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# The overexpression of the switchgrass (*Panicum virgatum* L.) genes *PvTOC1-N* or *PvLHY-K* affects circadian rhythm and hormone metabolism in transgenic *Arabidopsis* seedlings

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## Abstract

Switchgrass (*Panicum virgatum* L.) is a perennial C4 warm-season grass known for its high-biomass yield and wide environmental adaptability, making it an ideal bioenergy crop. Despite its potential, switchgrass seedlings grow slowly, often losing out to weeds in field conditions and producing limited biomass in the first year of planting. Furthermore, during the reproductive growth stage, the above-ground biomass rapidly increases in lignin content, creating a significant saccharification barrier. Previous studies have identified rhythm-related genes *TOC1* and *LHY* as crucial to the slow seedling development in switchgrass, yet the precise regulatory functions of these genes remain largely unexplored. In this study, the genes *TOC1* and *LHY* were characterized within the tetraploid genome of switchgrass. Gene expression analysis revealed that *PvTOC1* and *PvLHY* exhibit circadian patterns under normal growth conditions, with opposing expression levels over time. *PvTOC1* genes were predominantly expressed in florets, vascular bundles, and seeds, while *PvLHY* genes showed higher expression in stems, leaf sheaths, and nodes. Overexpression of *PvTOC1* from the N chromosome group (*PvTOC1-N*) or *PvLHY* from the K chromosome group (*PvLHY-K*) in *Arabidopsis thaliana* led to alterations in circadian rhythm and hormone metabolism, resulting in shorter roots, delayed flowering, and decreased resistance to oxidative stress. These transgenic lines exhibited reduced sensitivity to hormones and hormone inhibitors, and displayed altered gene expression in the biosynthesis and signal transduction pathways of abscisic acid (ABA), gibberellin (GA), 3-indoleacetic acid (IAA), and strigolactone (SL). These findings highlight roles of *PvTOC1-N* and *PvLHY-K* in plant development and offer a theoretical foundation for genetic improvements in switchgrass and other crops.

**Keywords** Circadian rhythm, LHY, Seedling development, Switchgrass (*Panicum virgatum* L.), TOC1

## Introduction

Switchgrass (*Panicum virgatum* L.) is a C4 perennial warm-season grass that thrives on marginal soils, making it an exemplary bioenergy crop due to its high-biomass production, high cellulose content, and broad environmental adaptability [1]. This plant also possesses high nitrogen use efficiency and can grow under rainfed conditions [2]. However, switchgrass

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experiences a slow-growing seedling stage, resulting in relatively low biomass yield during the planting year [3]. Without meticulous field management, the seedlings are often outcompeted by weeds, complicating large-scale cultivation [4]. In addition, rapid lignin accumulation post-flowering reduces the saccharification efficiency of the biomass [5]. Enhancing the quality and quantity of switchgrass biomass can be achieved by delaying flowering and extending the vegetative growth period, which is a highly desirable trait for a bioenergy crop [6]. Previous studies have indicated that the slow seedling development in switchgrass is linked to circadian rhythm genes such as *TIMING OF CAB EXPRESSION 1* [*TOC1*] and *LATE ELONGATED HYPOCOTYL* [*LHY*]), which also regulate the transition from vegetative to reproductive growth [7, 8], but little is known about the precise regulatory functions of these genes. Therefore, it is crucial to understand the specific functions of these genes in switchgrass to identify potential targets for crop breeding.

The genes *TOC1* (also known as *PSEUDO-RESPONSE REGULATOR 1* [*PRR1*]) and *LHY* are central components of the plant circadian rhythm system. Together with *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), they form a negative feedback regulatory loop that governs seedling morphogenesis [9]. *TOC1* is a part of the *PRR* family, whose members are expressed sequentially throughout the day, approximately every 2–3 h: *PRR9*, *PRR7*, *PRR5*, *PRR3*, and *TOC1* [10, 11]. Sequence similarity between these proteins is found in two specific regions: the N-terminal pseudoreceiver (PR) region and the C-terminal CCT domain [12]. *LHY* is part of the CCA1-like subfamily within the MYB-related family and includes a MYB domain, which typically has three conserved tryptophan residues, and a conserved amino acid motif (SHAQKFF) characteristic of the CCAl-like subfamily. *LHY* is generally co-expressed with *CCA1*, and these two proteins have some overlapping functions [13, 14].

Studies have demonstrated that *LHY* regulates carbon and nitrogen metabolism, directly influences seedling morphology, and modulates seedlings responses of seedlings to light and temperature [15, 16]. In addition, it contributes to yield hybrid vigor and is involved in mitochondrial retrograde signaling, impacting seedling development rates [17, 18]. *LHY* can self-regulate its expression, with structural overexpression inhibiting the transcription of both *CCA1*, resulting in significant disruptions to biological rhythms [19–21]. Furthermore, *LHY* and *CCA1* bind to the promoter of the evening-expressed gene *TOC1* to negatively regulate its expression. In this feedback loop, *TOC1* functions as a positive regulator since the morning activation of *CCA1*/*LHY* depends on *TOC1* [22]. However, overexpression of *TOC1* leads to reduced expression of *CCA1*/*LHY* because it acts as a general transcriptional repressor, negatively regulating not only *CCA1*/*LHY* but also several other clock-related genes [19, 23, 24]. This complexity highlights *TOC1*'s multifaceted role in the core circadian feedback pathway. Both *TOC1* and *LHY*, key circadian rhythm genes, exhibit diverse functions, and further investigation is needed to understand the effects of their functions and interactions on the plant development.

Circadian rhythm genes not only govern the circadian rhythms in plants but also orchestrate growth and development by regulating the expression of genes involved in plant hormone biosynthesis and signal transduction. Genome-wide studies using RNA extracted from seedlings have revealed that approximately 30% of all expressed genes are under the influence of the circadian rhythm [25–27]. Genes regulated by hormones such as abscisic acid (ABA), brassinosteroids (BRs), cytokinins (CKs), ethylene (ET), gibberellins (GAs), auxin, jasmonic acid (JA), and salicylic acid (SA) are particularly likely to be influenced by the circadian rhythm. In *Arabidopsis thaliana*, approximately 35–46% of circadian rhythm-regulated genes are associated with hormone signaling [28]. Chromatin immunoprecipitation studies have revealed that circadian-regulated proteins such as CCA1, TOC1, and PRRs bind to the promoters of hundreds of genes, including those influenced by plant hormones [29–32]. In this study, two *TOC1* genes and two *LHY* genes were identified in the switchgrass genome, designated as *PvTOC1-K* (Pavir.1NG350900), *PvTOC1-N* (Pavir.1KG385300), *PvLHY-K* (Pavir.6KG070500), and *PvLHY-N* (Pavir.6NG060600). The expression patterns of these genes were analyzed across multiple tissues under various stress and hormone treatments. Further, the roles of *PvTOC1-N* and *PvLHY-K* in seedling development were investigated in *Arabidopsis.* Results indicated that *PvTOC1-N* and *PvLHY-K* share partial homology with *AtTOC1* and *AtLHY*, respectively, and their overexpression can alter circadian rhythm and hormone metabolism in *Arabidopsis*. These findings offer new insights into the role of circadian genes in switchgrass, particularly in seedling development and vegetative growth, and help identifying strong candidates for future genetic enhancement of switchgrass and other crops.

## Materials and methods

### Plant materials and growth conditions

The *Arabidopsis* ecotype Columbia 0 (Col-0) was used for the experiments in this study. *Arabidopsis* seeds were incubated in sterile water at 4 °C for 3 days, then sown in soil and transferred to a growth chamber set at 25/22 °C day/night with a 16/8-h light/dark cycle (LD) for cultivation. The switchgrass variety used was the

lowland tetraploid 'Alamo' from our laboratory. 'Alamo' was planted in the field at Northwest A&F University in Yangling, Shaanxi, China (east longitude 108°–108° 7′, north latitude 34° 12′–34° 20′) to study gene expression in seedling tissues.

*PvTOC1* and *PvLHY* expression levels were detected in various switchgrass tissues (roots, stems, leaves, stem nodes, and leaf sheaths) at the E4 stage. Ears were collected when they reached 50 cm in length, and seeds were gathered during the late grain-filling period. All collected materials were flash-frozen in liquid nitrogen and stored at − 80 °C until further processing.

To study gene expression under different treatment conditions, 'Alamo' seedlings were grown hydroponically using Hoagland liquid medium. Seeds were first germinated for 7 days on filter paper in Petri dishes, then transferred to Hoagland liquid medium. Seedlings were grown in a growth chamber at 27/25 °C day/night with a 16/8-h light/dark cycle. Samples were collected from plants 36 days after germination treated with 100 μM gibberellic acid (GA), 50 μM abscisic acid (ABA), 200 mM sodium chloride (NaCl), or 20% (w/v) polyethylene glycol (PEG). Leaves from treated plants were collected at 6 h after treatment initiation. Untreated samples were collected simultaneously to serve as controls. The seedlings used in the photoperiod treatment were the same. Seedlings were acclimated to a 12-h light/12-h dark cycle at 22 °C for 10 days. After this period, they were switched to a continuous 12-h light/12-h light (LL) cycle. Samples were then collected every 4 h for 2 days, starting from the second full day under LL conditions [33].

#### Total RNA extraction and cDNA generation

Total RNA was isolated from plant samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration and purity of the extracted RNA were measured using a NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). cDNA was synthesized from the RNA using a PrimeScript™ RT Kit (6210A and RR047A; TaKaRa, Dalian, China) following the manufacturer's instructions.

#### Sequence analysis and isolation of *PvTOC1* and *PvLHY*

The full-length coding sequences (CDSs) of *PvTOC1* and *PvLHY* were amplified from cDNA synthesized from 'Alamo' seedlings. Primers specific to the two genes, incorporating *Xba*I and *Xho*I sites, were designed for one-step cloning based on the switchgrass reference genome available on the Phytozome database (https://phytozome-next.jgi.doe.gov/info/Pvirgatumvar_AP13HAP1_v6_1, Table A.1). Multiple sequence alignment was performed using Clustalx [34]. The isoelectric points of the proteins were calculated using the Expasy website (http://web.expasy.org/compute_pi/).

#### Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was extracted and cDNA was generated as previously described. Quantitative real-time PCR (qRT-PCR) was then performed on a QuantStudio™ 3 Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using the SYBR Premix Ex Taq™ II Kit (RR820A; TaKaRa) with three technical replicates. *EUKARYOTIC ELONGATION FACTOR 1α* (*PveEF-1α*) and *AtACTIN2* were used as positive controls to assess cDNA quality and as internal controls for gene expression normalization in switchgrass and *Arabidopsis*, respectively [35], using the $\Delta\Delta C_T$ method [36]. Primers were designed using Primer Premier 6 (http://www.premierbiosoft.com/primerdesign/index.html) and are listed in Additional file 1.

#### *Arabidopsis* transformation with *PvTOC1*- and *PvLHY*-overexpression vectors

The CDSs of *PvTOC1* and *PvLHY* containing *Xba*I and *Xho*I sites were inserted into the plant expression vector mCherry-pGreenII OE, which harbored the mCherry and Bar genes for selection. The resulting recombinant plasmids were then introduced into *Agrobacterium tumefaciens* strain GV3101, followed by transformation of *Arabidopsis* plants using the floral-dip method [37]. Transformed seeds emitting red light were selected using the LUYOR-3415RG fluorescent protein excitation light source or a fluorescence microscope with a 580 nm excitation wavelength. Transgenic plants were confirmed by spraying with a 0.05% glufosinate-ammonium solution and through PCR using primers specific for BarF/BarR. Phenotypic observations were conducted for 10 randomly selected plants per homozygous line. Three independent homozygous transgenic lines were generated for each gene.

#### *Arabidopsis* phenotypic analysis

Wild-type (WT) and transgenic seedlings at the 30-day-old stage were used in the phenotypic analysis. The expression of *PvTOC1-N* or *PvLHY-K* was detected and the number of rosette leaves were counted. Relative chlorophyll content in the leaves was measured using a SPAD-502 chlorophyll meter. Root tip cells of 6-day-old seedlings were observed via confocal microscopy. For this observation, *Arabidopsis* seeds were surface-sterilized, incubated at 4 °C in the dark for 3 days, then grown on ½×MS plates. After 4 days of growth, uniformly sized seedlings were selected and transferred to new ½×MS plates. After 2 days, root tips were stained with 10 μg/

mL propidium iodide (PI) for 2–3 min. The morphology of root apex cells was examined using a laser confocal microscope with an excitation wavelength: 543 nm. The length of the root apical meristem was measured, and the cell morphologies of the root apex, quiescent center, meristem, and mature zone were observed.

Wild-type (WT) and transgenic seedling root lengths were measured following stress treatments in ½×MS medium. The medium was supplemented with NaCl (150 mM, 200 mM, or 250 mM), mannitol (250 mM or 300 mM), ABA (30 μM, 60 μM, or 90 μM), or varying nitrogen levels (½×MS medium without nitrogen or with three times the nitrogen content). Surface-sterilized seeds were incubated on ½×MS medium for 4 days. Seedlings of uniform size were then selected and transferred to the treated medium for 7 days. Root length and survival rates were assessed in 20 seedlings per line, with untreated roots serving as controls for each treatment. Each treatment was performed in three biological replicates.

Seedling root lengths were also measured after growth in ½×MS medium supplemented with various hormones or hormone inhibitors. The seedlings were grown as described previously for the stress treatments. The hormone treatments included 1-naphthylacetic acid (NAA) at concentrations of 0.1 nM, 0.25 nM, or 1 nM; gibberellic acid (GA) at 30 μM, 60 μM, or 90 μM; *N*-(phenylmethyl)-9*H*-purin-6-amine (6-BA) at 0.05 μM, 0.5 μM, or 2 μM; and *rac*-GR24 (GR24) at 1 μM, 10 μM, or 100 μM. The hormone inhibitor treatments were 2,3,5-triiodobenzoic acid (TIBA) at 0.1 μM, 0.5 μM, or 2.5 μM; paclobutrazol (PAC) at 0.15 μM, 0.3 μM, or 0.6 μM; and lovastatin at 0.1 μM, 0.5 μM, or 2.5 μM.

### Chlorophyll fluorescence measurements

Well-grown leaves from WT and transgenic seedlings were wrapped in wet gauze for 15 min to become dark-adapted, and chlorophyll fluorescence parameters were then measured using a FluorCam multispectral fluorescence imaging system (Eco-tech, Beijing, China). To assess responses to oxidative stress, leaves were soaked in a 200 mM $H_2O_2$ solution in the dark for 2 h before additional chlorophyll fluorescence measurements. Post-oxidative stress treatment, the leaves were divided into two groups; one group was exposed to light for 0.5 h and the other for 1 h (both at 24,000 lx and 23 °C). Following the light treatment, the leaves were dark-adapted for 15 min before measuring chlorophyll fluorescence parameters. Measurements were taken from 20 leaves per line, with 3 biological replicates for each experiment.

Statistical analysis was performed on various photosynthetic parameters to assess plant photosynthetic capacity and oxidative stress-induced damage. The parameters analyzed included maximum quantum efficiency of photosystem II (QYmax), steady-state quantum efficiency of photosystem II (QY_Lss), steady-state light-adapted photochemical quenching (qL_Lss) which indicates fluorescence quenching caused by photosynthesis and steady-state non-photochemical quenching (i.e., fluorescence quenching caused by heat dissipation) (NPQ_Lss).

### Statistical analysis

The data were analyzed using SPSS Statistics version 22.0 (IBM, Armonk, NY, USA) to determine significant differences between genotypes and treatment groups. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were employed to calculate significance, with thresholds set at $p < 0.05$ and $p < 0.01$. Mean values from biological triplicate experiments were displayed and plotted using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) and Excel 2019.

## Results

### Identification and sequence analysis of *PvTOC1* and *PvLHY*

In a previous study analyzing transcriptomic data from switchgrass seedlings with different growth rates, we observed that *TOC1* was upregulated while *LHY* was downregulated in slow-growing seedlings [7]. The homologs of *PvTOC1* and *PvLHY* were found on the switchgrass K and N genomes. The amino acid sequence of PvTOC1s showed 93% similarity with OsTOC1 and 66% similarity with AtTOC1 (Additional file 2). The nucleic acid sequences of Pavir.1NG350900 (*PvTOC1-N*) and Pavir.1KG385300 (*PvTOC1-K*) had a 97% similarity, and their amino acid sequences had over 98% similarity. Due to the high similarity in both DNA and amino acid sequences, we selected *PvTOC1-N*, for further functional studies. The full-length coding sequence (CDS) of *PvTOC1-N* is 1566 bp long, encoding a protein of 522 amino acids with a molecular mass of 127.6 kDa and an isoelectric point of 4.97.

Similar to *TOC1*, switchgrass contained two homologous *LHY* genes on the K and N genomes: Pavir.6KG070500 (*PvLHY-K*) and Pavir.6NG060600 (*PvLHY-N*). Sequence analysis revealed a 42-bp deletion beginning at position 732 in the *PvLHY-N* sequence compared to *PvLHY-K* (Additional file 3). Both proteins contain a complete MYB domain, and the conserved SHAQKFF domain of the CCA1-like subfamily. The deletion in *PvLHY-N* does not cause a frameshift mutation, suggesting that both homologs may be functional. The amino acid similarity of PvLHYs proteins is approximately 90% with OsLHY and 74% with AtLHY (Additional file 3). *PvLHY-K* was selected for further study due to its higher sequence similarity with orthologs in rice.

The full-length CDS of *PvLHY-K* is 2163 bp long, encoding a protein of 720 amino acids with a molecular mass of 79.0 kDa and an isoelectric point of 6.12.

### *PvTOC1* and *PvLHY* expression in switchgrass

The expression profiles of the two *PvTOC1* genes from the Phytozome database were consistent with each other, showing high expression levels in both the floret and the vascular bundle (Additional file 4A). During the E4 stage in vegetative organs and in reproductive organs, expression analysis revealed that both *PvTOC1* genes were highly expressed in seeds, with *PvTOC1-K* showing higher expression (Fig. 1A). The genes most strongly co-expressed with each *PvTOC1* gene varied; Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis indicated that those co-expressed with *PvTOC1-K* were enriched in the "ribosome biogenesis in eukaryotes" and "circadian rhythm" pathways, while those with *PvTOC1-N* were enriched in the "RNA transport" and "ribosome biogenesis in eukaryotes" pathways (Additional file 5A). Both *PvLHYs* genes showed high expression in stems, leaf sheaths, and nodes, with *PvLHY-K* being more highly expressed than *PvLHY-N* (Fig. 1D, Additional file 4B). Similar to *PvTOC1*, the genes most strongly co-expressed with each *PvLHY* gene differed. For *PvLHY-K,* these genes were enriched in the "circadian rhythm" and "glyoxylate and dicarboxylate metabolism" pathways, while for *PvLHY-N*, they were enriched in the "circadian rhythm" and "carbon fixation in photosynthetic organisms" pathways (Additional file 5B).

Under various treatments, the *PvTOC1* genes exhibited similar expression profiles, and were both inhibited by ABA ($p < 0.05$, Fig. 1B). Conversely, both *PvLHY* genes were significantly induced by PEG treatment, with *PvLHY-K* expression being inhibited by GA ($p < 0.05$, Fig. 1E). As key components of rhythmic oscillations, these genes displayed consistent cycles of increased and decreased expression levels over time. Notably, *PvTOC1* and *PvLHY* genes showed opposing expression patterns over time, although the two copies of each gene had similar expression patterns (Fig. 1C, F). Overall, *PvLHY-K* was expressed at higher levels than *PvLHY-N*.

### Effects of *PvTOC1-N* or *PvLHY-K* overexpression on *Arabidopsis* growth and development

Based on the analysis of endogenous *PvTOC1* and *PvLHY* genes, the more highly expressed gene from each pair (*PvTOC1-N* or *PvLHY-K*) was transformed into *Arabidopsis* to study their effects on plant growth and development. Transgenic lines expressing *PvTOC1-N*

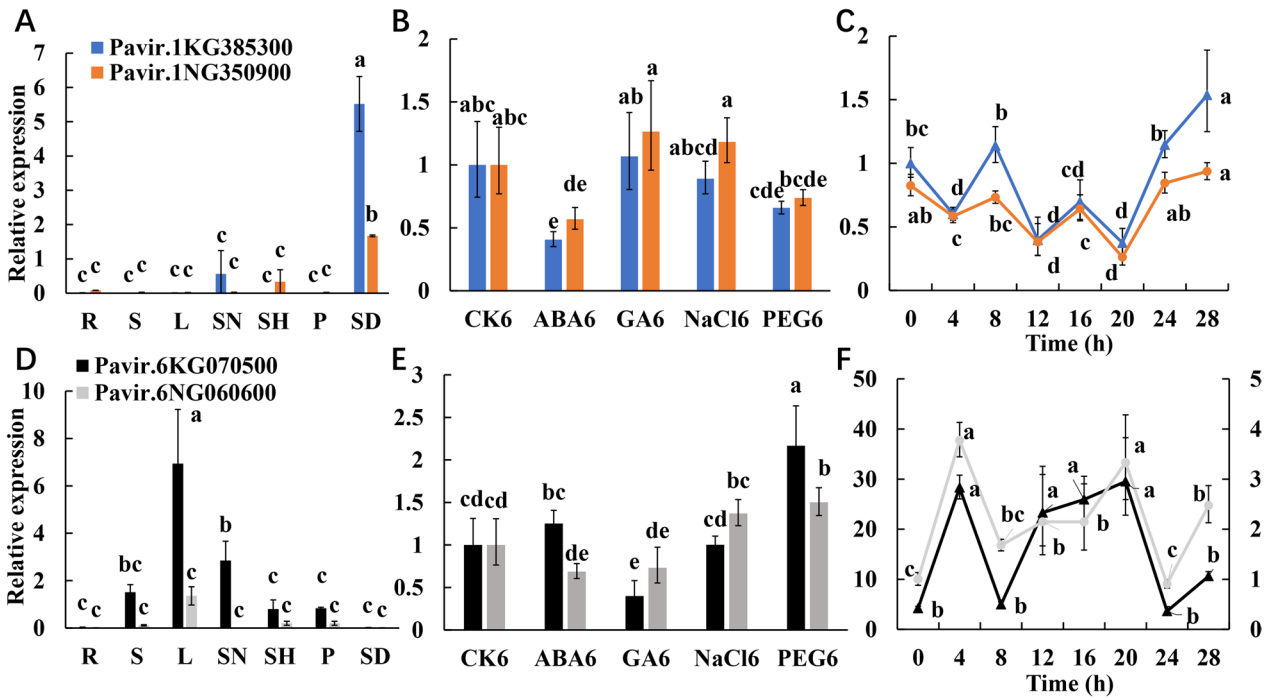


**Fig. 1** Expression pattern analysis of *TOC1s* and *LHYs* in switchgrass. **A** Expression patterns of *PvTOC1s* in multiple organs. **B** Expression patterns of *PvTOC1s* under various treatments for 6 h. **C** Expression patterns of *PvTOC1s* in switchgrass over time. **D** Expression patterns of *PvLHYs* in multiple organs. **E** Expression patterns of *PvLHYs* under various treatments for 6 h. **F** Expression patterns of *PvTOC1s* in switchgrass over time. R: root; S: stem; L: leaf; SN: stem node; SH: leaf sheath; P: panicle; SD: seed. The lowercase letters indicate statistically significant groups at $p < 0.05$

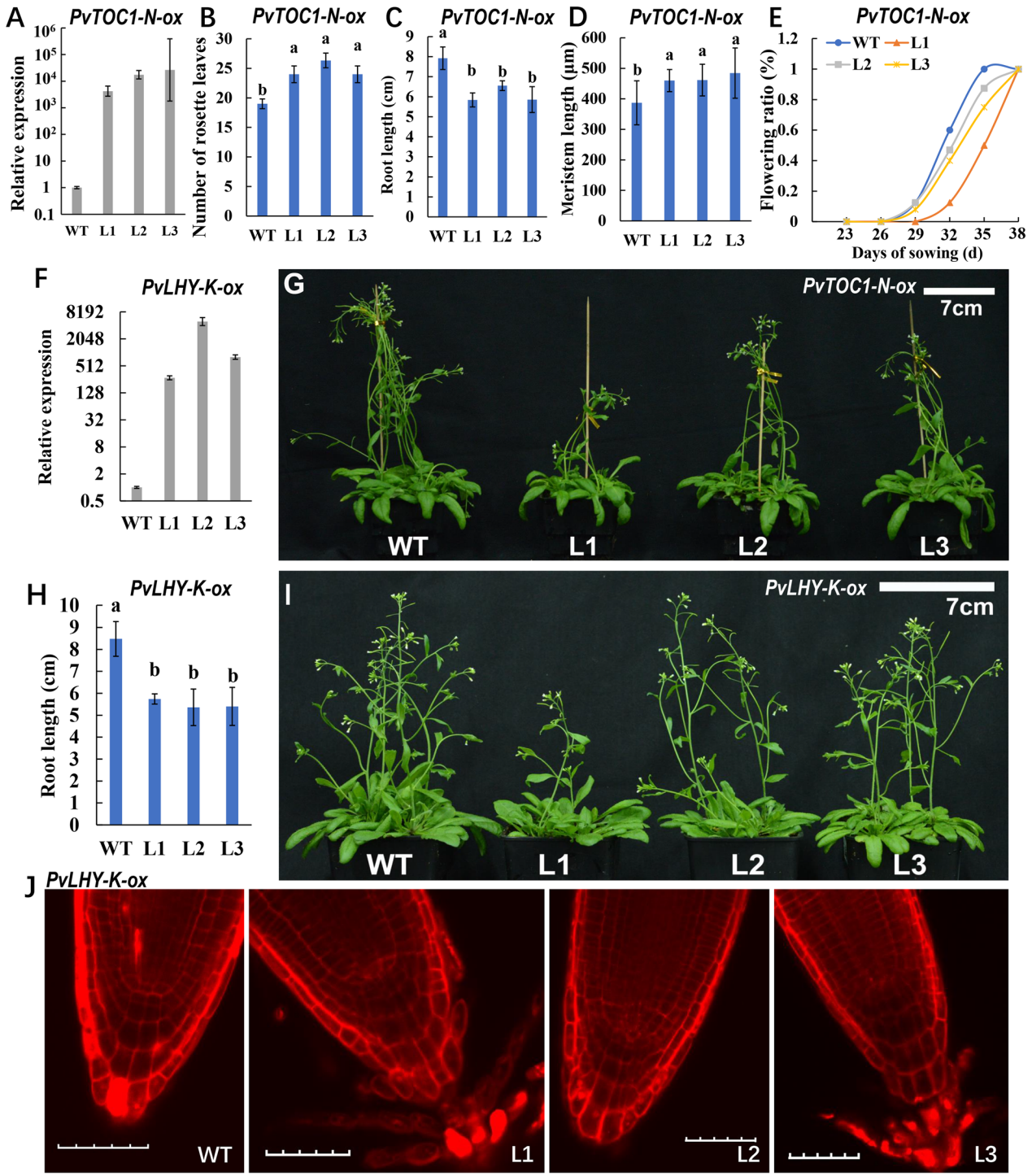


**Fig. 2** Phenotypic analysis of plants overexpressing *PvTOC1-N* and *PvLHY-K*. **A** *PvTOC1-N* expression in wild-type (WT) and *PvTOC1-N-ox* plants. **B** Rosette leaf number in WT and *PvTOC1-N-ox* plants. **C** Root lengths of WT and *PvTOC1-N-ox* plants grown on a ½ × MS culture medium. **D** Root meristem length in WT and *PvTOC1-N-ox* plants. **E** Flowering ratios of WT and *PvTOC1-N-ox* plants. **F** *PvLHY-K* expression in WT and *PvLHY-K-ox* plants. **G** Phenotypes of WT and *PvTOC1-N-ox* plants 45 days after sowing. Scale bar = 7 cm. **H** Root lengths of WT and *PvLHY-K-ox* plants on ½ × MS culture medium. **I** Phenotypes of WT and *PvLHY-K-ox* plants 45 days after sowing. Scale bar = 7 cm. **J** Root tip phenotypes of WT and *PvLHY-K-ox* plants. Scale bar = 50 μm. The lowercase letters indicate statistically significant groups at $p < 0.05$

or *PvLHY-K* were confirmed via PCR, showing high expression levels of these genes (Fig. 2A, F).

To investigate the effects of *PvTOC1-N* or *PvLHY-K* overexpression on *Arabidopsis* seedling growth and development, various phenotypic indicators were measured, including rosette leaf number, flowering time, root length, and root tip morphology (Fig. 2, Additional file 6). Plants overexpressing *PvTOC1-N* (*PvTOC1-N-ox*) had a significantly higher number of rosette leaves ($p < 0.05$, Fig. 2B) and flowered late, with one of the three transgenic lines flowering approximately 3 days later than the WT (Fig. 2E, F). In addition, *PvTOC1-N-ox* plants had significantly shorter roots after 7 days of growth on ½ × MS medium compared to the WT ($p < 0.05$, Fig. 2C). PI staining of the roots revealed that *PvTOC1-N-ox* plants had longer root apical meristems ($p < 0.05$, Fig. 2D, Additional file 6).

Compared to WT plants, those overexpressing *PvLHY-K* (*PvLHY-K-ox*) showed no significant differences in leaf shape, leaf number, or chlorophyll content, but they did bloom slightly later (Fig. 2I). *PvLHY-K-ox* plants had shorter roots than WT plants after 7 days of growth on ½ × MS medium ($p < 0.05$, Fig. 2H). PI revealed that the root apical meristem was longer in two of the transgenic lines (L1 and L3) compared to WT ($p < 0.05$, Additional file 6B), and the cell arrangement in the quiescent center of the root tip differed from that of WT in one transgenic line (L2) (Fig. 2J).

### *PvTOC1-N-ox* and *PvLHY-K-ox* plants responded differently to stress treatments compared to WT

We next evaluated the stress tolerance of *PvTOC1-N-ox* and *PvLHY-K-ox* plants by adding ABA, NaCl, mannitol, or nitrogen to the ½ × MS growth plates. Compared with the WT, *PvTOC1-N-ox* plants displayed longer roots under exogenous ABA treatment ($p < 0.05$ at 30 μM and 60 μM, Fig. 3A, C). However, we found no significant differences between WT and *PvTOC1-N-ox* plants under any of the remaining stress treatments tested.

*PvLHY-K-ox* plants were less sensitive to ABA than WT plants ($p < 0.05$ at 60 μM and 90 μM, Fig. 3D, F). On nitrogen-free ½ × MS medium (0 N), *PvLHY-K-ox* roots were longer than those of WT plants, indicating that *PvLHY-K-ox* plants were less affected

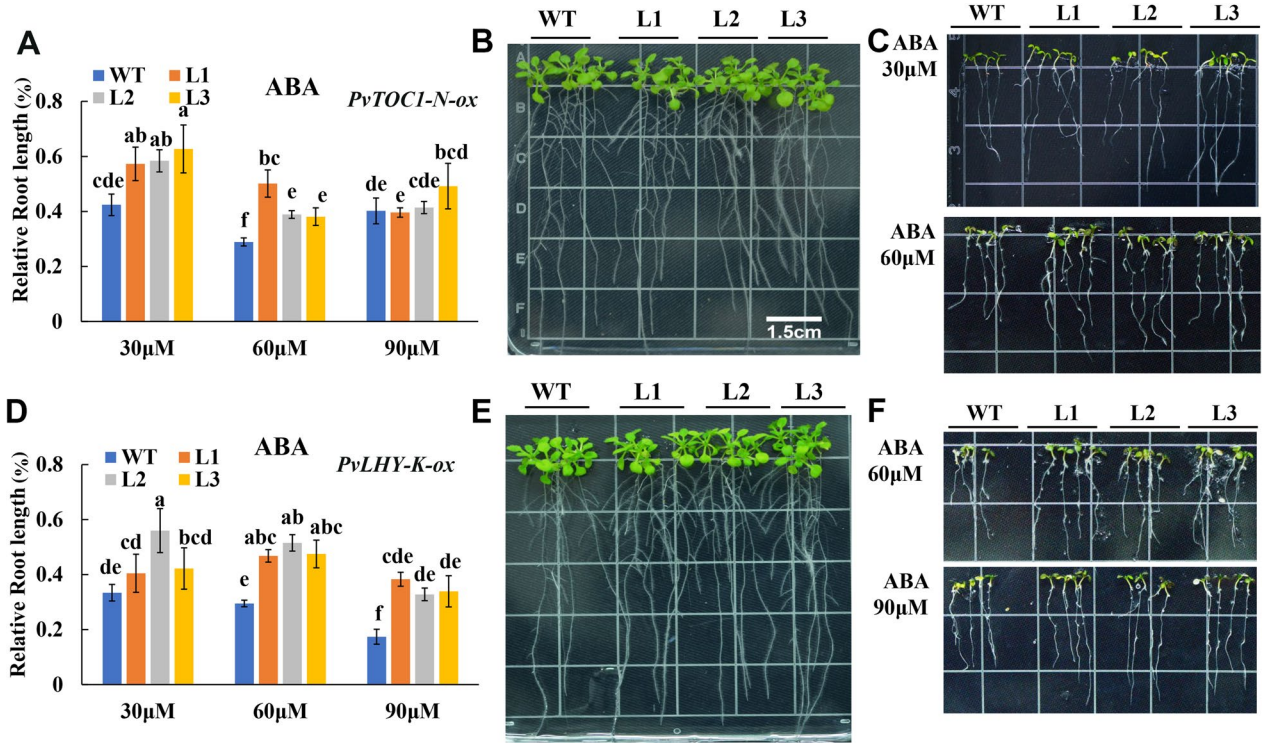


**Fig. 3** Stress tolerance in plants overexpressing *PvTOC1-N* and *PvLHY-K*. **A** Relative root length of wild-type (WT) and *PvTOC1-N-ox* plants after abscisic acid (ABA) treatment. **B** Root growth phenotypes of WT and *PvTOC1-N* transgenic lines on a ½ × MS culture medium. The diameter of the grid above the plate corresponds to 1.5 cm. **C** Phenotypes of WT and *PvTOC1-N-ox* plants after 30 μM or 60 μM ABA treatment. **D** Relative root lengths of WT and *PvLHY-K-ox* plants after ABA treatment. **E** Root growth phenotypes of WT and *PvLHY-K* transgenic lines on a ½ × MS culture medium. **F** WT and *PvLHY-K-ox* plant phenotypes after treatment with 60 μM or 90 μM ABA. The lowercase letters indicate statistical significance groups at $p < 0.05$. The relative root length corresponds to the ratio of the root length of the line on the stress treatment culture medium to the root length on the ½ × MS culture medium

by nitrogen-starvation stress (Additional file 7). When grown on medium with triple the standard nitrogen concentration, growth was severely inhibited in all genotypes. After 7 days of 250 mM NaCl treatment, both WT and *PvLHY-K-ox* plants died; however, under 150 mM NaCl conditions, *PvLHY-K-ox* plants had a higher survival rate than WT plants ($p < 0.05$, Additional file 7), indicating higher salt tolerance. There were no significant differences between *PvLHY-K-ox* and WT plants under mannitol treatment.

#### *PvTOC1-N-ox* and *PvLHY-K-ox* plants had low resistance to oxidative stress

Photosynthesis plays a crucial role in seedling growth. Therefore, we measured chlorophyll fluorescence in *PvTOC1-N-ox*, *PvLHY-K-ox*, and WT plants. Under normal growth conditions, QYmax was >0.8 in WT and *PvTOC1-N-ox* plants, indicating healthy growth. Compared to WT plants, qL_Lss was slightly higher in *PvTOC1-N-ox*, suggesting slightly stronger photosynthetic activity in the transgenic plants; NPQ_Lss values, indicating light protection in the seedlings, were low in both WT and *PvTOC1-N-ox,* suggesting high photosynthetic activity and healthy growth. Treatment with $H_2O_2$ for 2 h did not significantly alter the chlorophyll fluorescence parameters of WT and *PvTOC1-N-ox* plants. However, QYmax, QY_Lss, and qL_Lss were significantly reduced in $H_2O_2$-treated WT and *PvTOC1-N-ox* plants after 0.5 h or 1 h of light exposure. At this point, QYmax, QY_Lss, and qL_Lss were lower in *PvTOC1-N-ox*, indicating higher sensitivity to oxidative stress and decreased photosynthetic activity. The increased NPQ after light exposure suggested activation of light protection in the seedlings following oxidative stress. NPQ was also lower in *PvTOC1-N-ox* compared to WT plants, indicating slightly lower photoprotective ability in *PvTOC1-N-ox* (Fig. 4A).

Under normal growth conditions, the chlorophyll fluorescence parameters of *PvLHY-K-ox* plants were similar to those of *PvTOC1-N-ox* plants. Photosynthetic parameters in *PvLHY-K-ox* plants did not significantly change after 2 h of treatment with $H_2O_2$ in the dark. However, after the $H_2O_2$-treated WT and *PvLHY-K-ox* plants were exposed to light for 0.5 h or 1 h, QY_Lss decreased significantly ($p < 0.05$) and NPQ_Lss increased significantly ($p < 0.05$) in both lines. This indicated that the plants were under oxidative stress, and photoprotection was activated in response to the light treatment. After 1 h of light exposure, the chlorophyll fluorescence parameters of *PvLHY-K-ox* significantly differed from those of WT plants ($p < 0.05$, Fig. 4B), indicating severe oxidative stress in *PvLHY-K-ox*. Thus, *PvLHY-K* overexpression made seedlings more sensitive to oxidative stress and affected their photosynthetic efficiency and activity.

#### *PvTOC1-N-ox* and *PvLHY-K-ox* plants displayed distinct responses to hormones and their respective inhibitors

To understand the effects of *PvTOC1-N* or *PvLHY-K* overexpression on *Arabidopsis* seedling growth and development, WT and transgenic seedlings were treated with various hormones, including NAA, 6-BA, GA, or GR24; and hormone inhibitors, such as TIBA, lovastatin, or PAC. Following these treatments, we measured root growth in each respective seedling (Figs. 5 and 6). We found that NAA (0.1–1 nM) slightly promoted WT and *PvTOC1-N* root growth compared to untreated plants, but there were no differences between both (Additional file 8). In addition, our results showed that TIBA, which inhibits the polar transport of auxin, prevented *Arabidopsis* root growth [38, 39], while *PvTOC1-N-ox* plants were less sensitive to 0.5 μM TIBA than WT plants ($p < 0.05$, Fig. 5A). Treatment with exogenous 6-BA or lovastatin (an inhibitor of cytokinin synthesis) also inhibited *Arabidopsis* root growth in a concentration-dependent manner [40, 41]. Importantly, we found that *PvTOC1-N-ox* plants were less sensitive to 6-BA and lovastatin treatments than WT plants, and that this difference was significant at 0.05 μM 6-BA (Figs. 5B, G; Additional file 8B). Previous studies showed that the concentration of GA required to regulate root growth is lower than that necessary to regulate bud development, and 30–90 μM GA did not significantly promote root elongation [42]. We found that this concentration slightly promotes root elongation in *PvTOC1-N-ox* but not in WT plants (Fig. 5C). The GA biosynthesis inhibitor PAC also inhibited *Arabidopsis* root growth [43, 44], but our observations showed that *PvTOC1-N-ox* plants were less sensitive to PAC compared to WT plants ($p < 0.05$ at 0.15 μM, Fig. 5D, G). GR24, the most widely used synthetic strigolactone (SL), inhibited WT root growth at concentrations of 1–100 μM, but promoted *PvTOC1-N-ox* root growth, with an inverse correlation between GR24 concentration and root growth ($p < 0.05$ at 100 μM, Fig. 5E, G).

*PvLHY-K-ox* and *PvTOC1-N-ox* plants exhibited similar responses to hormone and inhibitor treatments. *PvLHY-K-ox* plants displayed a slight decrease in root lengths with increasing concentrations of NAA (Fig. 6A). GA treatment promoted root elongation in *PvLHY-K-ox* plants, although this promotion effect decreased with higher GA concentrations ($p < 0.05$ at 60 μM, Fig. 6B). *PvLHY-K-ox* plants exhibited reduced sensitivity to 6-BA/lovastatin treatment compared to WT plants, particularly at lower concentrations ($p < 0.05$, Fig. 6C,

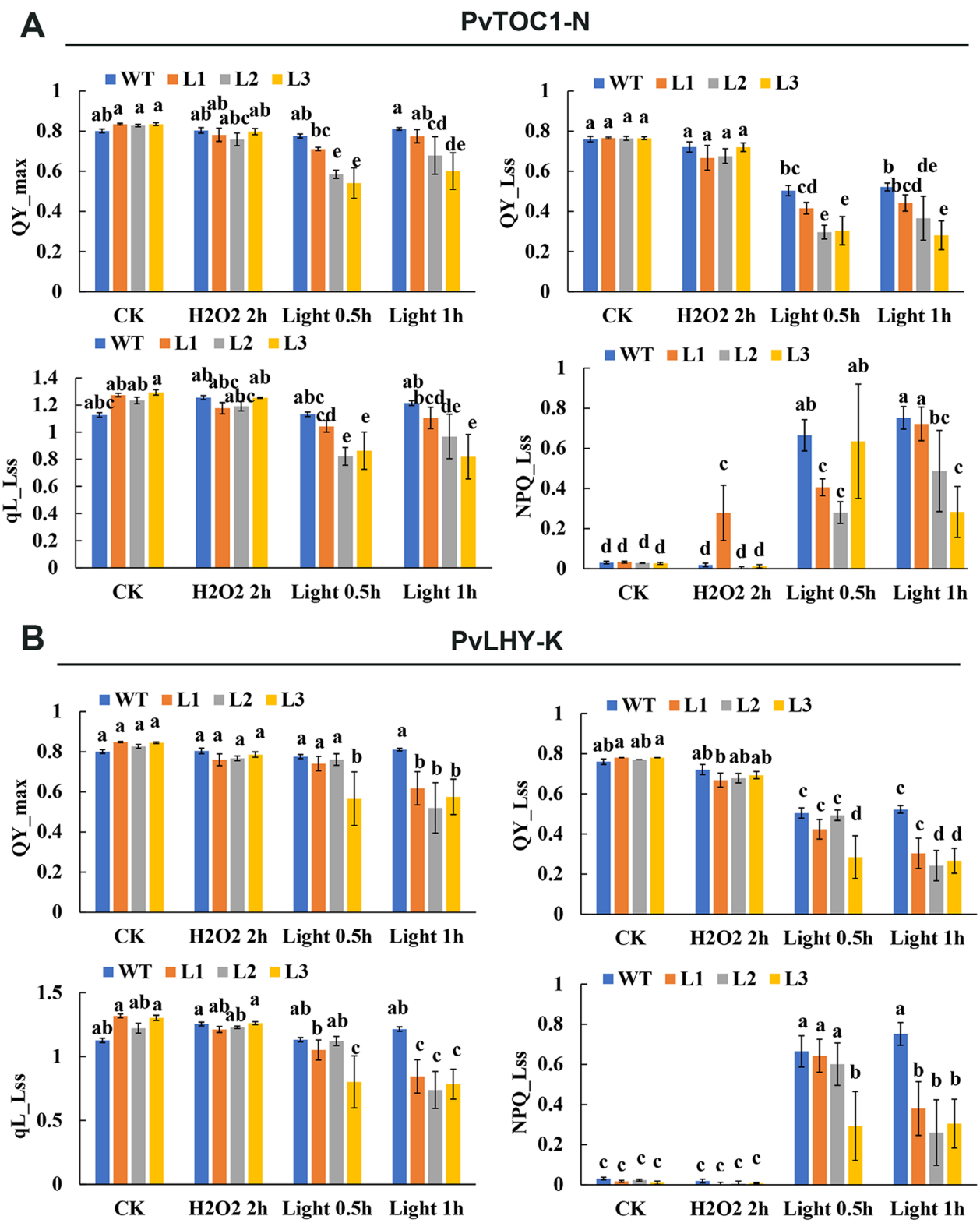


**Fig. 4** Chlorophyll fluorescence parameters in wild-type, *PvTOC1-N-ox* and *PvLHY-K-ox* plants. **A**, **B** Maximum quantum efficiency of photosystem II (QYmax), steady-state quantum efficiency of photosystem II (QY_Lss), steady-state light-adapted photochemical quenching (qL_Lss), and steady-state non-photochemical quenching (NPQ_Lss) in **A** WT and *PvTOC1-N-ox*; and **B** WT and *PvLHY-K-ox* plants subjected to oxidative stress conditions. The lowercase letters indicate statistically significant groups at $p < 0.05$

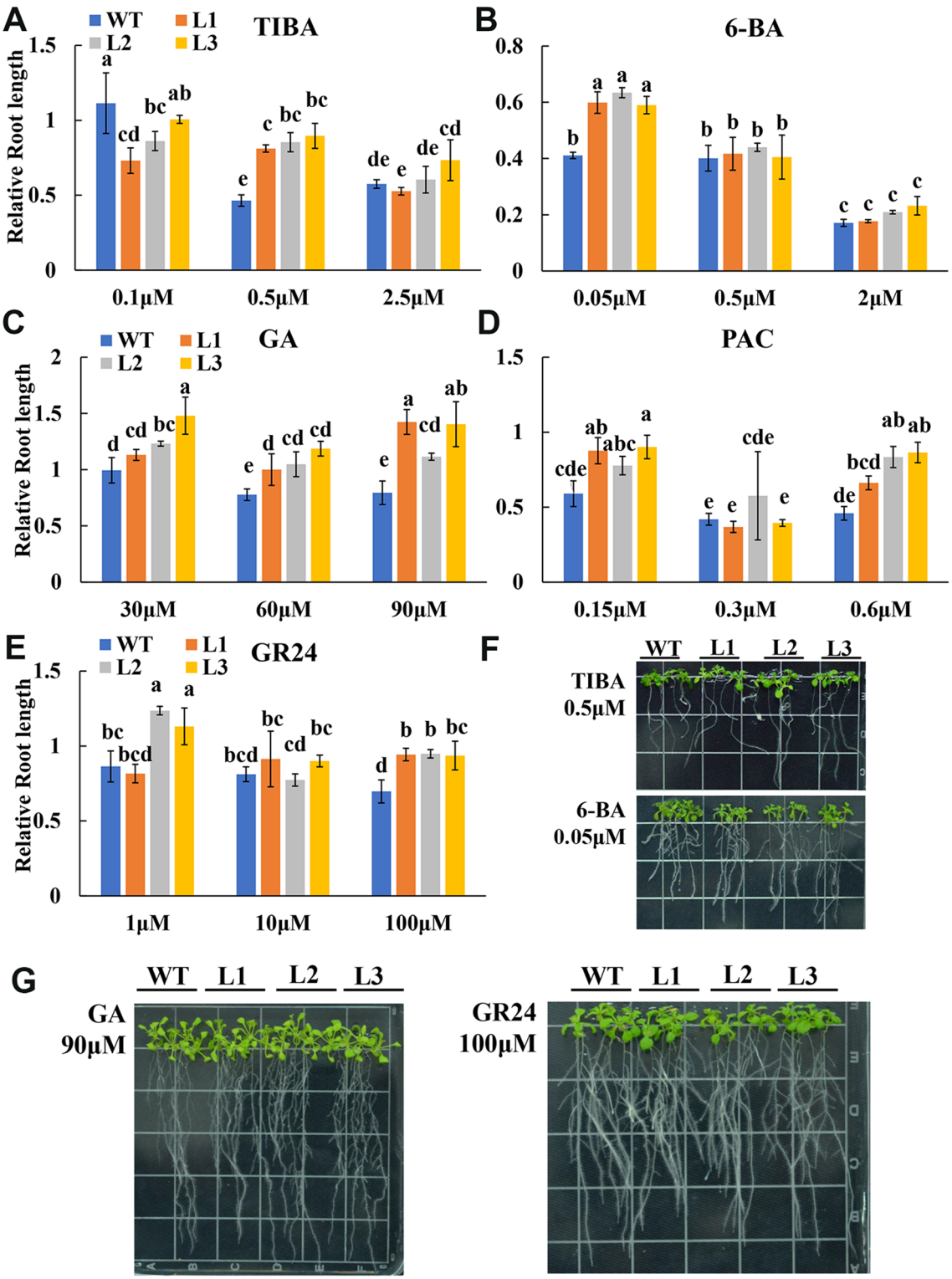


**Fig. 5** Effects of hormone and hormone inhibitor treatments on wild-type (WT) and *PvTOC1-N* overexpression (*PvTOC1-N-ox*) plant roots. **A**–**E** Comparison of relative root lengths between WT and *PvTOC1-N-ox* plants following treatment with **A** 2,3,5-triiodobenzoic acid (TIBA), **B** *N*-(phenylmethyl)-9*H*-purin-6-amine (6-BA), **C** gibberellin (GA), **D** paclobutrazol (PAC), or **E** *rac*-GR24 (GR24). **F**, **G** Root length phenotypes observed in WT and *PvTOC1-N-ox* plants treated with **F** 0.5 μM TIBA and 0.05 μM 6-BA or **G** 90 μM GA and 100 μM GR24. The lowercase letters indicate statistically significant groups at $p < 0.05$. Relative root length is defined as the ratio of root length measured on hormone or hormone inhibitor treatment culture medium to that observed on ½ × MS culture medium

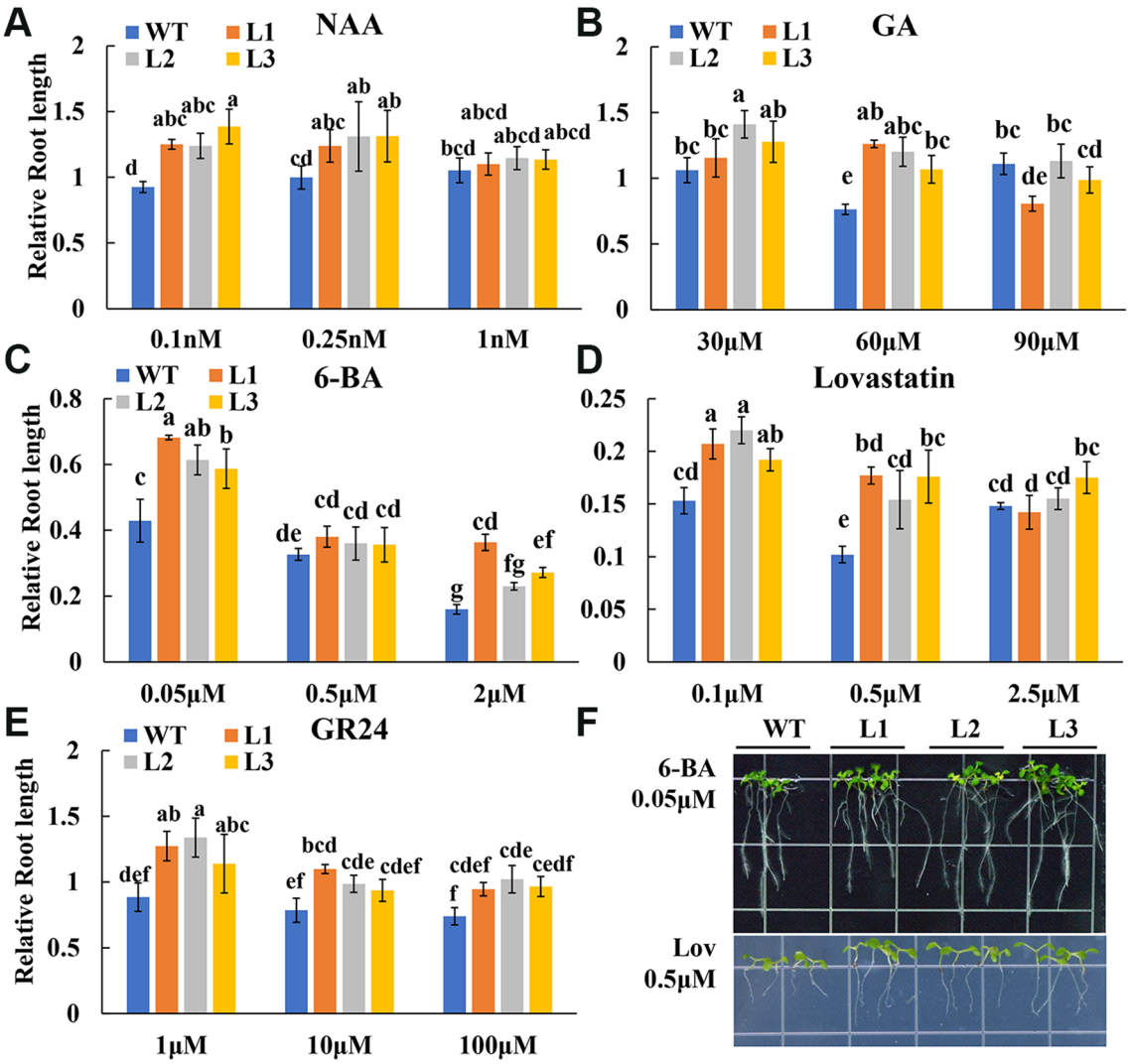


**Fig. 6** Effects of hormone and hormone inhibitor treatments on wild-type (WT) and *PvLHY-K* overexpression (*PvLHY-K-ox*) plants. **A**–**E** Relative root lengths of WT and *PvLHY-K-ox* plants following treatment with **A** 1-naphthylacetic acid (NAA), **B** gibberellin (GA), **C** *N*-(Phenylmethyl)-9*H*-purin-6-amine (6-BA), **D** lovastatin, or **E** *rac*-GR24 (GR24). **F** Root length phenotypes observed in WT and *PvLHY-K-ox* plants treated with 0.05 μM 6-BA and 0.5 μM lovastatin. Lowercase letters denote statistically significant groups at $p < 0.05$. Relative root length is defined as the ratio of the root length measured on the hormone or hormone inhibitor treatment culture medium to that observed on ½ × MS culture medium

D, F). Application of 1 μM GR24 significantly stimulated root growth in *PvLHY-K-ox* plants but not in WT plants ($p < 0.05$, Fig. 6E). No significant differences were observed between WT and *PvLHY-K-ox* plants following TIBA or PAC treatment (Additional file 8C, D).

### Effects of *PvTOC1-N* or *PvLHY-K* overexpression on hormone-related genes

The results above suggest that the transgenic overexpression lines responded differently to hormone or hormone inhibitor treatments compared to WT plants.

(See figure on next page.)

**Fig. 7** Expression of hormone- and circadian rhythm-related genes in wild-type (WT), *PvTOC1-N* overexpression (*PvTOC1-N-ox*) and *PvLHY-K* overexpression (*PvLHY-K-ox*) plants. **A**–**C** Expression of genes related to the **A** abscisic acid (ABA), **B** gibberellin (GA), and **C** auxin pathways in WT and *PvTOC1-N-ox* plants. **D**–**G** Expression of genes related to the **D** ABA, **E** auxin, **F** strigolactone, and **G** GA pathways in WT and *PvLHY-K-ox* plants. **H**, **I** Expression levels of *AtTOC1* and *AtLHY* in **H** WT and *PvTOC1-N-ox* and **I** WT and *PvLHY-K-ox* plants. The lowercase letters indicate statistically significant groups at $p < 0.05$

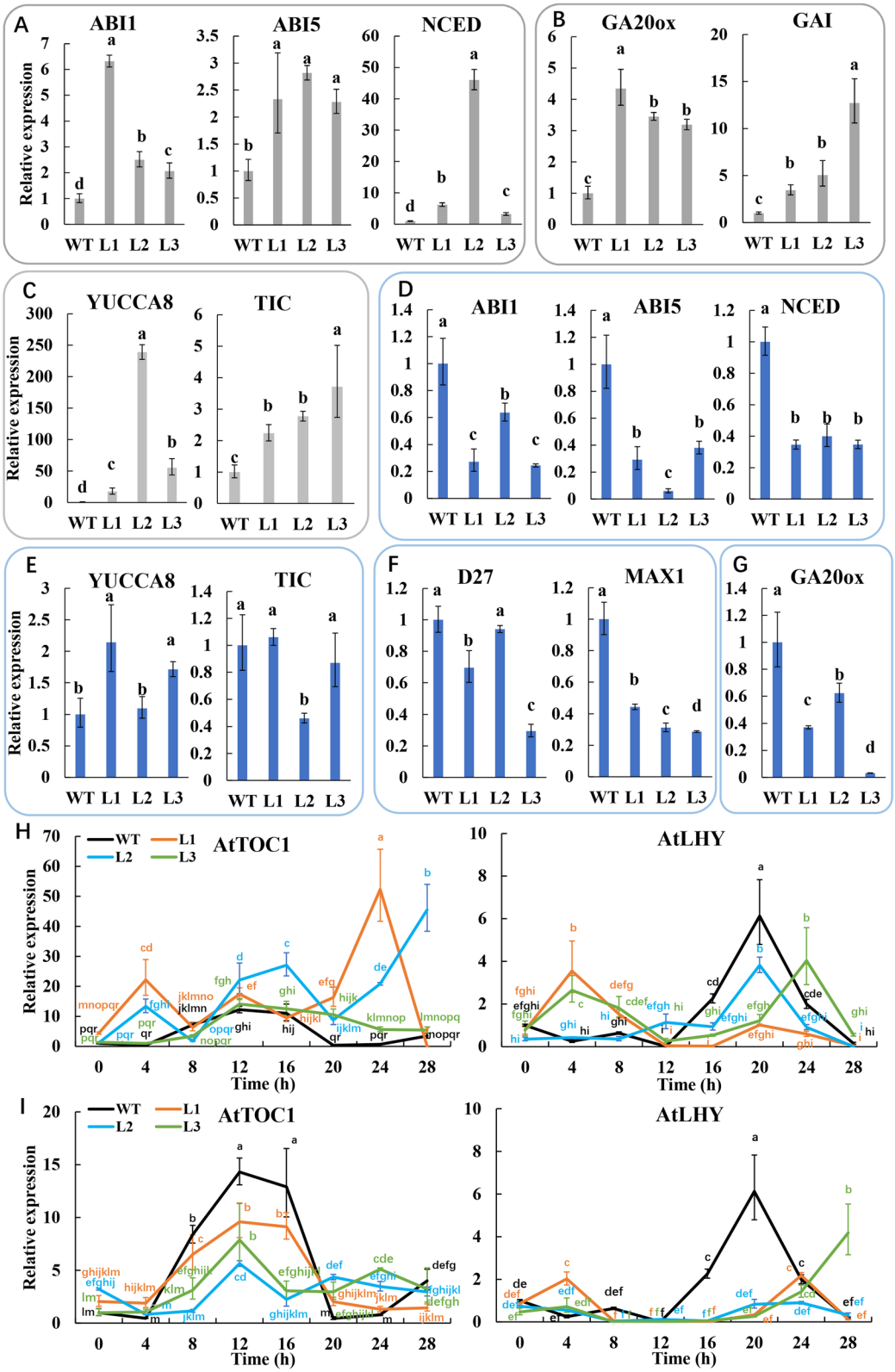


**Fig. 7** (See legend on previous page.)

Consequently, we investigated the effects of *PvTOC1-N* or *PvLHY-K* overexpression on genes involved in hormone biosynthesis and signal transduction pathways. In *PvTOC1-N-ox* plants, the rate-limiting ABA biosynthesis gene *9-CIS-EPOXY CAROTENOID DIOXYGENASE* (*NCED*) was upregulated ($p < 0.05$), along with negative ABA-response regulators *ABA-INSENSITIVE 1* (*ABI1*) and *ABA-INSENSITIVE 5* (*ABI5*) (Fig. 7A). In addition, *PvTOC1-N-ox* plants exhibited upregulation of a member of the DELLA family (*GA INSENSITIVE* [*GAI*]), a key enzyme in GA synthesis (*GA20 OXIDASE*, *GA20ox*), the auxin biosynthetic gene *YUCCA8*, and a gene controlling root meristem size, *TIME FOR COFFEE* (*TIC*), compared to WT plants ($p < 0.05$, Fig. 7B, C).

In *PvLHY-K-ox* plants, the expression of *NCED*, *ABI1*, *ABI5*, and *GA20ox*, were all significantly downregulated compared to the WT ($p < 0.05$, Fig. 7D, G). In addition, the canonical strigolactone (SL) biosynthetic enzymes *MORE AXILLARY GROWTH 1* (*MAX1*) (also known as *CYP711A*) and *DWARF 27* (*D27*) were downregulated in *PvLHY-K-ox* plants. *MAX1* showed significantly lower expression levels in all three transgenic lines compared to the WT (Fig. 7F). The differential expression of these hormone pathway-related genes may explain the varied responses to hormones observed in the transgenic lines.

#### Effects of *PvTOC1-N* or *PvLHY-K* overexpression on *Arabidopsis* circadian rhythm

Previous studies have established feedback regulation between *TOC1* and *LHY*/*CCA1*; *LHY* and *CCA1* negatively regulate *TOC1* expression, while *TOC1* overexpression inhibits *LHY* and *CCA1* expression [19, 24, 45]. To assess whether *PvTOC1-N* and *PvLHY-K* overexpression affected the circadian clock in *Arabidopsis*, WT and transgenic plants acclimated to a 12-h LD cycles were sampled every 4 h under LL conditions to measure *AtLHY* and *AtTOC1* expression. In *PvTOC1-N-ox* lines compared to WT, *AtTOC1* was highly expressed, whereas *AtLHY* was expressed at lower levels (Fig. 7H). A 180-degree phase shift was observed between *AtTOC1* and *AtLHY* expression. In *PvLHY-K-ox* lines, both *AtTOC1* and *AtLHY* were expressed at lower levels compared to WT (Fig. 7I). Thus, these two genes had different effects on the circadian rhythm when overexpressed.

## Discussion

Most organisms possess inherent time-keeping abilities; many circadian-related phenomena persist even when external time cues are absent, indicating that these phenomena arise from endogenous circadian rhythms [8]. Feedback regulation between *TOC1* and *LHY*/*CCA1*, identified as core components of the circadian rhythm in many plant species, plays a crucial role [20–22]. In bioenergy crops, such as switchgrass, the speed of seedling development and control over flowering time are essential for optimizing biomass production. A comprehensive understanding of the mechanisms governing plant circadian rhythms could be utilized to regulate reproductive growth, nutritional status, and ultimately achieve high-biomass crop varieties. It could also aid in improving seedling establishment in switchgrass. In this study, *TOC1* and *LHY* were identified as key circadian rhythm genes in switchgrass, and their functions in plant growth and development were analyzed in *Arabidopsis*.

#### The two *PvTOC1* and two *PvLHY* genes in switchgrass showed circadian-regulated expression patterns

Expression pattern analysis revealed that the two *PvTOC1* homologs exhibited similar expression patterns to each other, as did the two *PvLHY* homologs. However, comparison of the two *PvTOC1* genes to the two *PvLHY* genes showed opposing expression patterns. *PvTOC1* genes were highly expressed primarily in reproductive organs, such as florets and seeds, whereas *PvLHY* genes were highly expressed in vegetative organs (Fig. 1A, D, Additional file 4). Both *PvTOC1* and *PvLHY* genes showed circadian rhythmicity in switchgrass, with their expression levels upregulated and downregulated at opposite times of the day, consistent with previous findings that *LHY* negatively regulates *TOC1* expression (Fig. 1C, F) [24, 46]. Interestingly, the expression levels of *PvTOC1-K* and *PvLHY-K* were higher than their corresponding genes on the N chromosome. *PvTOC1* genes were repressed by ABA treatment, while *PvLHY* genes were induced by PEG treatment. The two *PvLHY*s responded differently to GA and NaCl treatments: *PvLHY-K* responded to GA treatment, whereas *PvLHY-N* responded to NaCl treatment (Fig. 1E). It suggests that *PvLHY-K* and *PvLHY-N* may share some overlapping circadian rhythm functions but also possess distinct roles in regulating switchgrass responses to different environmental factors. Each *PvLHY* and each *PvTOC1* gene exhibited a unique set of co-expressed genes, indicating functional differences between the homologs and suggesting that each gene may play different roles in developmental processes (Additional file 5).

#### *PvTOC1-N* or *PvLHY-K* overexpression affected *Arabidopsis* circadian rhythm

*CCA1* and *LHY* negatively regulate *TOC1* expression and can also regulate their own expression. Constitutive overexpression of either *LHY* or *CCA1* represses the transcription of both genes, leading to generalized circadian dysrhythmia [19–21]. Previously, it has been demonstrated that the overexpression of the maize (*Zea*

*mays*) genes *ZmCCA1b* or *ZmCCA1a* disrupts the circadian rhythm of *Arabidopsis* by inhibiting the expression of circadian rhythm-related genes [47, 48]. In this study, we analyzed the expression levels of *AtTOC1* and *AtLHY* in WT, *PvTOC1-N-ox*, and *PvLHY-K-ox* plants. We found that *PvLHY-K* overexpression significantly suppressed *AtTOC1* and *AtLHY* (Fig. 7I) indicating that *PvLHY-K* can regulate circadian rhythm in *Arabidopsis*. However, the effects of *TOC1* on *CCA1* and *LHY* are complex: *TOC1* is necessary to activate *CCA1* and *LHY* expression in the early morning, but *TOC1* overexpression in *Arabidopsis* inhibits *CCA1* and *LHY* expression [19, 22–24]. In this study, *PvTOC1-N* overexpression repressed *AtLHY*. Endogenous *AtTOC1* was expressed at higher levels in *PvTOC1-N-ox* than in WT plants (Fig. 7H, I). This might be attributed to reduced *TOC1* inhibition due to low *AtLHY* expression. Previous studies have shown that increased rhythmic *TOC1* expression delays the circadian rhythm, whereas constitutive *TOC1* overexpression completely disrupts rhythmicity [19]. In our study, *PvTOC1-N* overexpression in *Arabidopsis* did not completely abolish rhythmicity, likely due to the high rhythmic expression of endogenous *AtTOC1*.

#### *PvTOC1-N* or *PvLHY-K* overexpression altered hormone metabolism in *Arabidopsis*

The circadian rhythm directly influences plant hormone responses, and many hormone-related genes are regulated by circadian rhythm genes [49]. Therefore, we analyzed the expression levels of genes involved in hormone biosynthesis and signal transduction pathways in the transgenic overexpression lines. Genes related to the ABA, GA, and IAA pathways showed differential expression in *PvTOC1-N-ox* compared to WT plants, while genes related to the ABA, GA, and SL pathways exhibited differential expression in *PvLHY-K-ox* plants (Figs. 7, 8). Interestingly, both *PvTOC1-N-ox* and *PvLHY-K-ox* plants showed decreased sensitivity to exogenous ABA treatment compared to WT plants, consistent with previous studies [50–53]. Although plants overexpressing either gene exhibited differential expression of key genes in the ABA metabolic pathway, their expression profiles differed from each other and from WT plants (Fig. 8A). *LHY* can directly inhibit *NCED* expression [53], and overexpressing *PvLHY-K* significantly repressed *NCED*. *NCED* was upregulated in *PvTOC1-N-ox*, which may have been related to the low endogenous *AtLHY* expression. *TOC1* and *LHY* directly regulate separate sets of key genes in the ABA signaling pathway [50, 53], but they appeared to have different effects on *ABI1* and *ABI5*; *ABI1* and *ABI5* were downregulated in *PvLHY-K-ox* but showed a tendency to be upregulated in *PvTOC1-N-ox* plants. These findings suggest that *TOC1* and *LHY* play distinct roles in the ABA signaling pathway. *TOC1* may not only affect plant responses to ABA by regulating other ABA-related genes but also could indirectly affect ABA biosynthesis through interactions with *LHY*.

ABA and GA play crucial and often antagonistic roles in regulating plant growth and development [54, 55]. *ABI5* participates in an interaction with *DELLA* to regulate plant ABA homeostasis [56], and can also regulate *GA20ox* expression as part of the GA biosynthetic pathway [57]. In this study, *GAI* was found to be upregulated in *PvTOC1-N-ox* plants, whereas *GA20ox* was downregulated in *PvLHY-K-ox* plants (Fig. 8A). This suggests that overexpressing *PvTOC1-N* or *PvLHY-K* altered the circadian rhythm, influencing ABA and GA homeostasis in *Arabidopsis*.

In *PvTOC1-N-ox plants*, the auxin metabolism-related genes *YUCCA8* and *TIC* were upregulated, and the root meristem size (which can be altered by auxin accumulation) was increased. The enhanced root meristem size of *PvTOC1-N-ox* plants may result from auxin overproduction due to high expression of *YUCCA8* and *TIC*, which interact with *PIN* genes [58–60]. Moreover, *TIC* is known to interact with *MYC2* and negatively regulate JA signaling [61], suggesting that *TIC* upregulation may lead to changes in JA signaling in *PvTOC1-N-ox* plants.

#### *PvTOC1-N* or *PvLHY-K* overexpression reduced oxidative stress tolerance in *Arabidopsis*

*TOC1* acts as a molecular link between environmental information and circadian clock output; plants that overexpress *TOC1* have significantly enhanced light responsiveness [19]. Chlorophyll fluorescence assays showed that either *PvTOC1-N* or *PvLHY-K* overexpression enhanced photochemical quenching (qL_Lss) in *Arabidopsis* and improved photosynthetic characteristics, but the ability to cope with oxidative stress was significantly reduced. Following oxidative stress treatment, the photosynthetic characteristics and photoprotective capacity were notably decreased in transgenic lines. This finding aligns with previous studies indicating that altering the circadian rhythm can reduce plant adaptability to the external environment and decrease stress resistance [62]. Indeed, the importance of circadian rhythm has been demonstrated in phytoplankton and higher plants: organisms with circadian rhythms that match the external environment have competitive advantages [62–64]. Overexpression of *PvTOC1-N* or *PvLHY-K* altered the *Arabidopsis* circadian rhythm and external coordination, impacting the progression of seedling development. In switchgrass, *PvTOC1-N* and *PvLHY-K* play a role in circadian rhythm and receive environmental signals transmitted

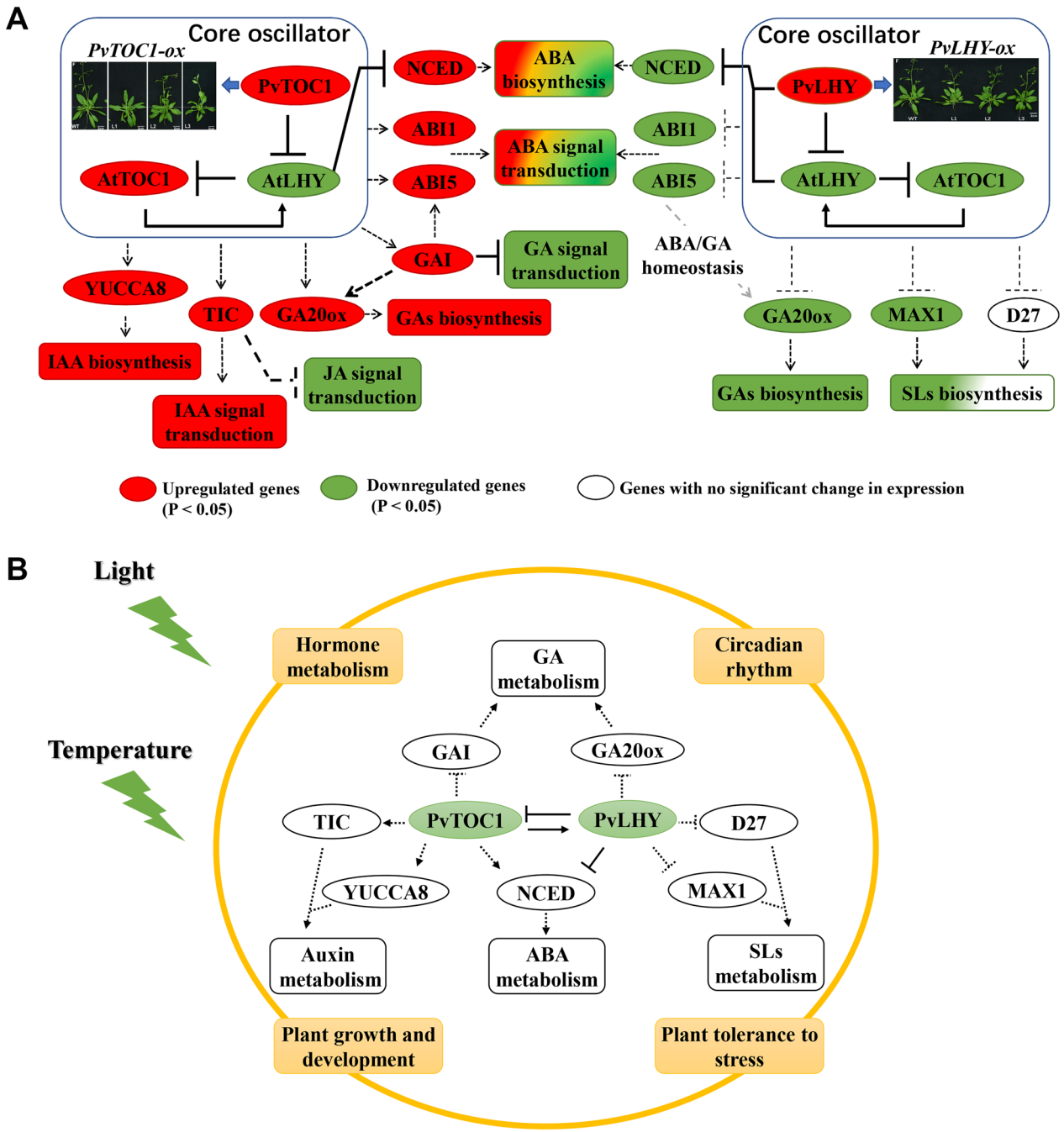


**Fig. 8** Gene interactions in wild-type (WT), *PvTOC1-N* overexpression (*PvTOC1-N-ox*), and *PvLHY-K* overexpression (*PvLHY-K-ox*) plants. **A** Gene expression levels in WT, *PvTOC1-N-ox*, and *PvLHY-K-ox* plants. **B** Functional patterns of *PvTOC1-N* and *PvLHY-K* expression in switchgrass. The arrows indicate the results of this study combined with previous studies

by receptors, influencing plant hormone homeostasis and stress resistance, thereby regulating seedling development and the flowering process (Fig. 8B).

In this study, it is evident that the functions of *PvTOC1-N* and *PvLHY-K* are relatively conserved in *Arabidopsis*, but there are also differences. Overexpression of *DhLHY* (*LHY* in *Doritaenopsis*) or *PbLHY* (*LHY* in Pear) in *Arabidopsis* significantly delays flowering; however, the inhibition of flowering caused by overexpression of *PvLHY-K* is not as pronounced (Fig. 2I) [65, 66]. This difference may

arise from distinct functions between *PvLHY-K* and *PvLHY-N*, or differences in flowering regulation between monocots and dicots. The enrichment analysis of co-expressed genes of the K chromosome group and N chromosome group reveals differences, and these genes respond to different stress treatment, indicating functional differences of *TOC1s* and *LHYs* on the two chromosome groups (Additional file 5, Fig. 1B, E). Therefore, further studies are needed to understand their functions in switchgrass.

## Conclusions

*PvTOC1* and *PvLHY* genes are core regulators of circadian rhythm in switchgrass, exhibiting opposing expression patterns. *PvTOC1* genes are highly expressed primarily in reproductive organs, while *PvLHY* genes are highly expressed in vegetative organs. Overexpressing *PvTOC1-N* or *PvLHY-K* in *Arabidopsis* resulted in delayed flowering, shorter roots, decreased resistance to oxidative stress, and lower sensitivity to hormone and hormone inhibitor treatment. Furthermore, *PvTOC1-N* or *PvLHY-K* overexpression disturbed the circadian rhythm and altered the expression of genes associated with hormone metabolism in *Arabidopsis*: in *PvTOC1-N-ox*, genes related to the ABA, GA, and IAA pathways were differentially expressed, whereas genes related to the ABA, GA, and SL pathways were differentially expressed in *PvLHY-K-ox* plants. *PvTOC1-N-ox* and *PvLHY-K-ox* plants exhibited the same response to exogenous ABA treatment, but overexpression of *PvTOC1-N* or *PvLHY-K* had different effects on genes involved in ABA biosynthesis and signal transduction pathways. These findings lay a theoretical foundation for the genetic improvement of switchgrass or other crops through the modulation of *TOC1* and *LHY* expression.

### Abbreviations

| | |
|---|---|
| ABA | Abscisic acid |
| ABI1 | ABA-INSENSITIVE 1 |
| ABI5 | ABA-INSENSITIVE 5 |
| ANOVA | One-way analysis of variance |
| BRs | Brassinosteroids |
| CCA1 | Circadian clock associated 1 |
| CDS | Coding sequence |
| CKs | Cytokinins |
| Col-0 | Columbia 0 |
| D27 | Dwarf 27 |
| eEF-1α | Eukaryotic elongation factor 1α |
| ET | Ethylene |
| GA | Gibberellin |
| GAI | GA insensitive |
| GA20ox | GA20 oxidase |
| GI | Gigantea |
| GR24 | *rac*-GR24 |
| IAA | 3-Indoleacetic acid |
| JA | Jasmonic acid |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| LD | Light/dark |
| LHY | Late elongated hypocotyl |
| LL | Light/light |
| MAX1 | More axillary growth 1 |
| NAA | 1-Naphthylacetic acid |
| NaCl | Sodium chloride |
| NCED | 9-*cis*-Epoxy carotenoid dioxygenase |
| NPQ_Lss | Steady-state non-photochemical quenching |
| PAC | Paclobutrazol |
| PEG | Polyethylene glycol |
| PI | Propidium iodide |
| PRR1 | Pseudo-response regulator 1 |
| PvLHY-K-ox | PvLHY-K overexpression |
| PvTOC1-N-ox | PvTOC1-N overexpression |
| qL_Lss | Steady-state light-adapted photochemical quenching |
| qRT-PCR | Quantitative real-time polymerase chain reaction |
| QYmax | Maximum quantum efficiency of photosystem II |
| QY_Lss | Steady-state quantum efficiency of photosystem II |
| SA | Salicylic acid |
| SL | Strigolactone |
| TOC1 | Timing of cab expression 1 |
| TIBA | 2,3,5-Triiodobenzoic acid |
| TIC | Time for coffee |
| WT | Wild type |
| 6-BA | *N*-(Phenylmethyl)-9*H*-purin-6-amine (6-BA) |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13068-024-02574-6.

Additional file 1. The list of primers used in this study.

Additional file 2. Sequence alignment of TOC1 proteins.

Additional file 3. Sequence alignment of LHY proteins.

Additional file 4. Expression patterns of *PvTOC1s* and *PvLHYs* in different switchgrass plant organs, based on the data retrieved from the Phytozome database.

Additional file 5. Kyoto Encyclopedia of Genes and Genomesbiochemical pathway enrichment of genes showing the strongest co-expression levels with *PvTOC1s* or *PvLHYs*. A, B. KEGG biochemical pathway enrichment analysis of genes showing the strongest co-expression levels with *PvTOC1s* or *PvLHYs*. *PvTOC1* and *PvLHY* expression levels in various switchgrass tissues were obtained from the Phytozome database, along with the most strongly co-expressed genes.

Additional file 6. Root phenotypes of *PvTOC1-N-ox* plants and *PvLHY-K-ox* plants. A. Root tip phenotypes and root apical meristem length of wild-typeand *PvTOC1-N-ox*. Scale bars correspond to 50 μm and 100 μm, respectively. B. Root apical meristem length of WT and *PvLHY-K-ox*.

Additional file 7. Relative root lengths of wild-type *PvLHY-K-ox* plants under NaCl and Nitrogen treatment conditions.

Additional file 8. Relative root lengths of wild-type, *PvTOC1-N-ox* and *PvLHY-K-ox* plants under multiple treatment conditions. A, B. Relative root lengths of WT and *PvTOC1-N-ox* plants under1-naphthylacetic acid and lovastatin. C, D. Relative root lengths of WT and *PvLHY-K-ox* plants under 2,3,5-Triiodobenzoic acid and paclobutrazol treatments. The lowercase letters indicate statistically significant groups at $p < 0.05$. The relative root length corresponds to the ratio of the root length of the line on the hormone or hormone inhibitor treatment in the culture medium to the root length on the ½ × MS culture medium.

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### Author contributions

Y.X. made substantial contributions to the conception and design of this study. S.Z. experimented and drafted the manuscript. C.Z. and F.S. participated in its design and also revised the manuscript. J.M. and W.W. performed the

RT-PCR experiment and improved the data. All the authors have read and approved the manuscript.

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**Data Availability**
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

## Declarations

**Competing interests**
The authors declare no competing interests.

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