, and *CKX5* homologs were the most abundant among the *IPT* and *CKX* gene family members, respectively, suggesting their possible participation in the biosynthesis and degradation of CKs in aerial parts of *C. ficifolium.* Interestingly, *IPT2* and *IPT9* participate in *c*Z biosynthesis [26]. Therefore, their homologs may perform a similar function in *C. ficifolium*.

Also, the genes functioning in the CK signaling pathway, e.g., encoding the type-B Response Regulators (transcription factors activating CK target genes), were transcribed more under SD. This observation implies a more robust CK response in SD-grown plants of *C. ficifolium* than LD-cultivated plants. The interaction between ABA and CK is generally antagonistic. The high ABA levels repressed the expression of the *IPT* genes and the CK content in *A. thaliana* [62]. The observed downregulation of many genes associated with CK metabolism and signaling under LD implies similar crosstalk in *C. ficifolium*, too. Also, JA is known to impose a negative effect on CK levels [21].

CK elevation was described upon transition from vegetative to the generative stage in *Brassica napus* [87] or in *Triticum monococcum* upon vernalization [90]. The transition to flowering by elevated endogenous [7] and exogenous [19] CK levels were described in A. *thaliana*. High endogenous CK content was also observed during floral induction in apple trees [53]. The application of CKs resulted in floral induction in non-inductive SD in *Sinapis alba* Bernier, [9]. The increase of *t*Z, the most physiologically active CK in stimulation of cell division, observed in our experiments, correlated well with advanced development of apex under SD. It is, therefore, possible that higher concentrations of bioactive CKs under SD may be associated with floral induction in *C. ficifolium* 459.

GAs are frequently referred to as the stimulators of flowering in *A. thaliana* [28,70] and other plant species, e.g., *Lolium perenne* [57], particularly under non-permissive photoperiod. The GA levels in whole aerial parts of *C. ficifolium* seedlings were low, which may be explained by their preferential accumulation in small apical parts, which prevented the detection of their changes in the whole aboveground samples. The GA_1 concentration was a bit higher under SD. The expression of some gene homologs responsible for GA biosynthesis or degradation was higher under SD, unlike some other metabolic genes (e.g., the homologs of GA20ox1 or GA2ox2) activated by LD. Simultaneously, upregulation of expression of the transcription factor *GAMYB33* under SD indicated stimulation of GA signal transduction. These findings suggest a complex regulation of GA metabolism in C. *ficifolium*, which may be related to the transition from vegetative to generative stage.

Auxin and CKs regulate cell proliferation and differentiation by controlling cell division. They also act in synergy in the course shoot apical meristem formation [98]. The concentrations of IAA and PAA did not differ very much between LD and SD in *C. ficifolium*. The accumulation of auxin metabolites IAA-glutamate and 2-oxindole-3-acetic acid seems to suggest dynamic auxin regulation under SD.

Many genes putatively participating in the auxin metabolism, transport or signaling were differentially expressed between LD and SD, being both up- and downregulated. We observed the strong upregulation of several *SAUR* genes, responsible for fast response to auxin, under SD. This activation may be associated with a faster hypocotyl elongation under SD [84]. Our data on GAs and auxins support the presence of a dynamic regulatory network, controlling their metabolism and signal transduction in *C. ficifolium*.

4.4. The transcription profile of the FTL1 gene, but not of the SOC1 homolog, follows the pace of the floral induction in C. ficifolium 459

The global transcriptomic study confirmed the acceleration of flowering under SD in *C. ficifolium* 459 as published previously [82]. Floral induction was accompanied by a hundredfold increase of the *FTL1* expression under the short photoperiod, which agrees with its function as the floral inducer in *Chenopodium* Drabešová et al. [22]. This acceleration was followed by the activation of the floral identity gene homologs – *LFY*, *CAL*, *AP1*, *AG*, *AGL9*, *AGL11*, and *UFO*, suggesting their likely function at the onset of flowering. It should be kept in mind that we are inferring putative gene functions based on sequence similarity with well-known genes, which constrains our conclusions. However, the

increasing expression correlating with flowering and shared with *FTL1* indicates that these *C. ficifolium* genes may similarly participate in the floral transition as their *A. thaliana* counterparts.

Unlike *FTL1*, the expression of its putative interactor *FD* increased a bit more under LD than SD in *C. ficifolium*. The FD protein forms heterodimers with FT and TERMINAL FLOWER 1 (TFL1) and other proteins in SAM of *A. thaliana* to regulate flowering [89]. The activation of *FD* rising with plant age may be necessary for SAM competence to flower. Another function of FD was recently described in *A. thaliana*, where FD controlled the response to ABA in SAM [75]. The increase of *FD* transcription under LD at high ABA concentrations may also be related to this function in *C. ficifolium*.

The *AP2* gene performs a dual role in *A. thaliana*. It is involved in the development of sepals and petals together with *AP1*, but it also suppresses the floral transition [95,102]. Its upregulation under LD, increasing with time, may suggest a similarly complex response in *C. ficifolium*. The *SPL4* and *SPL7* homologs were both activated under SD, but their transcription profiles differed. The *SPL4* expression rose gradually, similarly to the putative floral organ identity genes, but *SPL7* was strongly upregulated each morning, independently on plant age. Whereas *SPL4* might contribute to the floral induction in *C. ficifolium* [43], the *SPL7* expression is rather compatible with its function to ensure copper availability [4] necessary for plastocyanins to restart photosynthesis after long nights.

The important floral integrator *SOC1* supports floral transition in *A. thaliana* [11,77]. However, it may also repress flowering, as described in woodland strawberry [60]. We measured the expression of the *SOC1* homolog invariant during floral induction in *C. ficifolium* 459, which did not provide a clear hint about its function. The slightly higher expression under LD may suggest the negative effect on flowering. Still, it is also possible that the *SOC1* homolog does not participate in floral transition in *C. ficifolium*, as described, e.g., in *Pharbitis nil* [96].

5. Conclusion

The global transcriptomic data supported the essential role of *FTL1* as the main floral inducer in *C. ficifolium* 459, but did not indicate the participation of *SOC1* homolog in floral induction. Long days induced the production of stress-related phytohormones. Unlike some other angiosperms (*Lemna*, *Pharbitis nil*), the elevated concentrations of ABA, JA, and SA did not accelerate flowering in *C. ficifolium.* Enhanced content of cytokinins as well as stimulation of their signaling pathway under SD correlated well with floral primordia formation, which may reflect their role in flower initiation. The Up-regulation of *GAMYB33* expression at SD indicates possible participation of GA signaling in transition to generative stage. The presence of dynamic auxin regulation under SD was documented by the accumulation of auxin metabolites. Our study confirmed the suitability of *C. ficifolium* as the diploid model not only for the research of flowering but also for the investigation of the phytohormone roles in the development in the genus *Chenopodium*.

Author contributions

DGL, MK and HŠ designed the research. HŠ, DGL and MK wrote the text, CB, DGL, OAJA performed and interpreted the experiments. PID and RV measured and interpreted phytohormone changes, KE and ZV analyzed plant growth and development. MK an MJ were involved in data analysis and interpretation. All authors read and approved the text.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2022.111279.

References

[1] J. Acevedo-Garcia, S. Kush, R. Panstruga, Magical mystery tour: MLO proteins in plant immunity and beyond, New Phytol. 204 (2014) 273–281.
[2] I.F. Acosta, M. Przybyl, Jasmonate signaling during Arabidopsis stamen maturation, Plant Cell Physiol. 60 (2019) 2648–2659.
[3] M.M. Alonso-Peral, J. Li, Y. Li, R.S. Allen, W. Schnippenkoetter, et al., The microRNA159-regulated GAMYB-like genes inhibit growth and promote programmed cell death in Arabidopsis, Plant Physiol. 154 (2010) 757–771.
[4] R. Araki, M. Mermod, H. Yamasaki, T. Kamiya, T. Fujiwara, et al., SPL7 locally regulates copper-homeostasis-related genes in Arabidopsis, J. Plant Physiol. 224 (2018) 137–143.
[5] K. Asada, Production and scavenging of reactive oxygen species in chloroplast and their functions, Plant Physiol. 141 (2006) 391–396.
[6] W. Baek, C.V. Lim, S. Luan, S.L. Lee, The RING finger E3 ligases PIR1 and PIR2 mediate PP2CA degradation to enhance abscisic acid response in Arabidopsis, Plant J. 100 (2019) 473–486.
[7] I. Bartrina, H. Jensen, O. Novák, M. Strnad, T. Werner, et al., Gain-of-function mutants of the cytokinin receptors AHK2 and AHK3 regulate plant organ size, flowering time and plant longevity, Plant Physiol. 173 (2017) 1783–1797.
[8] M.A. Bendevis, Y. Sun, S. Shabala, E. Rosenqvist, F. Liu, et al., Differentiation of photoperiod-induced ABA and soluble sugar responses of two quinoa (Chenopodium quinoa Willd.) cultivars, J. Plant Growth Regul. 33 (2014) 562–570.
[9] G. Bernier, My favourite flowering image: the role of cytokinin as a flowering signal, J. Exp. Bot. 64 (2013) 5795–5799.
[10] H.D. Bertero, Response of developmental processes to temperature and photoperiod in quinoa (Chenopodium quinoa Willd.), Food Rev. Int. 19 (2003) 87–97.
[11] R. Borner, G. Kampmann, G.J. Chandler, R. Gleissner, E. Wisman, et al., A MADS domain gene involved in the transition to flowering in Arabidopsis, Plant J. 24 (2000) 591–599.
[12] J. Browse, J.G. Wallis, Arabidopsis flowers unlocked the mechanism of jasmonate signalling, Plants 8 (2019) 285.
[13] M.L. Campos, J. Kang, G.A. Howe, Jasmonate-triggered plant immunity, J. Chem. Ecol. 40 (2014) 657–675.
[14] L. Citores, R. Iglesias, J.M. Ferreras, Antiviral activity of ribosome-inactivating proteins, Toxins 13 (2021) 80.
[15] C.F. Cleland, A. Ajami, Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid, Plant Physiol. 54 (1974) 904–906.
[16] M.E. Cobos, V. Barve, N. Barve, A. Jimenez-Valverde, C. Nuñez-Penichet, Rangemap: Simple Tools for Defining Species Ranges, R Package Version 0.1.18., 2021. ⟨https://CRAN.R-project.org/package=rangemap⟩.
[17] S. Collani, M. Neumann, L. Yant, M. Schmid, FT modulates genome-wide DNA-binding of the bZIP transcription factor FD, Plant Physiol. 180 (2019), 367-280.
[18] L. Corbesier, C. Vicent, S. Jang, F. Fornara, Q. Fan, et al., FT Protein movement contributes to long-distance signalling in floral Induction of Arabidopsis, Science 316 (2007) 1030–1033.
[19] M. D'Aloia, D. Bonhomme, F. Bouche, K. Tamseddak, S. Ormenese, et al., Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the FT paralogue TSF, Plant J. 65 (2011) 972–979.
[20] T.T.H. Dao, H.J.M. Linthorst, R. Verpoorte, Chalcone synthase and its functions in plant resistance, Phytochem. Rev. 10 (2011) 397–412.
[21] A. Dob, A. Lakehal, O. Novak, C. Bellini, Jasmonate inhibits adventicious root initiation through transcriptional repression of CKX1 and activation of RAP2.6L transcription factor in Arabidopsis, J. Exp. Bot. 72 (2021) 7107–7118.
[22] J. Drabešová, L. Černá, H. Mašterová, P. Kolouškova, M. Potocký, et al., The evolution of the FT/FTL1 genes in Amaranthaceae and their expression patterns in the course of vegetative growth and flowering in Chenopodium rubrum, G3-Genes Genomes Genet. 6 (2016) 3065–3076.
[23] D.S. Favero, Mechanisms regulating PIF transcription factor activity at the protein level, Physiol. Plant. 169 (2020) 325–335.
[24] S. Fjellheim, S. Boden, B. Trevaskis, The role of seasonal flowering responses in adaptation of grasses to temperate climates, Front. Plant Sci. 5 (2014) 431.
[25] M. Frank, A. Cortleven, O. Novák, T. Schmülling, Root-derived trans-zeatin cytokinin protects Arabidopsis plants against photoperiod stress, Plant Cell Environ. 43 (2020) 2637–2647.

[26] I. Frébort, M. Kowalska, T. Hluska, J. Frébortová, P. Galuszka, Evolution of cytokinin biosynthesis and degradation, J. Exp. Bot. 62 (2011) 2431–2452.
[27] L. Fu, D. Tan, X. Sun Z., Z. Ding, J. Zhang, Transcriptional analysis reveals potential genes and regulatory networks involved in salicylic acid-induced flowering in duckweed (Lemna gibba), Plant Physiol. Biochem. 155 (2020) 512–522.
[28] V.C. Galvão, D. Horrer, F. Küttner, M. Schmid, Spatial control of flowering by DELLA proteins in Arabidopsis thaliana, Development 139 (2012) 4072–4082.
[29] M. Ghorbel, F. Brini, A. Sharma, M. Landi, Role of jasmonic acid in plants: the molecular point of view, Plant Cell Rep. 40 (2021) 1471–1494.
[30] D.G. Gilbert, Longest protein, longest transcript or most expression, for accurate gene reconstruction of transcriptomes? BioRxiv (2019), 829184.
[31] M.G. Grabherr, B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson, Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq dat, Nat. Biotechnol. (2011) 644–652.
[32] Q. Guo, Y. Yoshida, I.T. Major, K. Wang, K. Sugimoto, JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis, Proc. Natl. Acad. Sci. USA 114 (2018) E10768–E10777.
[33] B.J. Haas, A. Papanicolaou, M. Yassour, M. Grabherr, P.D. Blood, De Novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis, Nat. Protoc. 8 (2013) 1494–1512.
[34] Q. Hao, W. Wang, X. Han, J. Wu, B. Lyu, et al., Isochorismate-based salicylic acid biosynthesis confers basal resistance to Fusarium graminearum in barley, Mol. Plant Pathol. 19 (2018) 1995–2010.
[35] T. Hatayama, K. Takeno, The metabolic pathway of salicylic acid rather than of chlorogenic acid is involved in the stress-induced flowering of Pharbitis nil, J. Plant Physiol. 160 (2003) 461–467.
[36] R.W.P. Hiron, S.T.C. Wright, The role of endogenous abscisic acid in the response of plants to stress, J. Exp. Bot. 24 (1973) 769–780.
[37] D.H. Huson, A.F. Auch, J. Qi, S.C. Schuster, Megan analysis of metagenomic data, Genome Res. 17 (2007) 377–386.
[38] T. Izawa, What is going on with the hormonal control of flowering in plants? Plant J. 105 (2021) 431–445.
[39] S.T. Jackson, J.T. Overpeck, Responses of plant populations and communities to environmental changes of the late Quaternary, Paleobiology 26 (2000) 194–220.
[40] S.E. Jacobsen, A. Mujica, C.R. Jensen, The resistance of quinoa (Chenopodium quinoa Willd.) to adverse abiotic factors, Food Rev. Int. 19 (2003) 99–109.
[41] D.E. Jarvis, Y.S. Ho, D.J. Lightfoot, S.M. Schmöckel, B. Li, et al., The genome of Chenopodium quinoa, Nature 542 (2017) 307–312.
[42] S. Jin, J.H. Ahn, Regulation of flowering time by ambient temperature: repressing the repressors and activating the activators, New Phytol. 230 (2021) 938–942.
[43] J. Jung, H. Lee, J.Y. Ryu, C. Park, SPL3/4/5 integrate developmental aging and photoperiodic signals into the FT-FD module in arabidopsis flowering, Mol. Plant 24 (2016) 3320–3332.
[44] K. Kazan, J.M. Manners, MYC2: the master in action, Mol. Plant 6 (2013) 686–703.
[45] E. Kopylova, L. Noé, H. Touzet, SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data, Bioinformatics 28 (2012) 3211–3217.
[46] B. Krajnčič, J. Kristl, I. Janžekovič, Possible role of jasmonic acid in the regulation of floral induction, evocation and floral differentiation in Lemna minor L, Plant Physiol. Biochem. 44 (2006) 752–758.
[47] M. Krüger, O.A.J. Abeyawardana, C. Krüger, M. Juříček, H. Štorchová, Differentially expressed genes shared by two distinct cytoplasmic male sterility (CMS) Types of Silene vulgaris suggest the importance of oxidative stress in pollen abortion, Cells 9 (2020) 2700.
[48] F. Krüger, F. James, P. Ewels, E. Afyounian, B. Schuster-Boeckler, FelixKrueger/TrimGalore: v0.6.7, 2021, DOI via Zenodo (0.6.7). Zenodo. ⟨https://doi.org/10.5281/zenodo.5127899⟩.
[49] T. Kuroha, H. Tokunaga, M. Kojima, N. Ueda, T. Ishida, et al., Funtional analysis of lonely guy cytokinin-activating enzymes reveal the importance of the direct activation pathway in Arabidopsis, Plant Cell 21 (2009) 3152–3169.
[50] L. Li, Z. He, G.K. Pandey, T. Tsuchiya, S. Luan, Functional cloning and characterization of a plant efflux carrier for multidrug and heavy metal detoxification, J. Biol. Chem. 277 (2002) 5360–5368.
[51] W., A. Li, A. Godzik, Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences, Bioinformatics 22 (2006) 1658–1659.
[52] B. Li, N. Fillmore, Y. Bai, M. Collins, J.A. Thomson, et al., Evaluation of de novo transcriptome assemblies from RNA-Seq data, Genome Biol. 15 (2014) 553.
[53] Y. Li, D. Zhang, X. Zhang, L. Xing, S. Fan, et al., A transcriptome analysis of two apple (Malus × domestica) cultivars with different flowering abilities reveals a gene network module associated with floral transitions, Sci. Hortic. 239 (15) (2018) 269–281.
[54] C. Liang, C. Yang, Identification of ICE1 as a negative regulator of ABA-dependent pathways in seeds and seedlings of Arabidopsis, Plant Mol. Biol. 88 (2015) 459–570.
[55] H.K. Lichtenthaler, The stress concept in plants: an introduction, in: P. Csermely (Ed.) Stress of Life. Annals of the New York Academy of Sciences, 851, 1998, pp. 187–198.
[56] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold changes and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 550.
[57] C.P. MacMillan, C.A. Blundell, R.W. King, Flowering of the grass Lolium perenne. Effects of vernalization and long days on gibberellin biosynthesis and signaling, Plant Physiol. 138 (2005) 1794–1806.
[58] C. Martinez, E. Pons, G. Prats, J. Leon, Salicylic acid regulates flowering time and links defence response and reproductive development, Plant J. 37 (2004) 209–217.
[59] M.G. Mitchum, S. Yamaguchi, A. Hanada, A. Kuwahara, Y. Yoshioka, et al., Distinct and overlapping roles of two gibberellin 3-oxidades in Arabidopsis development, Plant J. 45 (2006) 804–818.
[60] K. Mouhu, T. Kurokura, E.A. Koskela, V.A. Albert, P. Elomaa, et al., The Fragaria vesca homolog of suppressor of overexpression of constans1 represses flowering and promotes vegetative growth, Plant Cell 25 (2013) 3296–3310.
[61] S. Nicolas-Freytes, M. Canelo, P.D. Cerdan, Regulation of flowering time: when and where? Curr. Opin. Plant Biol. 63 (2021), 102049.
[62] R. Nishiyama, Y. Watanabe, Y. Fujita, D.T. Le, M. Kojima, et al., Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis, Plant Cell 23 (2011) 2169–2183.
[63] S. Nitschke, A. Cortleven, T. Iven, I. Feussner, M. Havaux, et al., Circadian stress regimes affect the circadian clock and cause jasmonic acid-dependent cell death in cytokinin-deficient Arabidopsis plants, Plant Cell 28 (2016) 1616–1639.
[64] S. Nitschke, A. Cortleven, T. Schmülling, Novel stress in plants by altering the photoperiod, Trends Plant Sci. 22 (2017) 913–916.
[65] R.Ad.C. Oliveira, A.S. Andrade, D.O. Imparato, J.G.S. Lima, R.V.M. Almeida, et al., Analysis of Arabidopsis thaliana redox gene network indicates evolutionary expansion of class III peroxidase in plants, Sci. Rep. 9 (2019) 15741.
[66] G.K. Pandey, J.J. Grant, Y.H. Cheong, B.G. Kim, L. Li, et al., ABR1, and APETALA2-Domain transcription factor that functions as a repressor of ABA response in Arabidopsis, Plant Physiol. 139 (2005) 1185–1193.
[67] D.S.R. Patinarage, E. Asare, N. Maldonado-Taipe, E. Rey, N. Emrani, et al., Haplotype variations of major flowering time genes in quinoa unveil their role in the adaptation to different environmental conditions, Plant Cell Environ. 44 (2021) 2565–2579.
[68] R. Patro, G. Duggal, M.I. Love, R.A. Irizarry, C. Kingsford, Salmon provides fast bias-aware quantification of transcript expression, Nat. Methods 14 (2017) 417–419.
[69] I. Pokotylo, V. Kravets, E. Ruelland, Salicylic acid binding proteins (SABPs): the hidden forefront of salicylic acid signalling, Int. J. Mol. Sci. 20 (2019) 4377.
[70] A. Porri, S. Torti, M. Romera-Branchat, G. Coupland, Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods, Development 139 (2012) 2198–2209.
[71] S. Přerostová, B. Zupková, I. Petřik, J. Simura, I. Našinec, et al., Hormonal responses associated with acclimation to freezing stress in Lolium perenne, Environ. Exp. Bot. 182 (2021), 104295.
[72] S.A. Quarrieh, H.G. Jones, Effects of abscisic acid and water stress on development and morphology of wheat, J. Exp. Bot. 28 (1977) 192–203.
[73] H. Renssen, H. Seppä, X. Costa, H. Goose, D.M. Roche, Global characterization of the holocene thermal maximum, Quat. Sci. Rev. 48 (2012) 7–19.
[74] M. Riboni, M. Galbiati, C. Tonelli, L. Conti, Gigantea enables drought escape response via abscisic acid-dependent activation of the florigens and suppressor of overexpression of constans1, Plant Physiol. 162 (2013) 1706–1719.
[75] M. Romera-Branchat, E. Severing, C. Pocard, H. Ohr, C. Vincent, et al., Function divergence of the Arabidopsis florigen-interacting bZIP transcription factors FD and FDP, Cell Rep. 31 (2020), 107717.
[76] D.A. Samac, C.M. Hironaka, P.E. Yallaly, D.M. Shah, Isolation and characterization of the genes encoding basic and acidic chitinase in Arabidopsis thaliana, Plant Physiol. 93 (1990) 907–914.
[77] A. Samach, H. Onouchi, S.E. Gold, G.S. Ditta, Z. Schwarz-Sommer, et al., Distinct roles of constans target genes in reproductive development of Arabidopsis, Nature 288 (2000) 1613–1616.
[78] M. Seppey, M. Manni, E.M. Zdobnov, BUSCO: Assesing genome assembly and annotation completeness, in: M. Kollmar (Ed.) Gene Prediction. Methods in Molecular Biology, vol. 1962, Humana, New York, NY, 2019, pp. 227–245. ⟨https://doi.org/10.1007/978-1-4939-9173-0_14⟩.
[79] K. Shu, W. Zhou, F. Chen, X. Luo, W. Yang, Abcisics acid and gibberellins antagonistically mediate plant development and abiotic stress responses, Front. Plant Sci. 9 (2018) 416.
[80] L. Song, L. Florea, Rcorrector: efficient and accurate error correction for illumina RNA-seq reads, GigaScience 4 (2015) 48.
[81] H. Štorchová, J. Drabešová, D. Cháb, J. Kolář, E.N. Jellen, The introns in flowering locus T-like (FTL) genes are useful markers for tracking paternity in tetraploid Chenopodium quinoa Willd, Genet. Resour. Crop Evol. 62 (2015) 913–925.
[82] H. Štorchová, H. Hubáčková, O.A.J. Abeyawardana, J. Walterová, Z. Vondráková, et al., Chenopodium ficifolium flowers under long days without upregulation of flowering locus T (FT) homologs, Planta 250 (2019) 2111–2125.
[83] H. Štorchová, The evolution of the flowering locus T-like (FTL) genes in the goosefoot subfamily Chenopodioideae, in: P. Pontarotti (Ed.), Evolutionary Biology – A Transdisciplinary Approach, Springer International Publishing, Cham, 2020, pp. 325–335.
[84] N. Stortenbeker, M. Bemer, The SAUR gene family: the plant's toolbox for adaptation of growth and development, J. Exp. Bot. 70 (2019) 17–27.
[85] M. Subedi, E. Neff, T.M. Davis, Developing Chenopodium ficifolium as a potential B genome diploid model system for genetic characterization and improvement of allotetrapoid quinoa (Chenopodium quinoa), BMC Plant Biol. 21 (2021) 490.
[86] K. Takeno, Stress-induced flowering: the third category of flowering response, J. Exp. Bot. 67 (2016), 4952-4934.

[87] D. Tarkowska, M. Filek, J. Biesaga-Koscielniak, I. Marcinska, I. Macháčková, et al., Cytokinins in shoot apices of Brassica napus plants during vernalization, Plant Sci. 187 (2012) 105–112.
[88] P.B.H.J. Thomma, W.F. Broekaert, Tissue-specific expression of plant defensin genes PDF2.1 and PDF2.2 in Arabidopsis thaliana, Plant Physiol. Biochem. 36 (1998) 533–537.
[89] H. Tsuji, K. Taoka, K. Shimamoto, Florigen in rice: complex gene network florigen transcription, florigen activation complex, and multiple functions, Curr. Opin. Plant Biol. 16 (2013) 228–235.
[90] R. Vaňková, K. Kosová, P. Dobrev, P. Vítámvás, A. Trávníčková, et al., Dynamics of cold acclimation and complex phytohormone responses in Triticum monococcum lines G3116 and DV92 differing in vernalization and frost tolerance level, Environ. Exp. Bot. 101 (2014) 12–25.
[91] B.M. Walsh, D. Adhikary, P.J. Maughan, E. Emshwiller, E.N. Jellen, Chenopodium polyploidy inferences from salt overly sensitive 1 (SOS1) data, Am. J. Bot. 102 (2015) 533–543.
[92] L. Wang, K. Tsuda, W. Truman, M. Sato, L.V. Nguyen, et al., CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling, Plant J. 67 (2011) 1029–1041.
[93] Z. Wang, F.X. Wang, Y.C. Hong, J.J. Yao, Z.Z. Ren, et al., The flowering repressor SVP confers drought resistance in Arabidopsis by regulating abscisic acid catabolism, Mol. Plant 11 (2018) 1184–1197.
[94] C. Wasternack B., B. Hause, Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany, Ann. Bot. 111 (2013) 1021–1058.
[95] L. Yant, J. Mathieu, T.T. Dinh, F. Ott, C. Lanz, et al., Orchestration of the floral transition and floral development in Arabidopsis by the bifunctional transcription factor APETALA2, Plant Cell 22 (2010) 2156–2170.
[96] M. Yamada, K. Takeno, Stress and salicylic acid induce the expression of PnFT2 in the regulation of the stress-induced flowering Pharbitis nil, J. Plant Physiol. 171 (2014) 205–212.
[97] T. Yoshida, A. Christmann, K. Yamaguchi-Shinozaki, E. Grill, A.R. Fernie, Revisiting the basal role of ABA – roles outside stress, Trends Plant Sci. 24 (2019) 625–635.
[98] Z. Zhao, S.U. Andersen, K.K. Ljung, K.A. Dolezal, A. Miotk, et al., Hormonal control of the shoot stem-cell niche, Nature 465 (2010) 1089–1092.
[100] X. Zhao, S. Chen, S. Wang, W. Shan, X. Wang, et al., Defensive responses of tea plants (Camellia sinensis) against tea green leafhopper attack: a multi-omics study, Front. Plant Sci. 10 (2019) 1705.
[101] Y. Zhu, P. Huang, P. Guo, L. Chong, G. Yu, et al., CDK8 is associated with RAP2.6 and SnRK2.6 and positively modulates abscisic acid signalling and drought response in Arabidopsis, New Phytol. 228 (2020) 1573–1590.
[102] C. Zumajo-Cardona, N. Pabón-Mora, B.A. Ambrose, The evolution of euAPETALA2 genes in vascular plants: from plesiomorphic roles in sporangia to acquired functions in ovules and fruits, Mol. Biol. Evol. 38 (2021) 2319–2336.