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SPATULA regulates floral transition and photomorphogenesis in a *PHYTOCHROME B*-dependent manner in *Arabidopsis*

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ABSTRACT

Light is the most important exogenous stimulus regulating plant growth and various developmental processes. Phytochromes, especially PHYTOCHROME B (PHYB) mediates the various light-mediated processes in *Arabidopsis*. SPATULA (SPT) is an important transcription factor, which has been reported previously to participate in temperature-mediated transition from seed dormancy to germination. Here we investigate the function of *SPT* in the floral transition under long day conditions and photomorphogenesis in *Arabidopsis*. In this study, *spt-2* shows significantly delayed flowering time. But mutation of *SPT* in the background of *phyb-1* rescues the phenotype of *spt-2*. The flowering time of double mutant of *spt-2/phyb-1* is similar with the wild type. These results indicate that *SPT* promotes the transition from vegetative stage to floral stage and it regulates this transition in a *PHYB*-dependent manner. With qRT-PCR analysis, it is found that *SPT* regulates flowering time via *FLC*, *SVP*, *FT* and *SOC1*. Furthermore, *SPT* also controls photomorphogenesis. *spt-2* displays shortened hypocotyls and increased chlorophylls contents compared with the wild type. These phenotypes are also rescued in the double mutant of *spt-2/phyb-1*. These results indicate that *SPT* is also involved in photomorphogenic development in *Arabidopsis* and SPT regulates photomorphogenesis in a *PHYB*-dependent manner. Collectively, SPT is not only a temperature responder but it is also an important light regulator during plant growth and development.

1. Introduction

In most plant species, the transition from vegetative stage to reproductive stage is a crucial step for the propagation of plants. The occurrence of the transition determines the flowering time and is affected by various environmental stimuli (day-length, light quality, temperature, water, and nutrition) and endogenous regulating factors (hormones, age) [1,2]. In *Arabidopsis*, flowering time is controlled by different mechanisms, including autonomous pathway which refers to endogenous regulators that are independent of the photoperiod and gibberellin pathways [3], gibberellic acid (GA) induced pathway, vernalization and photoperiod [4–7]. In *Arabidopsis*, the flowering pathways are mainly regulated by two regulators: *CONSTANS* (*CO*) which is a flowering promoter and *Flowering Locus C* (*FLC*) which is a flowering suppressor [8,9]. CO is a zinc finger protein which integrates environmental signals to trigger flowering by directly targeting *FLOWERING LCUS T* (*FT*) [2,10,11]. *FT* encodes the florigenic protein to promote flowering. *FLC* encodes a MADS-box protein and represses flowering through direct binding to the promoter of *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) which is a major flowering promoter [9,12]. Similar with FLC, SHORT VEGETATIVE PHASE (SVP) is also a MADS-box transcription factor and represses flowering. Furthermore, SVP interacts with FLC directly to form a complex and affects cascades of downstream targets which are flowering inducers [13,14].

Photoperiod also affects the time of flowering. Plants respond to light through different types of photoreceptors including phytochromes which are the red and far-red light receptors, cryptochromes (CRYs) which are the blue and UV-A absorbing receptors, and phototropins [15–17]. Phytochromes play essential roles in various light-mediated development and physiology in plants [18,19]. The phytochrome family consists of five members (PHYA,

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PHYB, PHYC, PHYD, and PHYE) in *Arabidopsis thaliana* [20,21]. PHYB is a key regulator of the light mediated alteration in the plant developmental processes. Previous reports have demonstrated that PHYB is involved in the circadian clock and activation of de-etiolation [22–24]. It was also reported that a group of phytochrome interacting factors (PIFs): (PIF1, PIF3, PIF4, PIF5, PIF6 and PIF7) can bind to phytochromes directly [25,26]. Whereas PIF-like (PIL) can not bind phytochromes directly, they can form heterodimers with true PIFs and modulate their functions [27]. SPATULA (SPT) is a member of PIF family [1,28–30]. *SPT* gene was first identified to be involved in the development of carpel [29,31]. Studies have shown that SPT is a multi-functional transcription factor involved in a series of physiological development, including seed germination, seedling development, leaf expansion, gynoecium and fruit development, seed dispersal and root growth [31–39]. In addition, overexpression of *SPT* resulted in a long-hypocotyl phenotype, closely resembling a *PHYB* loss-of-function mutant phenotype [38]. These observations indicate that SPT plays a role in phytochrome-mediated photomorphogenesis. In another study, the enlarged cotyledon phenotype of *spt* mutants was attenuated by *phyb-9* mutant in *Arabidopsis* [33]. Furthermore, it was shown that the gynoecium, silique and seed area phenotypes of the *spt* mutants were restored by the null mutation of *PHYB*, suggesting a cooperative role for SPT and PHYB in gynoecium development [27]. In this research, we studied the flowering time, lengths of hypocotyls and content of chlorophylls with *spt-2*, *phyb-1* and double mutant *spt-2*/*phyb-1*. It is interesting to find that *SPT* is a regulator of flowering transition and it regulates flowering time in a *PHYB*-dependent manner. The results of the lengths of hypocotyls and the contents of chlorophylls also confirmed that *SPT* responds to light in a *PHYB*-dependent manner.

2. Material and methods

2.1. Plant materials growth conditions

The wild type used in study was Landsberg erecta (*Ler*), the *spt-2*, *phyb-1* mutants (*Ler* alleles) have been described previously [27,31,37,40]. As previously reported, *spt*-2 has an arginine to lysine change in the basic region of the bHLH domain, but this arginine is absolutely conserved in bHLH proteins [31]. The double mutant *spt-2/phyb-1* were obtained by cross-pollination and were confirmed by PCR and sequencing. The plants were grown in a growth room under a 16 h light (100 μmol m^{-2} s^{-1}, 22 °C) and 8 h dark (20 °C) photoperiod [41,42]. For each line, more than 60 were planted and recorded. The flowering data was recorded when the stalks come out. Seeds were sown on 1/2 MS media and kept under long day conditions (16 h/light, 20 °C, 8 h/dark, 22 °C) for 7 days. The lengths of hypocotyls were measure by Image Pro 7.0. The data shown are the means of at least 15 seedlings for each line.

2.2. RNA extractions and real-time quantitative PCR

Total RNAs were isolated from 9-day-old grown seedlings according to the protocol described previously [43]. For each line, more than 60 seedlings were planted and leaves were collected from more than 20 plants for each. cDNA was synthesized using the M-MLV Reverse Transcriptase (Promega) from 2 μg of total RNA in a 25 μl reaction and diluted 4-fold with water. Quantitative real-time PCR was performed using SYBR-green as described in a previous study [44,45]. Primers used in RT-qPCR were described previously *FLC* [9], *SOC1* [12], *FT* [10], *SVP* [13], *CHLI* [46], *CHLD* [46], *GUN5* [47]. The PCR program was as follows: 30 s at 95 °C, followed by 40 cycles of 5s at 95 °C, 30 s at 60 °C. The data was analyzed by ΔΔCT method. The data was repeated three times.

2.3. Chlorophyll content determination

Chlorophylls were extracted from small pieces of fresh cutting leaves with the reaction solution in small tubes. The reaction solution was mixture of pure acetone, pure ethanol and Distillated water (4.5: 4.5: 1). Put the cutting leaves into tubes and add 1 ml solution to each one. Keep the tubes in dark until the color of leaf pieces converted into white. Take the reading at $OD_{663\ nm}$ and $OD_{645\ nm}$. The contents of chlorophylls were counted as the formula:

$$\text{Chlorophyll } a = 12.71 \times OD_{663\ nm} - 2.59 \times OD_{645\ nm}$$

$$\text{Chlorophyll } b = 22.88 \times OD_{645\ nm} - 4.67 \times OD_{663\ nm}$$

$$\text{Total chlorophylls} = \text{Chlorophyll } a + \text{Chlorophyll } b \text{ [42]}.$$

2.4. Statistical analyses

All data shown in the figures were tested by means of Analysis of Variance for significance by using the Statistic program Version 3.5, Analytical Software, St. Paul, Mn, U. S. A. A Student's t-test was calculated at the probability at 5% (P value < 0.05) as we described before [41,48].

3. Results

3.1. SPT promotes flowering in a PHYB-dependent manner

In our work, it was found that mutation of *SPT* significantly delayed flowering time under long day (16 h/light and 8 h/dark) conditions. The mutant of *spt-2* showed delayed flowering compared with the wild type (Fig. 1A and B). The rosette leaves of *spt-2* were more than the wild type (Fig. 1C). *phyb-1* showed early flowering and decreased number of rosette leaves compared with wild type (Fig. 1A, B and C). However, mutation of *SPT* in the *phyb-1* null allele rescued the delayed flowering phenotype in *spt-2* (Fig. 1A, B and C). This suggested that *SPT* regulates flowering time in a *PHYB*-dependent manner.

3.2. SPT regulates flowering via FLC, SVP, FT and SOC1 in a PHYB-dependent manner

In order to elucidate the signaling pathways of *SPT* regulating flowering time in a *PHYB*-dependent manner, the expression levels of major flowering genes including *FLC*, *SVP*, *FT*, and *SOC1* were checked in all the lines. As shown in the results, the expressions of the flowering repressor genes *FLC*, *SVP* were significantly higher in *spt-2* compared with the wild type (Fig. 2A and B). The expression levels of *FLC* and *SVP* were significantly lower than the wild type in *phyb-1* (Fig. 2A and B). The expression of flowering promoter genes *FT* and *SOC1* were significantly lower in *spt-2*, but the expression of *FT* and *SOC1* were significantly higher in *phyb-1* (Fig. 2C and D). However, the expression of *FLC*, *SVP*, *FT* and *SOC1* in *spt-2/phyb-1* were similar with the wild type. These results confirmed that mutation of *SPT* on *phyb-1* background rescue the expression of *FLC*, *SVP*, *FT* and *SOC1* to the same level with wild type. So *SPT* regulates flowering time via *FLC*, *SVP*, *FT* and *SOC1* in a *PHYB*-dependent manner in *Arabidopsis*.

3.3. SPT regulates the accumulation of chlorophylls in a PHYB-dependent manner

While chlorophylls are required for the photoautotrophic growth in plants, the contents of chlorophylls were checked in the

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