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Research Article

Keywords: Photoperiod, Microgravity, F lowering, HSP17.4, FLOWERING LOCUS T, Transcriptome

Posted Date: April 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-400169/v1

Involvement of *FLOWERING LOCUS T* in microgravity response of *Arabidopsis thaliana* plants under long- and short-day conditions

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Number of words in main text: 4341;
Number of figures: 9
Number of tables: 2
Number of supplementary tables:11

Abstract

Microgravity have an impact on growth and development of higher plants in space at both vegetative stage and reproductive stage. A great deal of information has been available on the vegetative stage in space, but relatively little is known about the influence of microgravity on plants at the reproductive stage. In this study, we constructed a transgenic *Arabidopsis thaliana* plants expressing flowering control gene, *FLOWERING LOCUS T* (*FT*), together with green fluorescent protein gene(*GFP*) under control of a heat shock-inducible promoter (*HSP17.4*), by which we induced *FT* expression inflight through remote controlling heating shock treatment. Inflight photography data showed that induction of *FT* expression in plants in space could counteract the impact of microgravity and promote flowering. Whole-genome microarray analysis of gene expression changes in leaves of wild-type and these transgenic plants grown under different photoperiod conditions in space indicated that the function of the photoperiod-related microgravity response genes are mainly involved in protein synthesis and post-translation protein modulation, notably protein phosphorylation. In addition, changes of circadian component gene expression in response to microgravity under different photoperiod indicated that role of circadian oscillator could act as integrators of microgravity response and photoperiodic signals in Arabidopsis plant grown in space.

Key words: Photoperiod; Microgravity; Flowering; *HSP17.4*; *FLOWERING LOCUS T*; Transcriptome.

Introduction

Microgravity by spaceflight could cause an impact on growth and development of higher plants at both the vegetative stage and the reproductive stage. A great deal of information is available on the vegetative stage in space. For example, alteration of auxin polar transport in etiolated pea seedlings and maize coleoptiles in space (Ueda et al., 2000), inhibition of cell division and mitosis as well as significant karyological disturbances in root-tip cells of oat, mung bean and sunflower seedlings grown in space, modification of cell wall metabolism (Krikorian and O'Conner, 1984; Rasmussen et al., 1994, Sago et al., 2002). Reduction in fresh weight of shoot and photosynthetic function of wheat plants grown onboard space shuttle (Tripathy et al., 1996). The plants grown in space was often smaller than comparably aged ground controls (Kiss et al., 2000; Paul et al., 2012; Wang et al., 2018), while others grew faster in space (Matía et al., 2010; Hoson et al., 2014). However, relatively little is known about the influence of microgravity on plants at the reproductive stage. Some early experiments reported failure in seed formation under spaceflight conditions (Nechitatio and Maskinsky, 1993; Kuang et al., 1996; Strickland et al., 1997; Soga et al., 1999; Levinskikh et al, 2000; Campbell et al., 2001). As hardware improvement, the completion of the seed-to-seed cycle of several plants in space were reported (Stankovic, 2001; Link et al., 2003; Sychev et al., 2003; Link et al., 2014). These results indicate that plants could adapt to microgravity for seed-to-seed growth, but reproductive fitness is often reduced in space (De Micco, et al., 2014). Interruption of the reproductive process, delay in completion of single reproductive phase, lowering of reproductive success and alteration of seed reserves are still major bottlenecks to maximize the efficiency of plant growth and reproduction in space and to be used to support life in long-term

manned missions (Hoson, 2014; reviewed by Zheng 2018).

The reproductive success of plants is often dependent on their flowering time being adapted to the growth environment. A number of studies suggest that both biotic and abiotic stress factors play key roles in controlling to alter flowering time in plants. For example, plants often acceleration the flowering process under drought stress (Sherrard and Maherali, 2006;Galbiati et al., 2016) and delays flowering time by salt stress (Achard et al., 2006; Ma et al., 2015). Heat and cold stress can also have a dramatic effect on flowering. In addition, the other stresses, such as, nutrient, sugar budget, geomagnetic field and simulated microgravity, have significant effects on plant development including flowering process time (Lee et al., 2008; Posé et al., 2013; Agliassa et al., 2018; Xie et al., 2020). Increasing evidences document that microgravity is a novel stress for plants grown in space (Paul et al., 2001; De Micco et al., 2014; Zhang et al., 2015; Karahara et al., 2020), which cause changes at the physiological, morphological and molecular levels, including altered transcription patterns of many genes. In the space-grown Mizunna, a total of 20 in 32 ROS oxidative maker genes were up-regulated, including common genes response to abiotic and biotic stress (Sugimoto et al., 2014). In Arabidopsis culture cells grown in space, genes associated with heat shock, salt, drought, metals, wounding, phosphate, ethylene, senescence, terpenoids, seed development, cell walls, photosythesis, and auxin were up-regulated by five fold in comparison with their ground controls (Paul et al., 2012; Kwon et al., 2015). The endogenous systems that measure day length was found to interact with stress responses and override interpretation of the signals in plants on ground (Becker et al., 2005). It is however unclear how the photoperiod influence the signals in plants in space.

The developmental rate of Arabidopsis plants on ground is directly related to

daylength, because Arabidopsis is a long-day (LD) plant, an increase in photoperiod results in an increase in development rate. How photoperiod affect plant development in space has yet known. No space experiments had been carried out to compare the effects of different photoperiod on plant growth and development so far. To exmamine effects of photoperiod signals on the microgravity response of plants in space, we conducted the space experiment by growing Arabidopsis plants under the LD and the short-day (SD), respectively, on board the Chinese recoverable satellite SJ-10. A transgenic plants expressing *FLOWERING LOCUS T* (*FT*) and the reporter gene green fluorescent protein (GFP) under control of a heat shock-inducible promoter (*HSP17.4*) was constructed to invesitigate the role of FT in integation of microgravity into photoperiod controlling floral pathway. In addition, a full-genome analysis of RNA derived from the leaves of Arabidopsis plants in space under the LD and the SD, respectively, were also performed in comparison with their controls on ground.

Results

Gene switch for flowering induction in space experiment

To address the effects of microgravity on the *FT* regulating flowering pathway, we generated transgenic Arabidopsis plants that stably harbor *FT* gene and *GFP* gene, under control of the *HSP17.4* promoter, which have been utilized to establish a highly efficient regulatory system in plants through heat-shock treatment (Czarnecka et al., 1990). Under the LD condition, this *pHSP*::*FT, pHSP*:*GFP* (FG) transgenic plants grown on ground at normal temperature (22±2℃) exhibit a phenotype like wild-type (WT), except the size is slightly smaller (Supplementary Fig. S1 and S2). In the absence of heat shock (HS), no GFP fluorescence in leaves of FG plants was observed (Supplementary Fig. S1C and G), while heating at 37°C

for 30 min resulted in a clear induction of GFP expression in the leaves of FG plants (Supplementary Fig. S1D and H), but didn't in the leaves of WT plants (Supplementary Fig. S1B and F). Early floral development and apparent increase of *FT* gene expression in FG plants under the SD conditions (Supplementary Fig. S2) were also observed after HS induction, while the control plants (WT and *pHSP*::*GFP*) with or without exposure HS treatments showed negligible levels of background *FT* expression and little GFP fluorescent under the same condition (Supplementary Fig.S1; Supplementary Fig. S2G).

For space experiment on the satellite SJ-10, seeds of WT and FG were germinated and grown in the root modules on ground under the LD condition for 20 days (Fig. 1E ; corresponding to stage 1.06, Boyes et al., 2001). At this age, the plants had formed about 5-6 rossette leaves (Fig.1E and F), when they were loaded into the plant growth unit (PGU) less than 24h prior to take off. Under the LD condition, floral shoots of WT plants on ground appeared at day 4 after satellite launched, while plants in space initiated floral shoots on day 6 (Fig. 2A and B). For FG plants, floral shoots appeared at day 2 under the LD on ground were earier than those in space at day 4 (Fig. 2A and C), slightly earier that WT under the same condition. No apparent GFP signal was detected in leaves of WT and FG plants before 37°C HS induction (Fig.3 D and E). A strong transient expression of the transgenic GFP fluorescence in leaves of FG plants in space and on ground were detected under the SD condition after 24h HS-treatment, while no signal appeared in leaves of WT plants under the same conditions (Fig.3H-O). The highest abundance of GFP signal in the leaves of HS-treated plants grown under SD in space was obseved at day 8 (Fig. 3P). Flowering of FG plants under the SD both in space and on ground exhibited 2~3 days earler than that of WT plants (Fig. 2D, E and F). These results

indicated that the *pHSP::FT, pHSP:GFP* system we constructed in this study could mediate an "on/off" situation of *FT* gene activity in FG plants by HS treatment and could be used as gene switch for flowering induction both in space and on ground.

Identification of differentially expressed genes in response to microgravity in space under different photoperiodic conditions

To identify the molecular basis on FT integrates microgravity in controlling flowering pathway, the global transcriptional effects of microgravity were monitored in leaves using whole-genome Arabidopsis GeneChips (Affymetrix). RNA was extracted from leaves of WT and FG grown in microgravity (*μg*) on spaceflight under the LD (LD, μg) and the SD (SD, μg) , and their controls on ground under the LD (LD,1g) and the SD(SD,1g), respectively. The estimated mean level of gene expression in WT or FG in space were significantly different compared with the estimated mean of controls on ground when controlling the false discovery rate (FDR) at the level of 0.05 using the method of storey and Tibshirani (2003). Of the genes that met these criteria, we rank ordered them by fold change (FC). That expression level changed more than 2 (FC $\geq$ 2) were selected as differential expression gene (DEG). The DEGs were comparatively analyzed and divided into five steps (Fig.4 A). **Step 1**, expression of genes in leaves grown under the SD on ground was compared with those under the LD on ground (SD, 1g vs LD,1g, namely SD-1*g*). This approach allowed us to overveiw the influence of photoperiod on gene expression on ground without alteration of gravity in WT and FG, respectively (Supplementary Table S1 and S2). **Step 2**, genes altered expression levels in response to microgravity under the LD. For this, gene expression in WT and FG plants grown in space under the LD were compared with those on ground under the LD (LD, *μg vs* 1*g*, namely LD-*μg*), respectively (Supplementary Table S3

and S4). **Step 3**, changes of gene expression in response to μg occurred in WT and FG plants under the SD condition (SD, μg *vs* 1g, namely SD-μg), respectively (Supplementary Table S5 and S6). **Step 4**, Photoperiod related μg response genes are selected by comparisons between μg-LD and μg -SD response genes in WT and FG plants, respectively(Supplementary Table S7 and S8). **Step 5**, comparison of altered expression of photoperiod related μg response genes between WT and FG was performed (Supplementary Table S9). For this, "FT related photoperiod controlling μg response genes" could be identified.

A major impact of microgravity on global transcription

Figure 4B showed than 427 genes (7.3% of total 5863 DEGs) in WT and 477 genes (8.5%) in FG on ground were altered in transcript abundance under the SD compared to those under the LD on ground (Supplementary Table S1 and S2). Expression of 4432 genes (75.6%) in WT and 3922 genes (74.5%) in FG were altered in response to microgravity under the LD (Supplementary Table S3 and S4), while 2571 genes (43.9%) in WT and 2031genes (38.6%) in FG changed expression levels by subjected to microgravity under the SD (Supplementary Table S5 and S6). The proportion of genes up-regulated by microgravity was always higher than the down-regulated ones (Fig. 4C). Principal component analysis (PCA) of the samples demonstrated a strong difference between transcriptomes of samples grown on ground and in space under both the LD and the SD (Fig.4D).These results indicated that the number of genes defferential expression in response to microgravity was overall greater than the number of genes controlled by day length, suggesting a major impact of microgravity in space on global transcription in the leaves of WT and FG plants under both LD and SD conditions.

To validate the microarray data, we generated sequence-specific primers and performed real-time RT-PCR on a third independent replicate. Real-time PCR with isoform specific primers for calcium sensing receptor (At5g23060), haloacid dehalogenase-like hydrolase superfamily protein (At3g48420), constans-like 2(At3g02380) and TIMELESS (At5g52910) confirmed the relative abundance changes for the transcript levels of these genes of WT and FG in response to microgravity in space under LD and SD conditions (Fig.5).

Daylength related microgravity response genes

To characterize the gene categories in response to microgravity under different photoperiod, two groups of genes were divided based on their transcriptional behaviors (Table 1). The genes responding to microgravity under LD and SD conditions with similar behaviors were named ‘μg-common ’ genes, whereas those in response to μg specific to LD or SD were named ‘μg-daylength-related ’ genes. Among‘μg-common ’ genes, transcript levels of 1018 genes in WT and 720 genes in FG showed similar changes in response to microgravity under the LD and the SD (Fig. 6A; Table 1; Supplementary Table S7 and S8). In contrast, a relative large number of ‘μg-daylength-related ’ genes were found with total 4512 genes in WT and 4201 genes in FG (Table 1; Fig. 6A; Supplementary Table S7 and S8). Overall changes in gene expression pattern in WT and FG for ‘μg-daylength-related ’ genes were apparently more than those of ‘μg-common ’, indicating that daylength is an important factor to regulate response of plants to microgravity in space. GO categories representing ‘μg-common’ and ‘μg- daylength related ’ genes exhibit similar behaviors in the down-regulation of ribosome biogenesis and RNA processing (i.e. ncRNA, rRNA metabolism and processing), amino acid metabolic process. However, compared with ‘μg-common ’ genes, a significantly enriched GO terms were identified for ‘μg-daylength-related’ genes, among which the most overrepresentation was protein phosphorylation GO category in both WT and FG (Fig.6B and C). The function of proteins encoded by these overrepresented

178 ‘μg-daylength-related’ genes are involved in light and ethylene signaling, calcium
179 signaling, cell wall-associated receptor kinase-like proteins, phosphatase and protein
180 kinase and others (Supplementary Table S10).

Table 1 List of categories of differentially expressed genes in response to microgravity under different photoperiod

Group	**Transcriptional behavior**		**Nu. of genes**		**Description**
	LD-*μg*	SD-*μg*	WT	FG	
μg-common	YES	YES	1018	702	Genes differentially expressed (DE) and their expression levels under SD-μg are similar to those under LD-μg
μg-daylength-related	YES	YES	455	348	Genes DE under LD-μg and SD-μg, but with different behavior
	YES	NO	2959	2872	Genes DE only when μg is applied under LD
	NO	YES	1098	981	Genes DE only when μg is applied under SD

181 To test whether there are potential common cis-acting elements among the
182 ‘μg-daylength-related’ genes, we performed analyses using Plant Regulomics
183 (bioinfo.sibs.ac.cn/plant-regulomics) to find overrepresented motifs in the 1-kb
184 upstream sequence of the overrepresented genes in protein phosphorylation GO
185 terms with ‘μg-daylength-related’ behaviors in WT and FG plants (Fig.6B and C;
186 Supplementary Table S10). Thirty-one of coregulated genes (Fig. 7B) were
187 identified to share four common motifs, of which occurrence were significantly high
188 as compared to that in random genomic regions (Fig. 7A). These include
189 A(G/T)ATTC, which is identical to the AtNIGT1/HRS1(AT1G13300) motif that is
190 present in the promoters of nitrate and phosphate signaling genes (Medice et al.,
191 2015), GAATATTC, which represents to KAN4 motif that provides boundary
192 maintenance and promotes the laminar growth of the inner ovule integument
193 (Gomez et al., 2016) and GGGACCAC, which is identifical to the transcription
194 factor TCP5 that controls plant thermomorphogenesis by positively regulating
195 PHYTOCHROME INTERACTING FACTOR 4(PIF4) activity (Han et al., 2019).
196 Furthermore, the AAAG, which is similar to the dof zinc finger protein MNB1A
197 element that has been suggested to regulate photosynthetic gene expression in *Zea*

198 *may* (Cavalar et al., 2007). This result indicate that common regulators might be
199 involved in adaptation of plants to microgravity under different photoperiod
200 conditions.

201 *Expression of FT could altered daylenth-related microgravity response of leaves*
202 To explain whether plants change response to microgravity after *FT* expression
203 under different daylength conditions, we compared expression of ‘μg-daylength
204 related ’genes in FG with those in WT. The direction comparison of SD-1g genes in
205 FG on ground with those in WT showed no significant correlation (Fig. 8A). In
206 constrast, comparison of LD-μg genes in FG with those in WT showed a strong
207 positive correlation (Fig. 8B), while SD-μg genes in FG showed a strong negative
208 correlation with those in WT (Fig.8C), suggesting that the response of FG to
209 microgravity was similar to those in WT under the LD, but different under under the
210 SD, in which *FT* had expressed by heating induction.
211 To further study response of plants to microgravity after *FT* expression under the
212 SD, we focused on genes in three clusters. Cluster 1(C1) comprises 534 genes
213 which expression in WT under both the LD and the the SD were modified as well as
214 in FG under the LD , but unchanged in FG under the SD (Fig.8D and E). Analysis
215 of this cluster to assess overrepresented GO terms with the Biological Networks
216 Gene Ontology tool (BINGO) indicated the link with cellular metabolic process,
217 notably amino acid, amine, oxoacid metabolic process (Fig. 8F). Cluster 2(C2) is
218 represented by 467 genes. Their expression did not change in WT under the LD and
219 the SD as well in FG under the LD but was significantly up- or down-regulated in
220 FG under the SD. BINGO analysis of the induced genes revealed that this cluster
221 include many of the abotic stimulus response-associated genes noted in Fig.8G, as
222 well as a number of genes involved in post-translational protein modification
223 (Supplementary Table S9). Cluster 3 include 251 genes which expression altered in
224 response to microgravity in both WT and FG under the LD, but didn’t change in
225 WT under the SD. BINGO analysis of this cluster genes suggested that the induced
226 processes include metabolic process notably chlorophyll biosynthetic process, and

response to stimulus, such as , high light, temperature and ethylene stimulus (Fig. 8H; Supplementary Table S9).

Impact of microgravity on daylength flowering pathways

To further explore the impact of microgravity on daylength flowering-time pathways, we investigate expression pattern of the 49 core flowering control genes (http://wikipathways.org) in response to microgravity in WT and FG under the LD and the SD, respectively. Thirty-seven of them showed altered expression levels in response to microgravity in WT and/or FG under at least one of daylength conditions (Supplementary Table S11). More than one-third of these core flowering control genes are circadian clock genes, including, *LATE ELONGATED HYPOCOTYL* (*LHY*), *REVEILLE 1*(*RVE1*), *RVE2*, *CLOCKASSOCIATED1* (*CCA1*), *PATHOGEN AND CIRCADIAN CONTROLLED 1*(*PCC1*), *EARLY FLOWERING 4*(*ELF4*), *ELF4-L4*, *GIGANTEA*(*GI*), *CONSTITUTIVE PHOTOMORPHOGENIC1*(*COP1*), *CONSTANS* (*CO*), *CONSTANS-LIKE9*(*COL9*), *ARABIDOPSIS PSEUDO-RESPONSE REGULATOR 5*(*APRR5*). The differential expression of these core flowering control genes between WT and FG in response to microgravity was observed (Fig.9A and B). For example, expression of *PCC1* is down-regulated in FG on ground, while up-regulated in space, in comparison with that in WT. Expression of *FT* in FG were apparent higher under both LD and SD conditions on ground and in space than that in WT(Fig. 9A), consistenting with GFP signal in FG observed by inflight images (Fig. 3P). The gene *CO*, which is necessary for the daylength regulating of flowering, exhibited up-regulated by 4.5-fold in WT under LD, while down-regulated by 0.1-fold in WT under SD in space in comparison with their ground controls. By contrast, FG showed no change of CO expression to microgravity under both LD and SD conditions(Supplementary Table S11). In addition, several circadian clock genes were observed among FT interactome (Fig. 9 B). *REV2*, which is involved in regulating both photoperiod pathway and circadian processes, was up-regulated about 115 fold in FG and about 35 fold in WT under the LD in space in comparison with their control on ground,

but less increased under the SD (Fig.9C). This indicates a specific role of REV2 in regulating microgravity response under the LD condition in space. Expression level of *LHY* exhibited up-regulated under LD in WT and FG in space (5.82- and 15.56-fold, respectively), but more significantly increased under SD in space (9.05- and 26-fold, respectively). In contrast, expression level of *GI* was down-regulated under both LD and SD in WT and FG in space (Fig.9 C). These results indicated that circadian clock gene could play an important role for plant adaptation to microgravity in space during flowering.

Discussion

Day length and microgravity are both impact on growth and development of plants in space, but interaction between them remain unclear. In this study, we constructed a transgenic plants (*pHSP::FT*; *pHSP::GFP*) and setup a protocol to initiate the floral transition on orbital condition using HS treatment by remote control. Using this experimental system, we contributed to identify: (1) plant response to microgravity at transcriptional levels depend on the daylength conditions. (2) daylength-related microgravity response could be involved in alteration of transcriptional activity in protein sythesis and post-translation protein modulation, notably protein phosphorlation. (3) Expression of *FT* in Arabidopsis leaves by HS induction can change the response of plant to microgravity under the SD, possibly through modulating expression of circadian clock genes in photoperiod controlling flowering pathway.

Manipulate de novo transgene expression at key developmental stage using a gene switch, which can mediate an “on/off” situation of gene activity, will be very importance to design efficient production and resilient crop cultivation in bioregenerative life-support system (BLSS) for a long-term mission such as on earth orbit space station, moon and Mars in the future. A pervious study using transgenic

Arabidopsis containing the alcohol dehydrogenase (Adh) gene promoter linked to the β-glucuronidase (GUS) reporter gene has been used to evaluate the stress signal perception and transduction in Arabidopsis in spaceflight (Paul et al., 2001). In this study, we demonstrate that a *HSP17.4* promoter combined with *FT* and *GFP* could be used in flowering induction of transgenic Arabidopsis by simply heating the culture chamber (37°C, 1h), and expression of *GFP* gene in leaves, which could be directly monitored by robotic camerea. This system provide a possible approach to manipulate crops for high production in space through controlling plant flowering time.

Flowering is a prerequisite for crop production whenever seeds or fruits are harvested (Blümel et al., 2015; Shim et al., 2017). Various environmental stresses, such as drought, salt and temperature, were reported to interfered flowering (Galbiati et al., 2016; Fernández et al., 2016). Microgravity is a novel stress to plants, which were evolved on earth (Hampp et al., 1997; Zheng et al., 2008; Zhang and Zheng, 2015; Paul et al., 2017; Wu et al., 2020). Previous studies indicated that microgravity on spaceflight is a compound stress, imposing multiple constraints on plants by interation with other environmental factors. For example, roots of plants in space appeared to become hydortropically more sensitive to moisture gradients (Morohashi et al., 2017) and altered response to red- and blue-light phototropism (Valbuena et al., 2018; Herranz et al., 2019). In this study, we found that Arabidopsis plants grown under the LD exhibited more sensitive to microgravity in comparison those under the SD condition at transcriptional level. Down-regulation of expression in ribosome biogenesis and RNA processing (i.e. ncRNA, rRNA metabolism and processing) and amino acid metabolic process was observed among ‘μg-common’ genes as well as ‘μg- daylength related ’ genes. This result is

307 consistent with that regulation of ribosome biogenesis, which is linked to factors
308 controlling cell growth and proliferation, was decreased in Arabidopsis cell cultures
309 and seedlings in real or simulated microgravity(Matía et al., 2010; Manzano et al.,
310 2012; Kamal et al., 2018). The reason for down-regulation of ribosome biogenesis
311 under microgravity is unknown. A pervious study estimated that cells dedicate ~80%
312 of total trancriptional activity to the synthesis of rRNAs and proteins for ribosome
313 biogenesis (Wamer, 1999), making ribosome biogenesis a major nutrient and
314 energy-consuming process in growing cells (Lempiäinen and shore, 2009).
315 Down-regulation of ribosome biogenesis found in 'μg-common' genes suggest a
316 limitation in nutrient and energy supply to plants during the microgravity response
317 under both the LD and the SD condition. Another interesting finding is our
318 observation that microgravity trigger networks related to daylength signals altered
319 expression of genes involved in the GO category 'protein phosphorylation' in both
320 the WT and FG. These 'μg-daylength-related' genes, including protein kinase,
321 receptor-like protein kinase, phosphatase and signaling in light, ethylene and
322 calcium, were down-regulated expression in the μg-LD datasets and/or up-regulated
323 expression in the μg-SD datasets. This result is consistent with a recently study,
324 which indicated that protein phosphorylation plays a crucial role in gravisignaling,
325 and gravitropism and phototropism of plants (Yang et al., 2020). Among 'protein
326 phosphorylation' GO category genes, we found phytochrome interacting factor
327 4(*PIF4*) and phototropic-responsive *NPH3* family protein, and a blue light receptor
328 phototropin 1(*PHOT1*), which showed expression profiles in response to
329 microgravity were modulated different by day length. Similarly, expression of genes
330 involved in sensing the extracellular environment and triggering intracellular signals,
331 such as several wall-associated kinase (WAK), ethylene response sensors and

calcium-dependent protein kinases, were mostly downregulated in response to microgravity under the LD only, but didn't change expression level under the SD (Supplemantary Table S10).

Plant photoperiodic regulation can be divided into three parts: light input, circadian clock, and output. Previous study pointed that light-associated pathways in Arabidopsis showed significant down-regulation in microgravity (Valbuena et al., 2018; Vandenbrink et al., 2019). However, those experiments were all performed under the LD condition, but no one under the SD. Our study found that light-associated genes, such as *PHOT1* was down-regulated only under the LD, while expression of *PIF4* and *NPH3* didn't changed under the LD, but up-regulated specifically under the SD, suggesting that photoperiod apparently affect plant response to microgravity during light input. In addition, light information is integrated into innate photoperiodic timing mechanisms governed by the circadian clock to induce *FT* expression that trigger flowering (Shim et al., 2017). The plant circadian clock consists of multiple transcription-translation feedback loops that are influenced by environmental signals, linking the clock with plant stress adaptation. For example, phytochrome- and cryptochrome-mediated light signals mediate the induction of *CCA1*, *LHY*, and *PRR9* gene expression(Somers et al., 1998; Farré et al., 2005; Bieniawska et al., 2008). The expression of circadian clock components, such as, *CCA1*, *ELF3*, *GI*, *GRP7*, *PRR9*, TOC1, and ZTL, were affected by stress environment, including water use efficience of Arabidopsis (Simon et al., 2020), high light (Yakir et al., 2007) as well as the 3-D clinostat rotational simulated microgravity (Xie and Zheng, 2020). In this study, our data indicate that the circadian oscillator is important for regulating microgravity response of Arabidopsis. Up-regulation of morning components (*CCA1*, *LHY*, *REV1* and *REV2*) and

down-regulation of late day (*GI*) and evening (*ELF4-L4*) components of the circadian oscillator in space under the LD and/or the SD in both WT and FG. Additionally, *COL9* and *COP1* enhanced expression levels in response to microgravity specifically under the LD condition, while *APRR5* increase expression levels in response to microgravity specifically under the SD condition. Together, this results suggests that the circadian oscillator can be altered expression level in response to microgravity dependent on daylength condition, which could in particular interfere with flowering in space. Change circadian function by microgravity in space influence plant flowering and fitness offerig the hypothesis that optimizing circadian function will enhance crop productivity in space. In the future, more refinement of our understanding of the circadian clock mechanism under different photoperiodic conditions in space is necessary to inform manipulation towards the goal of enhacing crop productivity in bioregenerative life-support systems (BLSS).

Methods

Plant Materials and growth condition

Arabidopsis thaliana Columbia (Col-0) ecotype was used as the wild-type. Plants were germinated and grown in plastic cups under long-day (16h light /8h dark) at 120 μmol. $m^{-2}.s^{-1}$ conditions for 5 days, then set in the root modules (240×120×65 mm^3) containing a commercially available vermiculite immersed by a medium containing MS macronutrients (Murashige and Skoog, 1962) and cultived in greenhouse for 20 days prior to flight.

Construction of transgenic Arabidopsis plants

For the construction of *pHSP*::*FT*, the coding sequence (CDS) of *FT* was amplified

by PCR from a Col-0 cDNA using the primers with the restriction sites underlined 5'-ATCACTAGTATGTCTATAAATATAAGAGACCCTCTTA-3' and 5'-CGTTCTAGACTAAAGTCTTCTTCCTCCGCAGC-3' and ligated into a pBluescript KS minus vector. A 1109 bp DNA fragment, upstream from *HSP17.4*(AT3G46230) start codon corresponsing to the putative promoter, was amplfied by PCR with the primers 5'-ACTCTGCAGACCAGTCATACGTAGCGCAAT-3' and 5'-ATGACTAGTCGTTTCGCTTACTCTGTTTGC-3', and fused to the *FT* coding sequence in the pBluescript KS minus vector. For the construction of *pHSP*:: *GFP*, the *HSP17.4* promoter DNA fragment was fuse to a *GFP* in pBluescript SK minus vector. *pHSP::FT* or *pHSP::GFP* were then cleaved and ligated into the pCambia1301-NOS-3, vector. These two gene fusions were transferred to Arabidopsis (Col-0) plants through the *Agrobacterium tumefaciens* strain GV3101, respectively, by the floral dip method according to the methods of Clough and Bent (1998). After regeneration in the presence of hygromycin, transgenic plants were screened for expression of *FT* and *GFP* by heat shock (37°C for 1h) in an incubator. *pHSP*::*FT*, *pHSP*:*GFP* (FG) gene were co-expression in Arabidopsis by genetic crossing as described by Qi and Zheng (2013). F3 progeny homozyous for FG were used for space expriments.

Hardware design and the spaceflight experiment

The plant growth system used for the SJ-10 experiment consisted of four growth compartments, illumination, photograph , air-flowing heating and humidity controling system (Fig. 1A-C). Plants have about 4-6 rosette leaves when they loaded into the growth chamber (Fig. 1D). Two set of plants were prepared and placed in the plant growth units (PGUs) for spaceflight experiment (Fig.1A, B and

406 C) and the ground control, respectively. The flight PGU was positioned in the
407 capsual of satellite about 8 h prior to launch. The SJ-10 satellite was in orbit for
408 about 12 days and 15h (lauch: 1:38, April 6, 2016; landing: 16:30, April 18, 2016).
409 Illumination was provided by light banks made up of 200 solid state light emitting
410 diode (LED) lamps (400-700 nm white light and red light, 2:1) on a long-day (LD,
411 16h light/8h dark) or a short-day (SD, 8h light/16h dark) photoperiod. Inside the
412 chambers, temperatures were 22±2°C, relateve humidity was between 90% and
413 100%. The photosynthetically active photon flux density produced by LED lamps
414 was 120 μmol.m^{-2}.s^{-1} for Arabidopsis at surface of the first leaf of the experimental
415 plants. Temperature and humidity were recorded every 1min during flight. These
416 data were used to set the ground control in a control growth chamber. Three video
417 cameras were mounted in the PGU to allow recording of plant growth and
418 development plants in space. Photographic equipments were consisted of two video
419 cameras (image size 1280×1024 pixels) and one GFP fluorescence camera, which
420 were automatic and preprogrammed and allowed recording of plants in PGUs both
421 in visible light and in GFP fluorescence (Fig.1B and C). The photographs were
422 taken at 2-h intervals during the light period. Two video cameras were used for
423 photographed seedlings grown under the LD and the SD conditions, respectively.
424 The GFP fluorescence camera was used to follow expression of GFP in seedlings
425 after heating induced. All manipulations involved in the experiment were automated
426 or carried out by remote control.

427 After SJ-10 satellite return to Earth, the PGU was unloaded and received at a
428 temporary laboratory in the landing site about 2h post-landing. Plants were
429 harvested and fixed with RNAlater solution (ambion, Austin, TX, USA) at the
430 landing site. The samples were then brought to our Shanghai laboratory where they

were analyzed for transcriptional changes.

Sample processing and Analysis

Total RNA was extracted from the space samples and the ground controls and then purified using miRNeasy Mini Kit（Cat#217004, QIAGEN, GmBH, Germany) following the manufacturer's instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). RNA amplified, labeled and purified by using GeneChip 3' IVT PLUS Reagent Kit (Cat#902416, Affymetrix, Santa Clara, CA, US) following the manufacturer's instructions to obtain biotin labeled cRNA. Array hybridization and wash was performed using GeneChip® Hybridization, Wash and Stain Kit (Cat#900720, Affymetrix, Santa Clara, CA, US) in Hybridization Oven 645 (Cat#00-0331-220V, Affymetrix, Santa Clara, CA, US) and Fluidics Station 450 (Cat#00-0079, Affymetrix, Santa Clara, CA, US) following the manufacturer's instructions.

Analysis of microarray data

Slides were scanned by GeneChip® Scanner 3000 (Cat#00-00212, Affymetrix, Santa Clara, CA, US) and Command Console Software 4.0 (Affymetrix, Santa Clara, CA, US) with default settings. Raw data were normalized by MAS 5.0 algorithm, Affymetrix packages in R. Probe sets with signal values lower than the detectable range were adjusted to 75 and probe sets with the values of 75 for all conditions were removed from subsequent analysis. The averages of normalized ratios are calculated by dividing the average of normalized signal channel intensity by the average of the normalized control channel intensity. The standard deviation of the ground control (two biological replicates) was employed to identify genes of

455 significant changes relative to the ground controls (P value<0.05). Only genes that
456 showed transcript level changes in at least two folds in comparison with its ground
457 control and with the same tendency in both biological replicates were considered as
458 relevant for microgravity. Gene Ontology (GO) Overrepresentation was performed
459 using PANTHER (Fisher's Exact type with False Discovery Rate correction)
460 (http://www.pantherdb.org) (Mi et al. 2019). For motif enrichment, motifscan (Sun
461 et al., 2018) was used to determine whether the occurrence of a given motif in input
462 genes was significantly high as compared to that in random regions (Ran et al.,
463 2019).

464 **Real-time RT-PCR**

465 Total RNA was extracted from leaves of the space samples and the ground controls
466 as described. The genes and their qRT-PCR primers are presented in Supplementary
467 Table S1. The Arabidopsis ACTIN gene was used as the loading control for all
468 qRT-PCRs. At least three technical replicates of each biological replicate were used
469 for real-time PCR analysis.

Acknowledgments

The authors are indebted to Professor W.R. Hu for valuable and helpful suggestions and Professor T. Zhang's group for PGU construction and helping in the space experiment. This work was supported by the China Manned Space Flight Technology project Chinese Space Station, the National Natural Science Foundation of China (31870850), the Strategic Pioneer Projects of CAS (XDB37020104), the National natural fund joint fund project (U1738106).

Author Contributions: LW and JX carried out plant culture, data curation and investigated gene expression; YW, CM, YJ and YD participated in investigation, HZ

conceived of the study, participated in its design and coordination, and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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Figure legends

Figure 1 Experimental setup on board the Chinese recoverable satellite SJ-10.

(A) The external view of the plant growth unit (PGU) (H×W×D=370 ×270×270 mm) with the cover.

(B) The inside view of the PGU without the cover, as described in (C).

(C) Diagram of PGU used on board the Chinese satellite SJ-10. Showing the distribution and state of components and samples in PGU during space flight. The components included four root modules, three cameras, two fans and heating parts, and four LED banks with controlled long-day (LD) or short-day (SD) photoperiods, respectively. indicating LED plates; heating systems; video CCD cameras(Cam 1 and Cam 2); the GFP imaging camera (Cam f) and root modules (R1 and R3 with rice seedlings; R2 and R4 with Arabidopsis seedlings).

(D)An overview of the time process of the space experiment.

wild-type and transgenic Arabidopsis plants were germinated and grown in the root module in green house on ground for 20 d after sowing. At this age, the plants had formed four rosette leaves, when they were loaded into PGU less than 24h prior to take off. Heating treatment (red arrows point) to activated expression of p*HSP*::*FT*; p*HSP*::*GFP* was performed at day 2 on orbiter. The plants in PGU were grown under long-day (LD, 16 h light/8 h dark) and short-day(SD, 8 h light/16 h dark) conditions in space (μg) and on ground (1g), respectively.

(E and F) 20-day old wild-type (WT) and transgenic (*pHSP*::*FT*; *pHSP*::*GFP*, FG) Arabidopsis seedlings grown in the root modules before being loaded into the PGU.

(G and H) The situation of samples recovered from spaceflight under LD (G) and SD (H) conditions. Bars=1cm.

Figure 2 Floral transition time of wild-type and *pHSP*::*FT*; *pHSP*::*GFP* transgenic plants grown under the long-day condition on ground and in flight on board the SJ-10 satellite.

(A) example images of plants grown under long-day condition on ground(1g, on ground) and in space (μg, in space) at day 2 (d2) to day 9 (d9) after satellite took off.

(B and C) comparison of floral transition time and average length of stems of wild-type (WT) and *pHSP*::*FT*; *pHSP*::*GFP* (FG) transgenic plants under the long-day (LD) in space with their controls on ground. Data are determined from the living images of plants on orbit in space or on ground. n=4 plants per treatment.

(D) example images of plants of wild-type and transgenic plants under the short-day (SD) condition on ground and in space at d2 to d9 after satellite took off.

(E and F) comparison of floral transition time and average length of stems of WT and FG plants treated by heating shock (red asteres indicate) under the SD condition in space and on ground. Data are determined from the living images downloaded from the plant growth units in space and on ground. n=4 plants per treatment. Bars in (A) and (D) = 10 mm.

Figure 3 GFP expressing in short-day grown plants treated by 37°C heating on board SJ-10.

(A) *pHSP*::*FT*; *pHSP*::*GFP* transgenic plants and wild-type (WT) plants photographed in white light before launching. The framed region in (A) is detailed in (B).

(C) a represent GFP image showed the expression of GFP of leaves1-6 in (B)

(D-O) Example GFP images were captured by the GFP imager at day 2 to day 4 after heating treatment. The images were captured at four time points (15:50, 15:55, 23:50 and 23:55, on board SJ-10 satellite time) every day and reflect heating inducing of GFP expressing.

(P) Quantification of the intensity of GFP signal in plants under the short-day (8h light/16h dark) on oribit the SJ-10 and on ground, respectively. The GFP signals was measured as described in Materials and Methods. Values represent means for two time point images (at 15:50 and 15:55 dark period and 23:50 and 23:55

light period every satellite day, respectively). The red asteres indicate the time points of heating shock. White areas, light; dark grey panels, dark.

Figure 4 Transcriptional responses microgravity in Arabidopsis wild-type (WT) and trangenic plants (pHSP::FT; pHSP::GFP, FG) under the Long-day (LD) and the short-day (SD) conditions, respectively.

(A) Workflow of microarray data analysis. Analysis of twofold differentially expressed genes consists of five major steps: analysis of photoperiod response genes in WT and FG plants grown on ground(1g)(step 1), identification of genes in WT and FG plants in space involved in microgravity (μg) responses under LD (step 2) and SD (step 3) conditions, comparison of microgravity response genes in WT and FG plants specific to LD- or SD- conditions (Step 4) and selection of microgravity response genes in FT pathway by comparison of differetial expression genes (DEGs) in WT to those in FG (step 5).

(B) Venn diagram of transcriptomic data.

(C) Numbers of DEGs in WT and FG plants ($P<0.05$) under the LD and the SD conditions, respectively, in response to microgravity.

(D) Principal component analysis demonstrates a strong difference between the flight and the ground sample transcriptones, as well as the LD and the SD samples. Multidimensional scaling (MDS) of all DEGs in WT and FG in response to microgravity under the LD and the SD, respectively.

Figure 5 Relative transcript abundance changes in WT and FG under LD- and SD-conditions in space as compared to the ground controls, respectively, were analyzed by microarray and real-time PCR. Microarray data for *Cas* (At5g23060), *HAD* (At3g48420), *COL2*(At3g02380) and *ATM*(At5g52910) are shown as an average of two independent replicates. Real-time PCR with isoform-specific primers for those genes was performed on a third independent replicates.

Figure 6 Pair-wise comparison and GO terms analysis of changes in microgravity-mediated gene expression.

(A)Genes that are regulated in both daylength (fold changes ($-1<\log_2(\text{SD-}\mu g/\text{LD-}\mu g)<1$, common) or differentially regulated under the SD versus the LD [$\log_2(\text{SD-}\mu g/\text{LD-}\mu g)>1$ or <-1, daylength specific microgravity response] are depicted. The total number of genes at least twofold differentlly regulated in indicated.

(B and C) GO terms overrepresented in microgravity responses of WT (B) and FG (C) under the long-day (LD) and the short-day (SD). The significant gene ontology (PANTHER statitical overrepresentation test, GO-Slim biological precess, FDR P value<0.01) categories from up-regulated DEGs or down-regulated DEGs in WT and FG under the LD and the SD are depicted. WT, wild-type; FG, *pHSP*::*FT*; *pHSP*::*GFP* trangenic plants.

Figure 7 Potential regulator of coregulated genes in protein phosphorylation funcational cluster of 'μg-daylength-related' genes of WT and FG. (B)Four overreprented motifs enriched in upstream promoter sequences of genes in protein phosphorylation GO category in Figure 5 B and C, as detected by the plant regulomics (bioinfo.sibs.ac.cn/plant-regulomics). Indicated are the P-Value representing the statistical significance of the motif. (B) Clustering analysis of the selected corregulated genes in protein phosphorylation GO category in Figure 6B and C, which changed transcript abundance in response to microgravity in WT and FG plants under long-day (LD) and short-day (SD) conditions, respectively and have AT1G13300, KAN4, ARALYDRAFT_496250 and MNB1A binding sites in upstream promoter regions.

Figure 8 Expression of *FT* affected photoperiod-microgravity response transcriptome. (A-C) Scatterplot showing the microgravity response of DEGs between FG and WT under the LD and the SD, respectively.

(D) Venn diagrams summarizing the number of DEGs (FC>2 and P<0.05) in response to microgravity under the LD and the SD, respectively, among WT and FG samples.

(E) Selected DEGs in FG plants in response to microgravity under the LD (L-μg) and the SD (S-μg) are compared with those in WT plants in space.

(F-H) Enriched GO Terms in DEGs of selected clusters in D. The networks graphs show BiNGO visualization of the overrepresented GO terms for the selected clusters corresponding to cluster C1 to C3 indicating in E, respectively. Categories in GoslimPlants(Breeze et al., 2011) were used to simplify this analysis and the same nodes are shown on all three graphs. Uncolored nodes are not overreprensented, but they may be the parents of overrepresented terms. Colored nodes represent GO terms that are significantly overrepresented (Benjamini and Hochberg corrected P value <0.05), with the shade indicating significance as shown in the color bar.

Figure 9 The core photoperiod response genes altered expression levels by expouse to microgravity.

(A) Log_2 FC of the 20 core photoperiod genes in the FG under LD or SD on ground (1g) and microgravity in space (μg), respectively, in comparison with these genes in wild-type (WT) under the same condition (FG/WT).

(B) Diagram of the protein interaction networks of the photoperiod response genes. The genes, which altered expression level in response to microgravity in space in comparison with their controls on ground, were labelled by colour in yellow and log_2 FC of these highlighted genes are indicated in C.

(C) Log_2 FC of selected core photoperiod genes in WT and FG in response to microgravity under the LD and the SD condition, respectively.

Supplementary data

Supplementary Figure S1 Heat shock activation of *GFP* gene expresion in pHSP::FG,*pHSP*::*GFP* (FG)transigenic plants.

(A and E) 14-day-old wild-type (A) and FG transgenic seedlings (E) were treated by 37°C for 1h or under 20°C control conditions, respectively.

(B-D) Fluorescence images of leaves from 37°C heat treated seedlings and 20°C control plants, respectively.

(F-H) image of leaves under differential interence contrast optics microscope.

Supplementary Figure S2 Heat shock treatment induced FT expression and flowering. All plants were 20-day old and were subjected to heat shock (37°C) induction for 2h at day 15 after germination.

(A, D) Phonetype of wild-type (WT) plants were grown under the long-day (16 h light/8h dark, LD) and short-day (8 h light/16h dark, SD) conditions, respectively .

(B, E)Phonetype of transgenic plants *pHSP*::*GFP* were grown under the LD and the SD conditions, respectively.

(C, F) Phonetype of transgenic plants *pHSP*::*FT,pHSP::GFP* were grown under the LD and the SD conditions, respectively.

(G) qRT-PCR analysis of *FT* transcript levels in 16-day-old *pHSP::FT,pHSP::GFP* plants grown under the SD conditions with or without the 2h heat shock treatment (37°C).

Note that the plants *pHSP::FT,pHSP::GFP* under both LD- and SD-conditions (C and F) appeared early flowering in comparison with their controls of WT(A and D) and *pHSP::GFP* (B and E) under the same conditions.

Supplementary Table S1 Expression data of identified genes of wild-type (WT) plants grown under the short-day (SD) on ground was compared with those under the long-day (LD) on ground(WT- 1g-SD versus WT-1g-LD).

Supplementary Table S2 Expression data of identified genes of *pHSP::FT, pHSP:GFP* (FG) plants grown under the short-day (SD) on ground was compared with those under the long-day (LD) on ground(FG- 1g-SD versus FG-1g-LD).

Supplementary Table S3 Expression data of identified genes of wild-type (WT) plants grown under the long-day (LD) in microgravity (μg) in space were compared with those under the LD on ground(WT-μg-LD versus WT-1g-LD).

Supplementary Table S4 Expression data of identified genes of *pHSP::FT, pHSP:GFP* (FG) transgenic plants grown under the long-day (LD) in microgravity (μg) in space were compared with those under the LD on ground(FG-μg-LD versus FG-1g-LD).

Supplementary Table S5 Expression data of identified genes of wild-type (WT) plants grown under the short-day (SD) in microgravity (μg) in space were compared with those under the SD on ground(WT-μg-SD versus WT-1g-SD).

Supplementary Table S6 Expression data of identified genes of *pHSP::FT, pHSP:GFP* (FG) transgenic plants grown under the short-day (SD) in microgravity (μg) in space were compared with those under the SD on ground(FG-μg-SD versus FG-1g-SD).

Supplementary Table S7 Pair-wise comparison of altered expression of genes in response to microgravity in WT under the SD with those under the LD conditions.

Supplementary Table S8 Pair-wise comparison of altered expression of genes in response to microgravity in *pHSP::FT, pHSP:GFP* (FG) transgenic plants under the SD with those under the LD conditions.

Supplementary Table S9 Data for the selected clusters corresponding to cluster C1 to C3 indicating in Figure 7E.

Supplementary Table S10 Protein phosphorylation proteins encoded by day-length related microgravity-responsive genes.

Supplementary Table S11 Microgravity response of core photoperiod response genes in WT and/or FG plants grown in space. Genes identified with a significant (FC>2 and $p<0.05$) change in expersion level.

Figures

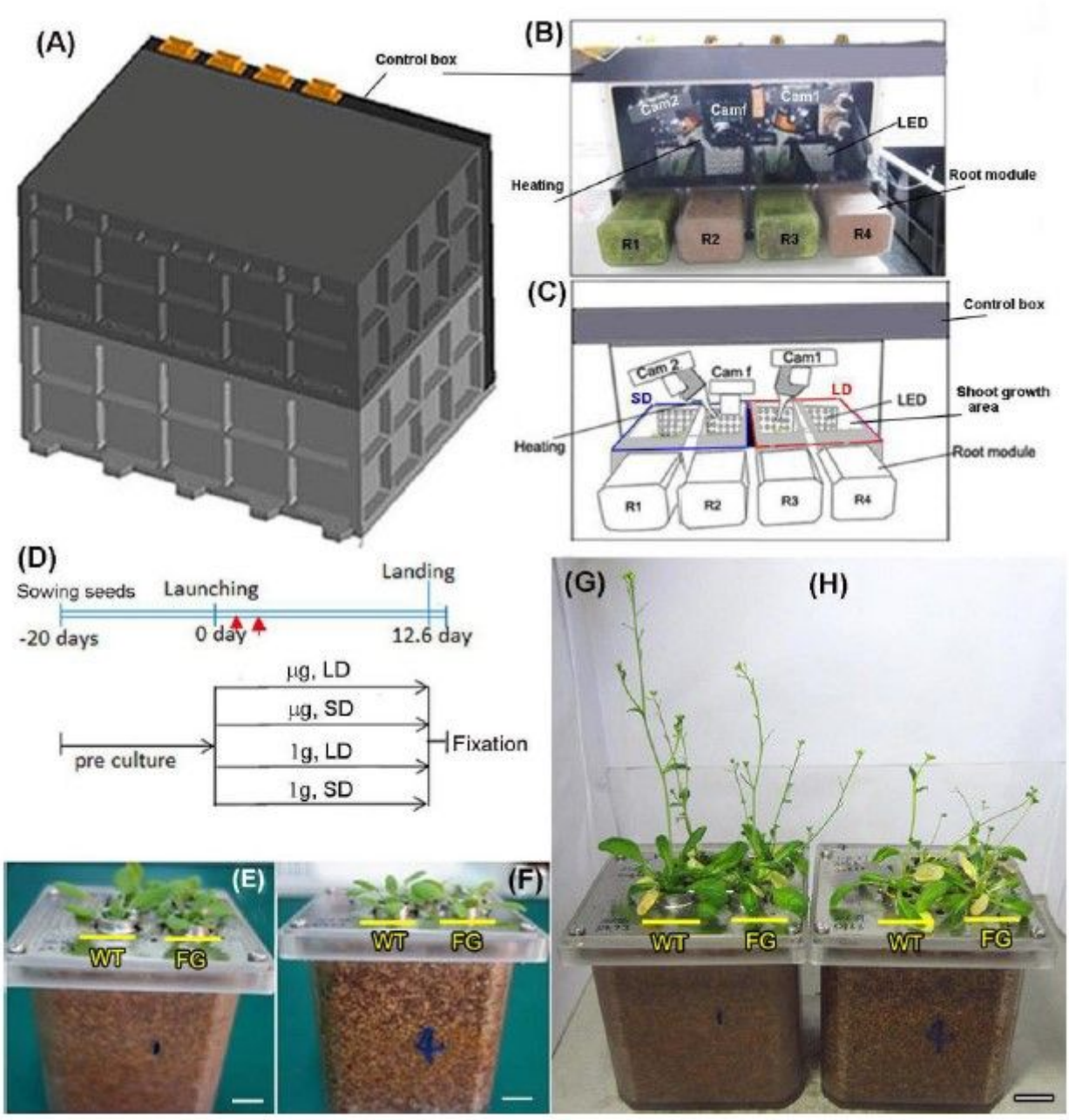


Figure 1

Experimental setup on board the Chinese recoverable satellite SJ-10. (A) The external view of the plant growth unit (PGU) (H×W×D=370 × 270×270 mm) with the cover. (B) The inside view of the PGU without the cover, as described in (C). (C) Diagram of PGU used on board the Chinese satellite SJ-10. Showing the distribution and state of components and samples in PGU during space flight. The components included four root modules, three cameras, two fans and heating parts, and four LED banks with controlled long-day (LD) or short-day (SD) photoperiods, respectively. indicating LED plates; heating systems; video CCD cameras(Cam 1 and Cam 2); the GFP imaging camera (Cam f) and root modules (R1 and R3 with rice seedlings; R2 and R4 with Arabidopsis seedlings). (D)An overview of the time process of the space

experiment. wild-type and transgenic Arabidopsis plants were germinated and grown in the root module in green house on ground for 20 d after sowing. At this age, the plants had formed four rosette leaves, when they were loaded into PGU less than 24h prior to take off. Heating treatment (red arrows point) to activated expression of pHSP::FT; pHSP::GFP was performed at day 2 on orbiter. The plants in PGU were grown under long-day (LD, 16 h light/8 h dark) and short-day(SD, 8 h light/16 h dark) conditions in space (μg) and on ground (1g), respectively. (E and F) 20-day old wild-type (WT) and transgenic (pHSP::FT; pHSP::GFP, FG) Arabidopsis seedlings grown in the root modules before being loaded into the PGU. (G and H) The situation of samples recovered from spaceflight under LD (G) and SD (H) conditions. Bars=1cm.

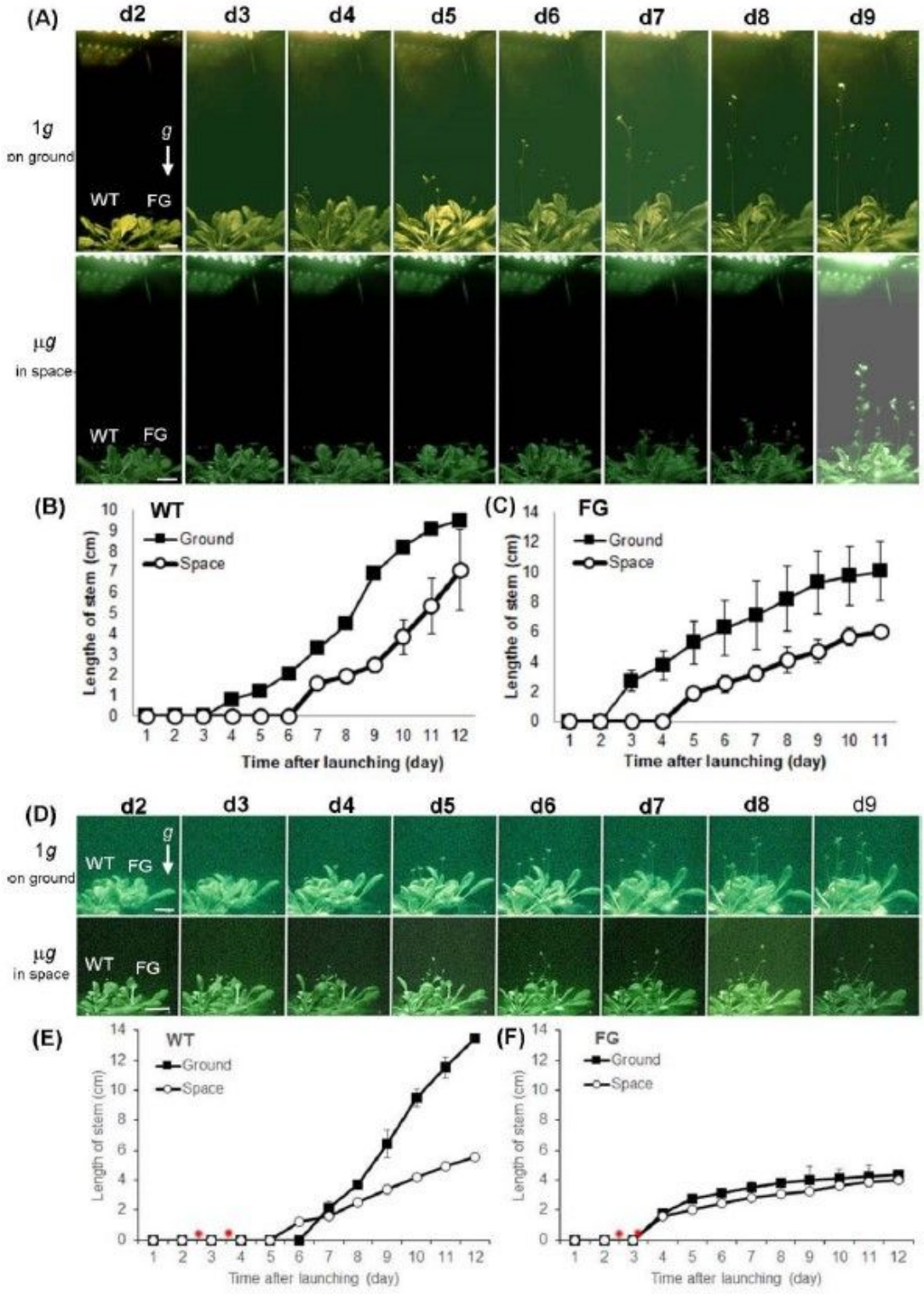


Figure 2

Floral transition time of wild-type and pHSP::FT; pHSP::GFP transgenic plants grown under the long-day condition on ground and in flight on board the SJ-10 satellite. (A) example images of plants grown under long-day condition on ground(1g, on ground) and in space (μg, in space) at day 2 (d2) to day 9 (d9) after satellite took off. (B and C) comparison of floral transition time and average length of stems of wild-type (WT) and pHSP::FT; pHSP::GFP (FG) transgenic plants under the long-day (LD) in space with their

controls on ground. Data are determined from the living images of plants on orbit in space or on ground. n=4 plants per treatment. (D) example images of plants of wild-type and transgenic plants under the short-day (SD) condition on ground and in space at d2 to d9 after satellite took off. (E and F) comparison of floral transition time and average length of stems of WT and FG plants treated by heating shock (red asteres indicate) under the SD condition in space and on ground. Data are determined from the living images downloaded from the plant growth units in space and on ground. n=4 plants per treatment. Bars in (A) and (D) = 10 mm.

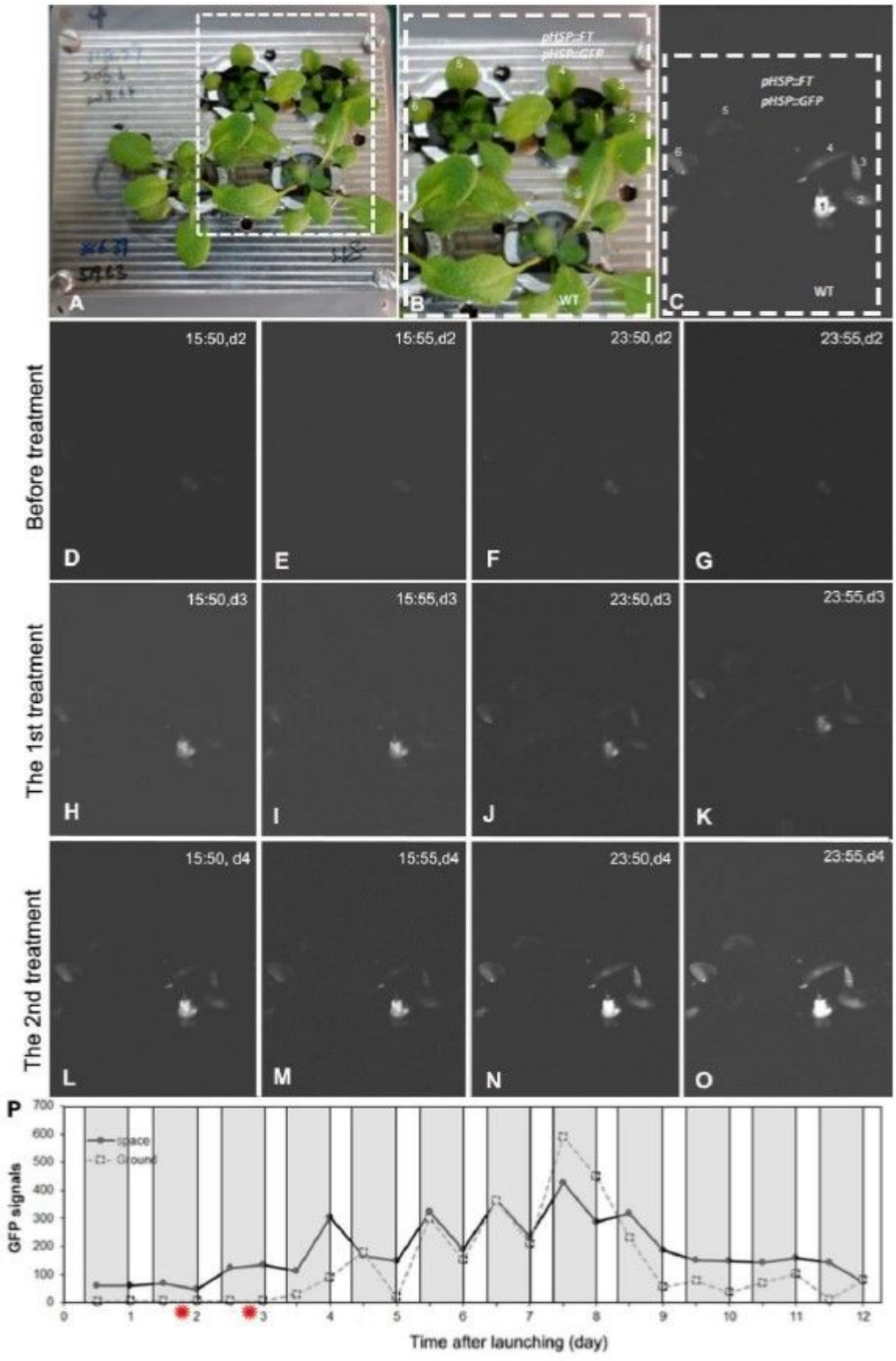


Figure 3

GFP expressing in short-day grown plants treated by 37°C heating on board SJ-10. (A) pHSP::FT; pHSP::GFP transgenic plants and wild-type (WT) plants photographed in white light before launching. The framed region in (A) is detailed in (B). (C) a represent GFP image showed the expression of GFP of leaves1-6 in (B) (D-O) Example GFP images were captured by the GFP imager at day 2 to day 4 after heating treatment. The images were captured at four time points (15:50, 15:55, 23:50 and 23:55, on board SJ-10 satellite time) every day and reflect heating inducing of GFP expressing. (P) Quantification of the intensity of GFP signal in plants under the short-day (8h light/16h dark) on oribit the SJ-10 and on ground, respectively. The GFP signals was measured as described in Materials and Methods. Values represent means for two time point images (at 15:50 and 15:55 dark period and 23:50 and 23:55 light period every satellite day, respectively). The red asteres indicate the time points of heating shock. White areas, light; dark grey panels, dark.

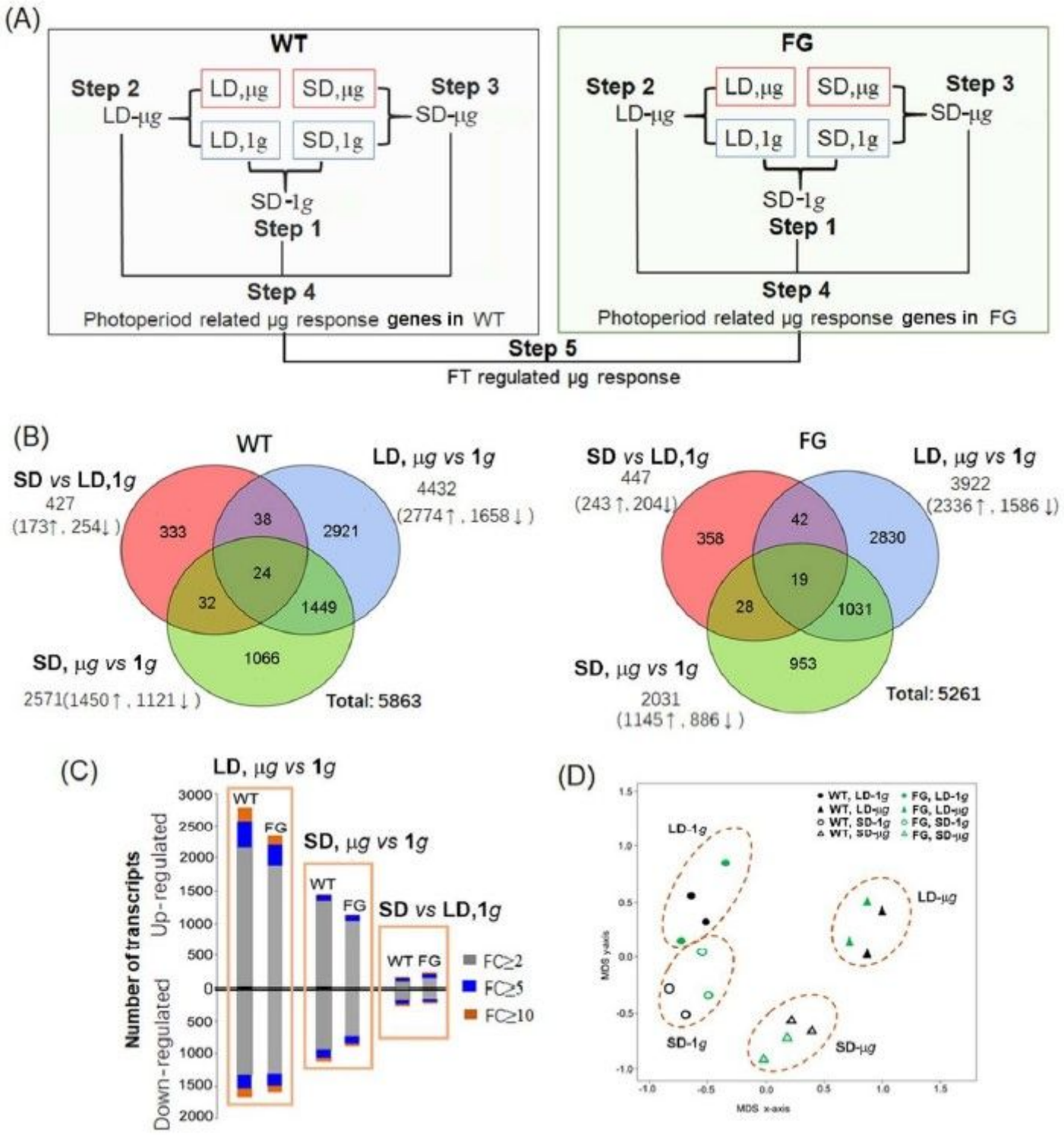
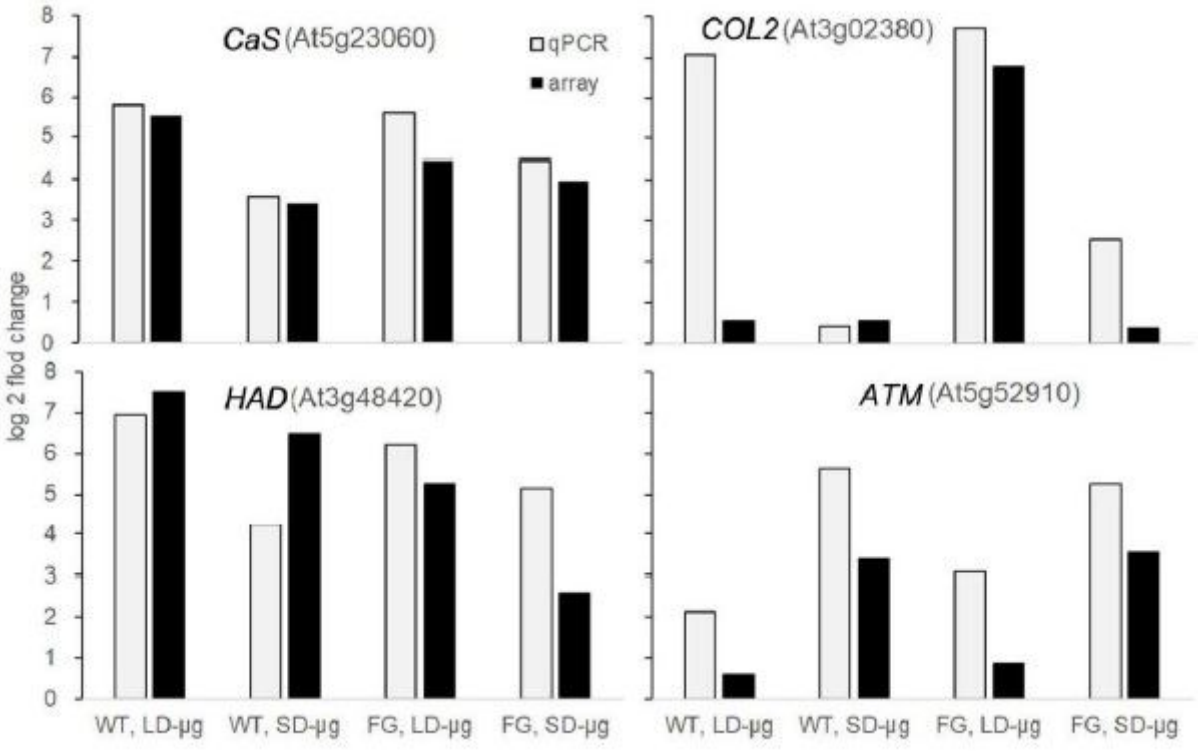


Figure 4

Transcriptional responses microgravity in Arabidopsis wild-type (WT) and trangenic plants (pHSP::FT; pHSP::GFP, FG) under the Long-day (LD) and the short-day (SD) conditions, respectively. (A) Workflow of microarray data analysis. Analysis of twofold differentially expressed genes consists of five major steps: analysis of photoperiod response genes in WT and FG plants grown on ground(1g)(step 1), identification of genes in WT and FG plants in space involved in microgravity (µg) responses under LD (step 2) and SD

(step 3) conditions, comparison of microgravity response genes in WT and FG plants specific to LD- or SD- conditions (Step 4) and selection of microgravity response genes in FT pathway by comparison of differetial expression genes (DEGs) in WT to those in FG (step 5). (B) Venn diagram of transcriptomic data. (C) Numbers of DEGs in WT and FG plants ($P<0.05$) under the LD and the SD conditions, respectively, in response to microgravity. (D) Principal component analysis demonstrates a strong difference between the flight and the ground sample transcriptones, as well as the LD and the SD samples. Multidimensional scaling (MDS) of all DEGs in WT and FG in response to microgravity under the LD and the SD, respectively.



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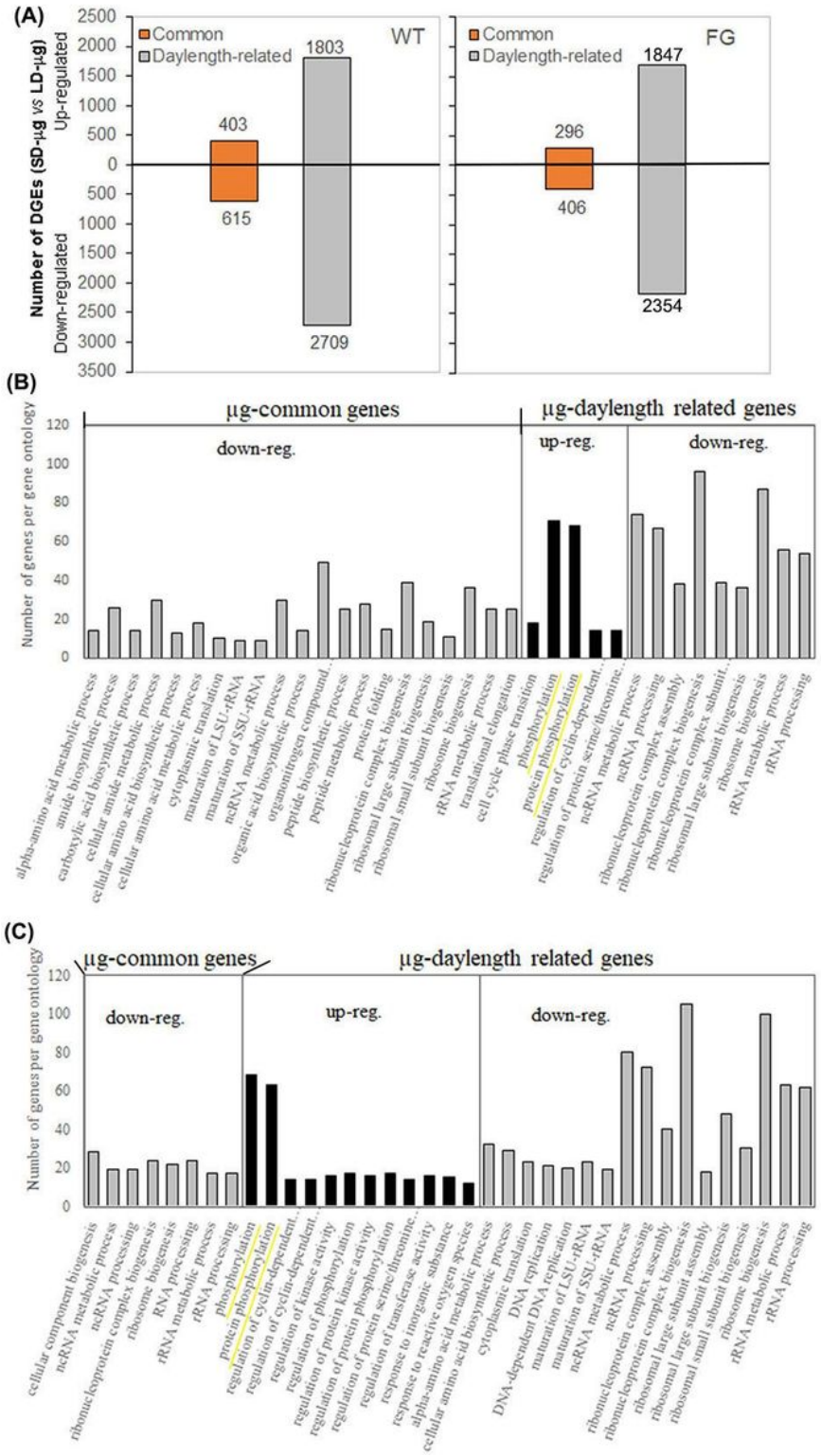

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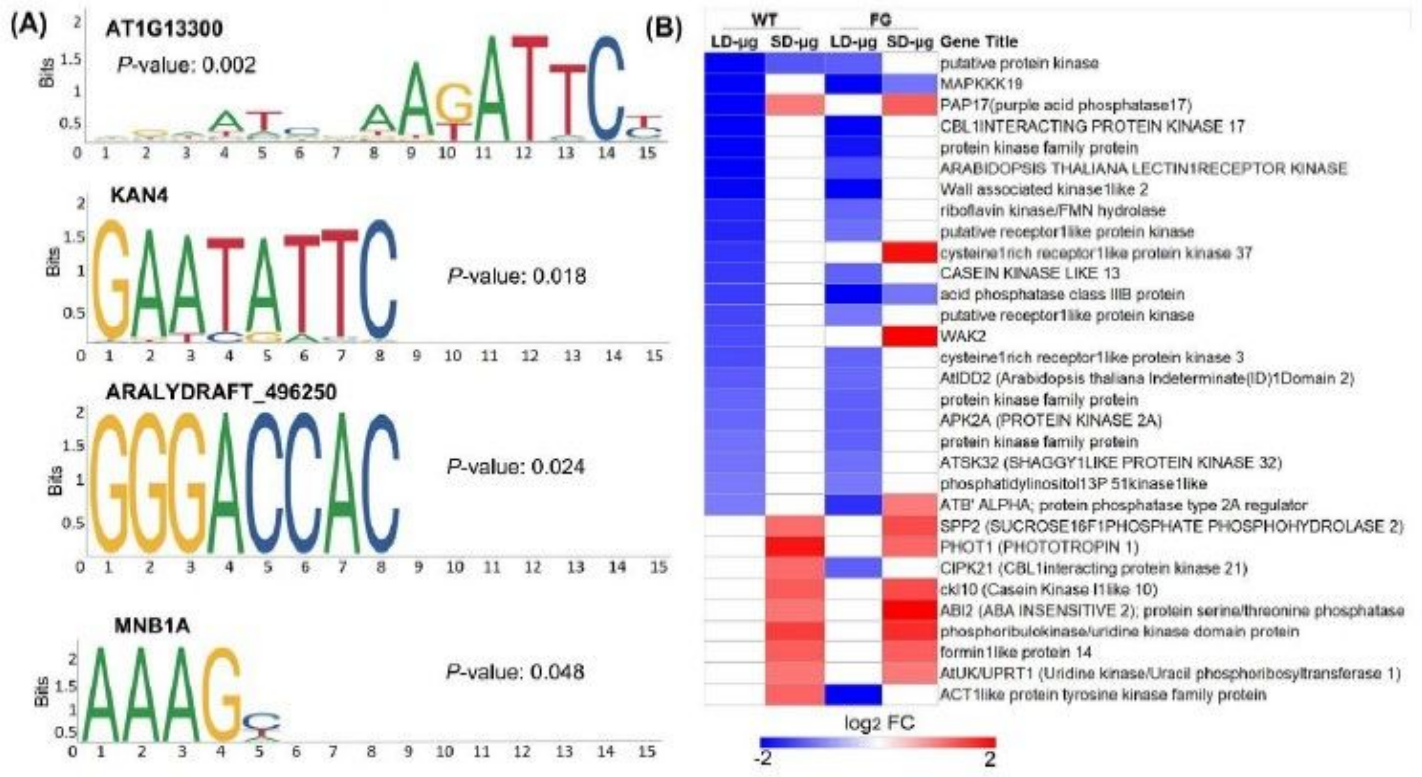

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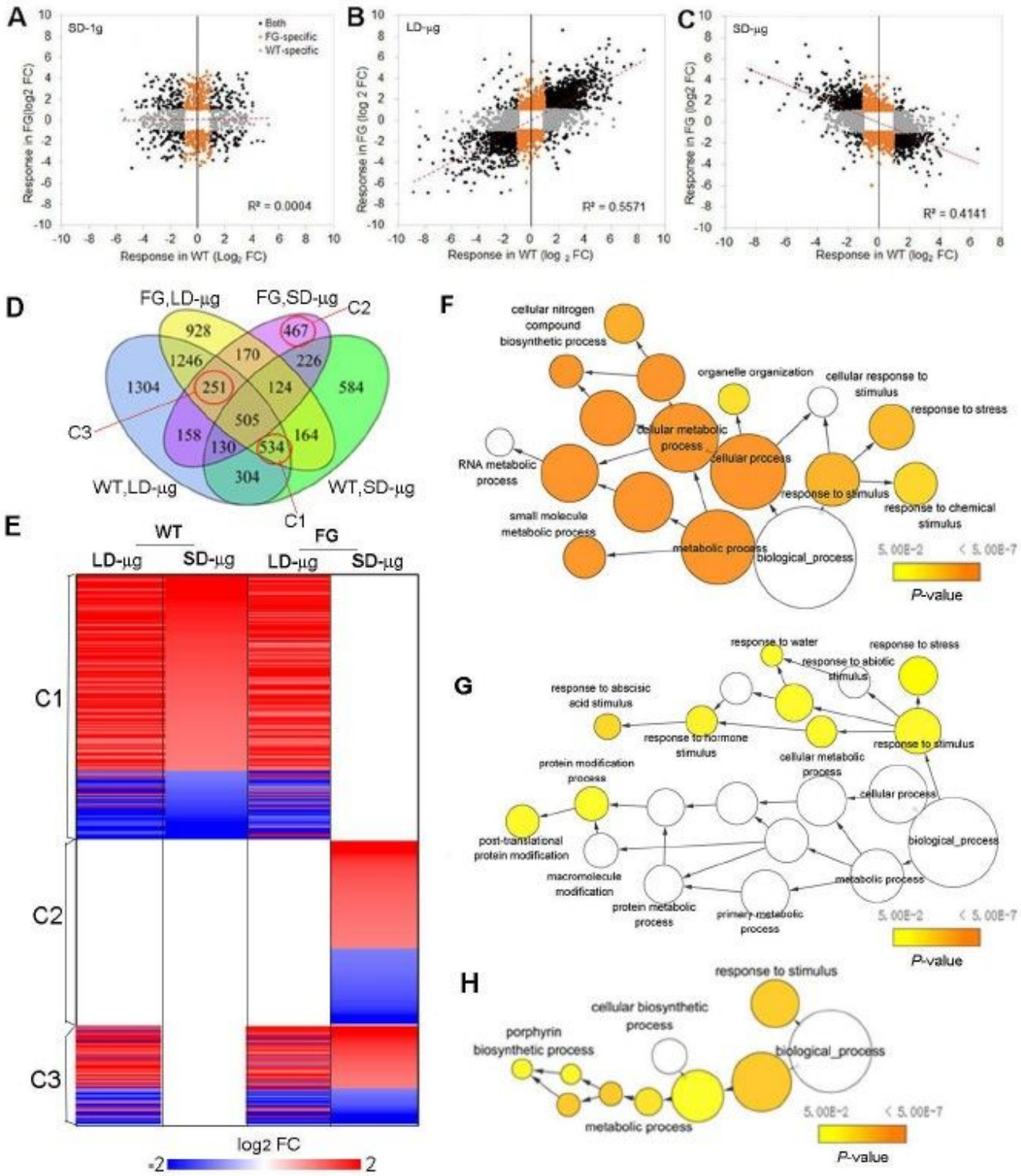

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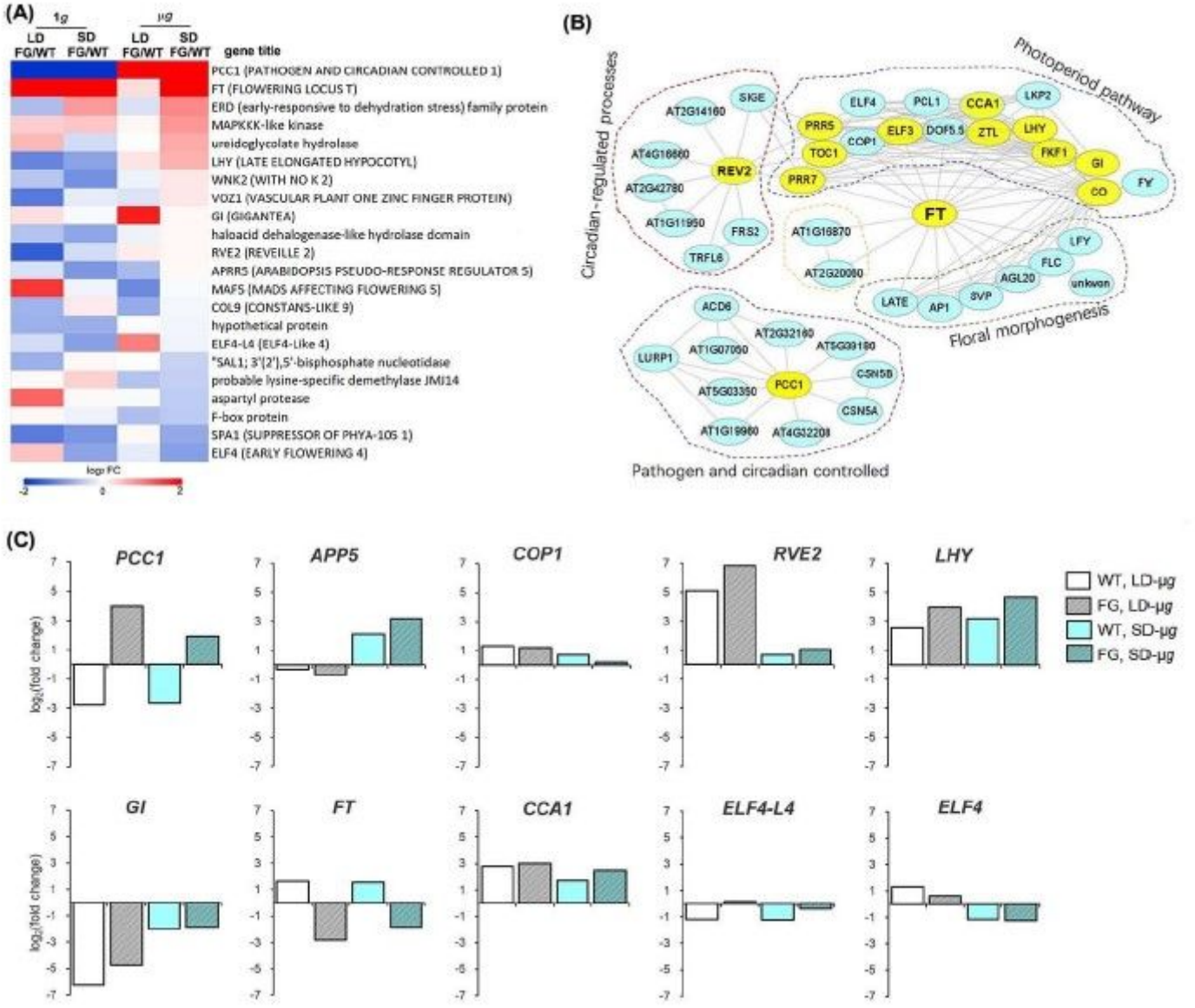

Figure 9

The core photoperiod response genes altered expression levels by expouse to microgravity. (A) Log2 FC of the 20 core photoperiod genes in the FG under LD or SD on ground (1g) and microgravity in space (μg), respectively, in comparison with these genes in wild-type (WT) under the same condition (FG/WT). (B) Diagram of the protein interaction networks of the photoperiod response genes. The genes, which altered expression level in response to microgravity in space in comparison with their controls on ground, were

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplTabS1SD1ginWT.xlsx
- SupplTabS2SD1ginFG.xlsx
- SupplTabS3LDuginWT.xlsx
- SupplTabS4LDuginFG.xlsx
- SupplTabS5SDuginWT.xlsx
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